PROSPECTS AND PROGRESS IN DRUG DESIGN BASED ON PEPTIDES AND PROTEINS

Organizers: Victor J. Hruby and Gary L. Olson

March 8-14, 1993; Taos, New Mexico

Plenary Sessions *Page*

Poster Sessions

Peptide/Protein Design

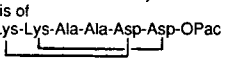
L 001 TEMPLATES DESIGNED FOR INITIATION OF SECONDARY STRUCTURE, Daniel S. Kemp, Room 18-582, Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139

Conformationally restricted templates that mimic the backbone geometry of helices or sheets act as initiation sites from which secondary structure can propagate into a covalently linked polypeptide. Reporter functions within the template permit monitoring of the stability of secondary structure as a function of solvent, temperature, and amino acid composition and sequence. Principles of template design are explored in the light of results from study of peptide conjugates of several structurally related helix and sheet templates. The roles of hydrogen bonding valence and geometry, hydrophobicity, and charge are considered. Sheet and helix propagation constants obtained in templated conjugates for natural and unnatural amino acids in water and other solvents are compared with stabilizing factors observed for model systems studied by other workers, and results of tests of site independence of these parameters are reported. The role of fluorinated alcohols in stabilizing secondary structure in water is discussed. Results of detailed NMR analyses of templated peptides in helix and sheet orientations are described.

L 002 MULTICYCLIC PEPTIDES AND GUEST PEPTIDES. John W. Taylor, Rutgers University, Department of Chemistry, Piscataway, NJ 08855-0939, USA.

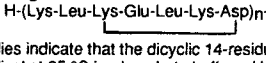
The conformational flexibility of most peptide hormones, and the current inaccessibility of their cell-surface receptors to standard methods for protein structure determination, make understanding the molecular details of peptide hormone-receptor recognition particularly challenging. The CD spectra of many flexible peptide hormones adsorbed onto hydrophobic coated quartz slides are indicative of induced amphiphilic α-helical structures that might also be present at biological interfaces. In order to test the functional importance of such structures, we are developing synthetic approaches to peptides incorporating multiple side-chain to side-chain amide (lactam) bridges, and we are using this synthetic methodology in order to investigate the helix-stabilizing properties of these bridges.

The synthetic approach is based on assembly of protected peptide segments on the Kaiser oxime resin for solid-phase synthesis, followed by intrachain peptide cyclization with concomitant cleavage from the resin, generating fully protected, side-chain bridged peptide segments. Multicyclic structures are then assembled by condensation of these segments in solution or on a solid support, using standard procedures. Furthermore, by combining FMOC/OFm protection of side-chain amines and carboxylic acids with the acid-labile protecting groups we have used in the oxime-resin cyclization *procedure, bicyclic* protected peptide building blocks may also be prepared and incorporated into segment condensation syntheses. As an example of this approach, the synthesis of



will be described.

Using the oxime resin cyclization method, we have prepared a series of peptides of general structure:



CD studies indicate that the dicyclic 14-residue (n = 2) peptide is approximately 50% helical at 25 °C in phosphate buffer, pH 7.0. The 28-residue (n = 4) peptide, with four lactam bridges, is essentially 100% helical and appears to aggregate as a dimeric coiled-coil structure. By studying analogues of the n = 2 and n = 3 peptides, we have also shown that lactam bridges linking Lys^{i},Asp^{i+4} residue pairs are more helix-stabilizing than bridges linking Lys^{i},Glu^{i+4} pairs and, surprisingly, that Orn^{i},Glu^{i+4} bridges are helix destabilizing relative to unbridged Gln residues. Application of this approach to the design of helix-stabilized analogues of neuropeptide Y and β-endorphin indicates, however, that both long-range and local structural effects can act to minimize or even negate the helix stabilization expected from a Lys^{i},Asp^{i+4} side-chain lactam bridge.

We are also tackling the complementary problem of defining the character and environment of the binding sites for peptide ligands on their cell-surface receptors. Our approach is a novel one consisting of genetically engineering the receptor-recognition epitopes of peptide hormones ("guest peptides") into surface-accessible loops of globular proteins., and then using these hormone-protein hybrids as multifunctional probes for the steric and electrostatic environments of the receptor binding sites. The initial applications of this approach to studying somatostatin receptors and the opioid-receptor selectivity of dynorphin will be described.

Computational Methods in Peptide Drug Design

L 003 INTUITION AND COMPUTER-ASSISTANCE IN THE DESIGN OF BIOLOGICALLY ACTIVE COMPOUNDS, Paul A. Bartlett, Georges Lauri, Bradley P. Morgan, Felicia A. Etzkorn, Tao Guo, Department of Chemistry, University of California, Berkeley, California 94720

A number of opportunities for a structure-based approach to molecular design can be identified, based on the information available. If the structure of a ligand binding site is known, de novo design techniques can be used to devise a molecule that complements the site geometrically and electronically. If the 3-dimensional structure of a ligand is available, mimics can be designed that incorporate the key points of interaction in a smaller or different framework. And if information on the bound conformation of the ligand or the structure of the ligand-receptor complex has been determined, modifications in the ligand structure can be made to enhance binding affinity.

In each case, a vector-based approach may be invoked in the design of the ligand. Such an approach necessitates a focus on the 3-dimensional relationship between bonds, in contrast to the attention paid to relationships between atoms in other methods. The program CAVEAT has been devised to take advantage of this insight. Examples of the vector-based approach in the successful design of a number of structure-based inhibitors will be described, along with the development and applications of CAVEAT and the companion structural databases TRIAD and ILIAD.

L 004 COMPUTER-BASED PEPTIDE AND PEPTIDOMIMETIC DESIGN. Garland R. Marshall, Gregory Nikiforovich, Krystof Kaczmarek, Krystyna Plucinska, Andrea Tafi, Fabrice Cornille, Urszula Slomczynska, Kaiming Li and Wei-Jun Zhang. Center for Molecular Design, Washington University, St. Louis, Missouri 63130

In deducing the biologically active conformation of a peptide in preparation to design peptidomimetics, two phases of research are necessary. First is the accumulation of structure-activity data on a series of analogs. Unusual amino acids and dipeptide derivatives provide the chemist with an appropriate vocabulary for perturbing the normal conformational ensemble available to biologically active peptides in order to probe the receptor-bound conformation. The requirement for appropriate sidechain functionality in order to preserve recognition and allow activation of the receptor has led to the development of chimeric amino acids in which conformational properties are combined with the sidechain requirements. One example is found with α, α-dialkylamino acids such as MeF, MeP, MeY, and MeA (Aib) where D-Ala has been hybridized with Phe, Pro, Tyr, and Ala, respectively. Chimeric amino acids which has proven exceptionally useful in determining the receptor-bound conformations of angiotensin II (AII) and bradykinin (BK) has been functionalized proline analogs, such as 4-mercaptoproline (Mpt, *trans* and Mpc, *cis*) and 4-aminoproline (Apt, *trans* and Apc, *cis*) which combines the conformational restrictions of Pro with the sulfhydryl and amino function groups, respectively, for [Supported by NIH grant GM24483]

introduction of sidechain cyclization. As a diverse set of modifications which retain significant biological activity become available, then exploration of the conformational space through molecular mechanics generates sets of low energy conformers. A common three-dimensional pattern of important sidechains can be the essential feature required for recognition by and activation of the receptor. These hypotheses can be tested by design and synthesis of novel sequences which can also present the same pattern.This approach has led to models of the receptor-bound conformation of AII,BK,CCK, etc. In addition to unusual amino acids, novel dipeptides which have predictable conformational effects are of great utility. An example is β-turn mimetics. Novel electrochemical cyclizations of dipeptides of Ser-Pro and other X-Pro and X-N-MeAA dipeptides have yielded bicyclic dipeptides which serve as conformational constraints. Analyses on the impact of these and other modifications on sterically allowed conformations offers a rationale for the choice of peptide modification. Analysis of the confomational impact of these analogs as well as the effects of sidechain cyclization provide the peptide chemists with a set of building blocks with predefined properties to allow testing of conformational hypotheses.

L 005 HYDROPHOBIC CLUSTERING, HYDROPHOBIC COLLAPSE AND THE EFFECT OF WATER ON THE BIO-ACTIVE CONFORMATIONS OF PEPTIDES AND PEPTIDOMIMETICS. D. H. Rich, School of Pharmacy and Department of Chemistry, University of Wisconsin-Madison. 425 N. Charter St. Madison, WI 53706.

Kinetic studies of the binding of cyclosporine (CsA) to the peptidyl prolyl *cis-trans* isomerase (PPIase) cyclophilin (Cyp) indicate that water and not the enzyme induces the bioactive conformation of the ligand [1-3]. A tight-binding CsA conformation that is close in structure to the enzyme-bound conformation of CsA [4-6] is formed when CsA is dissolved in water [7] or in LiCl/THF [8]. Literature reports indicate that other peptide and non-peptide derived systems may be affected in a similar manner [1]. The process wherein hydrophobic clustering of aromatic or aliphatic groups in peptide-derived inhibitors dramatically distorts the aqueous conformation from that observed in organic solution can be called hydrophobic collapse [1]. Extrapolations of ligand conformations determined in organic solvents or *in vacuo* to the conformations in aqueous media is likely to be incorrect when the molecules are flexible and contain multiple hydrophobic groups. This has led to our suggestion that mimetics of peptides should be designed either to exploit or resist hydrophobic collapse, depending upon the starting point in the design process. Furthermore, the effect of aqueous media on the conformations of peptides and mimetics with hydrophobic ligands must be considered in the design process [1].

[1] R. A. Wiley and D. H. Rich, *Medicinal Research Reviews*, in press.
[2] J. L Kofron, P. Kuzmic, V. Kishore, E. Colon-Bonilla, and D. H. Rich. *Biochemistry* **1991**, *30*, 6127.
[3] J. L. Kofron, P. Kuzmic, V. Kishore, G. Gemmecker, S. W. Fesik, and D. H. Rich. *J. Am. Chem. Soc.***1992**, *114*, 2670.
[4] S. W. Fesik, R. T. Gampe, Jr., T. F. Holzman, D. A. Egan, R. Edalji, J. R. Luly, R. Simmer, R. Helfrich, V. Kishore, and D. H. Rich. *Science* **1990**, *250*, 1406.
[5] S. W. Fesik, R. T. Gampe, H. L. Eaton, G. Gemmecker, E. T. Olejiniczak, P. Neri, T. F. Holzman, D. A. Egan, R. Edalji, R. Simmer, R. Helfrich, J. Hochlowski, M. Jackson. *Biochemistry* **1991**, *30*, 6574.
[8] C. Weber, G. Wider, B. Von Freyberg, R. Traber, W. Braun, H. Widner, K. Wüthrich. *Biochemistry* **1991**, *30*, 6563.
[13] M. Kock, H. Kessler, D. Seebach, and A. Thaler. *J. Am. Chem. Soc.* **1992**, *114*, 2676.
[7] D. Altschuh, O. Vix, B. Rees, and J.-C. Thierry. *Science* **1992**, *256*, 92.

Pharmaceutical Applications (Joint)

L 006 NMR STUDIES OF RAS P21 AND HIV PROTEASE, Sharon Campbell-Burk[1], Rich DeLoskey[1], Tom Van Aken[1], Peter Domaille[1], P. Weber[1], M. Hillman[1], Richard Yates[1], E. Laue[2], [1]Du Pont Merck Pharmaceutical Company, Wilmington, DE and [2]Cambride University, Cambridge, England.

We have been involved in NMR efforts directed toward understanding the structural and dynamic basis for protein-antagonist interactions in two systems: ras p21 and HIV protease. Both proteins are of wide spread pharmaceutical interest. The c-H-ras p21 protein is the product of the human ras proto-oncogene and is amongst the most prevalent of all oncogenes found in human tumors. HIV-1, on the other hand, is an aspartyl-protease responsible for proteolytic processing of gag and gag-pol fusion protein which is essential for HIV infectivity. Both systems have proven to be tractable NMR problems after considerable optimization. NMR progress on these systems and efforts involved in expression, purification, refolding, concentration, solubility as well as genetic engineering will be discussed.

L 007 HIGH RESOLUTION STRUCTURE, DYNAMICS AND FUNCTION OF THE GRAMICIDIN A CHANNEL, Timothy A. Cross, Christopher L. North, Steven M. Pascal, Randal R. Ketchem, Weidong Hu and Kwun-Chi Lee, Institute of Molecular Biophysics and Department of Chemistry, Florida State University, Tallahassee, FL 32306

The function of the gramicidin A dimer in a lipid bilayer is to selectively conduct monovalent cations. However, the atomic details of this function remain mysterious. The extent of cation solvation by the polypeptide backbone, which lines the channel, is undetermined. While the cations are in the channel they are largely stripped of their waters of hydration, however the carbonyl oxygens can play a solvating role. The location of the carbonyl oxygens relative to the channel axis is dependent upon knowing, not only the Φ and Ψ torsion angles, but also the ω torsion angle in the polypeptide backbone. Local dynamics of these carbonyl oxygens may also be critical for the functioning of the channel. For this it is important to develop a model for the local motions, rather than assuming a general model, and then solve for the local motional frequencies. Solid state NMR of isotopically labeled gramicidin channels in oriented bilayers are being used to determine a detailed model for the molecular motions of the polypeptide backbone. The axis about which local motions occur has been determined to be coincident with the axis joining adjacent alpha carbons and the amplitude which varies from one peptide plane to another has an RMSD of 12 to 17°. Furthermore, through ^{2}H lineshape analyses it has been clearly shown that the global correlation time is in the μsec timescale. In light of this motionally averaged model and the global correlation time it has been possible to analyze the relaxation data for ^{15}N sites at different field strengths and to show that the frequency for these local motions is in the nanosecond timescale. This raises the exciting possibility that the dynamics of the polypeptide backbone and the kinetic rate for cation transit which also occurs on the nanosecond timescale may be correlated.

Another functional question is centered on the role of the four indole rings in each gramicidin monomer. When these indole groups are replaced with phenyl groups the conductance decreases by a factor of 20. Through solid state NMR studies the orientation of the indole dipole moments have been determined and their potential import for conductance will be discussed.

L 008 MULTIDIMENSIONAL NMR STUDIES OF IMMUNOSUPPRESSANTS AND THEIR BINDING PROTEINS. Stephen Fesik, Andrew Petros, Tim Logan, Liping Yu, Robert Xu, Yves Theriault, David Nettesheim, Robert Meadows, Edward Olejniczak, Jay Luly, Thomas Holzman, Earl Gubbins, and Robert Simmer. Abbott Laboratories, Pharmaceutical Discovery Division, Abbott Park, IL 60064.

Using isotope-edited and isotope-filtered NMR techniques, the conformation and active site environment of protein-bound ligands can be rapidly determined. In addition, due to recent advances in heteronuclear three- and four-dimensional NMR methods, virtually complete ^{1}H, ^{13}C, and ^{15}N assignments and high resolution three-dimensional structures of protein/ligand complexes can be obtained. In principle, this type of structural information on a small molecule bound to its target site could aid in the design of analogs which are more suitable as pharmaceutical agents than the inital lead compounds. In order to aid in the design of improved immunosuppressants, we have been studying the three-dimensional structures of several immunosuppressants bound to their target proteins by NMR spectroscopy. Cyclosporin A (CsA) and FK506, two structurally different immunosuppressants, bind to two different target proteins. CsA binds to cyclophilin (165 aa); whereas, FK506 and the structurally related immunosuppressants, ascomycin and rapamycin, bind to FKBP (107 aa). Both proteins are peptidyl-prolyl cis-trans-isomerases, and both play an important role in T cell activation. In this presentation, the conformation and active site environment of several protein-bound immunosuppressants determined from isotope-filtered NMR experiments will be described. This information is used to explain the structure/activity relationships observed for these ligands. In addition, high resolution structures of the ascomycin/FKBP and CsA/cyclophilin complexes determined from a quantitative analysis of heteronuclear 3D and 4D NOE data will be presented. Our attempts to use these 3D structures in the design of novel immunosuppressants will be discussed.

L 009 STRUCTURE OF HNRNP C PROTEIN AND ITS COMPLEXES TO RNA OLIGONUCLEOTIDES, Luciano Mueller[1], Michael Wittekind[1], Mark S. Friedrichs[1], Donna Bassolino[1], Matthias Görlach[2], Gideon Dreyfuss[2], [1]Bristol-Myers Squibb Pharmaceutical Research Institute, 4000, Princeton, NJ 08543-4000, [2]Howard Hughes Medical Institute and Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA 19104-6148.

The hnRNP C1 and C2 are abundant nuclear proteins that bind to heterogeneous nuclear RNAs and appear involved with pre-mRNA processing. We have studied the RNA-binding domain of hnRNP C protein, which contains ninety three amino acid residues. Sequential resonance assignments were performed using heteronuclear multidimensional NMR methods using uniformly [^{15}N] and [^{15}N, ^{13}C]- enriched samples at a molar concentration of 2.5 mM. Distance constraints for structure calculations were extracted from three- and four-dimensional NOESY spectra by manual and automated procedures. Homo- and heteronuclear vicinal J-couplings provided torsion angle constraints. These couplings were obtained with various heteronuclear methods. A valuable source for inter proton J-coupling information were ^{13}C-correlated proton TOCSY spectra. The protein has a compact folded structure (babbab) with the RNA-binding pocket formed by the four-stranded anti-parallel b-sheet. Besides the free protein, we also characterized its complexes to putative target molecules i.e. RNA oligo nucleotides which contain a segment of multiple uracil residues. Our observations suggest that in addition to, the beta sheet structure of the domain,both the flexible N- and C- terminal ends of the peptides are also involved in the RNA binding.

Peptide Mimetic Design

L 010 REDUCTION OF PEPTIDE SAR TO A NON-PEPTIDE SCAFFOLD: DESIGNED INHIBITORS OF GPIIbIIIa / FIBRINOGEN BINDING, Brent K. Blackburn and Robert S. McDowell, Genentech, Inc., South San Francisco, CA.

The activation dependent platelet glycoprotein IIbIIIa is a heterodimer that binds to fibrinogen resulting in platelet aggregation. Inhibition of this interaction can be achieved with Arg-Gly-Asp (RGD) containing peptides. Molecular dynamic calculations and solution structure determination[1] coupled with a wealth of structure/activity studies of R-G-D containing cyclic peptides[2] established the structural and conformational criteria from which the design of a series of non-peptide RGD mimics may be attempted. Primary among these criteria is the shape which the cyclic peptide adopts. Translation of this shape information into a novel lead compound was subsequently accomplished. The importance of shape as a primary design criterion was addressed by the preparation of materials that remove that characteristic from the molecule without disturbing the principle binding determinants. An overview of this approach toward the identification of a small-molecule inhibitor of IIbIIIa/Fg binding will be presented. Examination of the structural and conformational requirements for selectivity over other RGD sensitive cellular receptors (eg. $\alpha^V\beta^3$) will also be discussed.

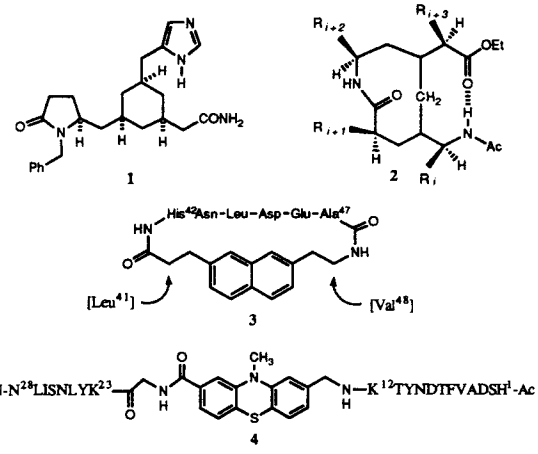
[1]McDowell, R.S.; Gadek, T.R., *J. Am Chem. Soc.* **1992**, *114*, 0000-0000. [2]Barker, P.B.; Bullens, S.; Bunting, S.; Burdick, D.; Chan, K.S.; Deisher, T.; Eigenbrot, C.; Gadek, T.; Gantzos, R.; Lipari, M.T.; Muir, C.D.; Napier, M.A.; Pitti, R.M.; Padua, A.; Quan, C.; Stanley, M.; Struble, M.; Tom, J.Y.K.; Burnier, J.P. *J. Med. Chem.* **1992**, *35*, 2040-2048.

L 011 TOWARD THE RATIONAL DESIGN OF ENDOTHELIN ANTAGONISTS, W.L. Cody, A.M. Doherty, J.B. Dunbar Jr., M.D.Reily, S.J.Haleen[1] and E.E. Reynolds[1], Departments of Medicinal Chemistry and Pharmacology[1], Parke-Davis Pharmaceutial Research, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, MI 48105.

The endothelins (ET's) and sarafotoxins (SRTX's) comprise a family of potent vasoconstricting peptides that contain 21-amino acids arranged in a unique bicyclic motif formed by two disulfide bridges between positions 1-15 and 3-11. All members of this family possess a hydrophobic C-terminal hexapeptide, culminating in tryptophan with a free carboxylate. We have performed a systematic examination of this C-terminal hexapeptide to develop potent functional antagonists of endothelin induced vascoconstriction. The critical modification that leads to a functional antagonist involves the incorporation of unnatural hydrophobic D-amino acids in the sixteen position, specifically D-3,3-diphenylalanine (D-Dip, PD-142893). Two subtypes of the endothelin receptor have been described and designated ET_A and ET_B. Other antagonists of endothelin have been described that are specific for the ET_A receptor subtype, but PD-142893 (Ac-D-Dip-Leu-Asp-Ile-Ile-Trp) is the *only* known functional antagonist of vasoconstriction in tissues containing either of the reported receptor subtypes with a pA_2 value of approximately 7.3 at each subtype. The structural parameters of PD-142893 and related analogues will be addressed in relation to its pharmacological profile and contrasted with other known endothelin antagonists.

L 012 SMALL MOLECULE MIMETICS OF PEPTIDES AND PROTEINS--AN OVERVIEW, Gary L. Olson, Elliot Chiang, Ho-Chuen Cheung, Michael Bös, Matthew Voss, Michael Kahn, Victoria Lombardo, Mary Pat Bonner, Ramakanth Sarabu, Kathleen Lovey, Vincent S. Madison, Charles Cook, David Fry, David Bolin, Margaret O'Donnell, George Vincent, Jerry Sepinwall, Hoffmann-La Roche, Inc., Nutley, NJ 07110.

Basic concepts of peptide mimetic design, and examples of the construction of templates which mimic architectural elements of peptide and protein structure will be discussed. This program includes mimetics of β-turns, α-helices, and Ω-loops. In addition, potent, orally active mimetics of the tripeptide neuroendocrine hormone, thyrotropin releasing hormone (TRH, p-Glu-His-ProNH$_2$) have been developed with the peptide backbone replaced by a cyclohexane ring framework (e.g., **1**, Ro 24-9975). The β-turn example to be discussed is the nine-membered lactam system **2**, shown to closely resemble protein β-turn geometry in model systems. A naphthalene-based spacer molecule has also been designed to constrain an Ω-loop segment of one of the prominent external loops of interleukin-1α. The naphthalene-bridged hybrid peptide **3** was shown by ^{1}H-NMR and molecular dynamics to mimic the loop structure well. The construction of a spacer to replace segments of the α-helical region of vasoactive intestinal peptide (VIP) will also be presented. VIP analogs incorporating the phenothiazine (e.g., **4**) exhibited full agonist activity, with potency approximating 10% of native VIP. Prospects for application of this methodology to design nonpeptide antagonists of protein-receptor interactions will be discussed.



Conformation-Biological Activity Relationships

L 013 CONFORMATIONAL CONSIDERATIONS IN THE DESIGN OF OPIOID RECEPTOR LIGANDS: AN OVERVIEW, Peter W. Schiller, Brian C.Wilkes, Brian J. Marsden, Thi M.-D. Nguyen, Grazyna Weltrowska, Carole Lemieux, and Nga N. Chung, Laboratory of Chemical Biology and Peptide Research, Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal, Que., Canada H2W 1R7.

Small linear neuropeptides are structurally flexible molecules, capable of existing in a number of different conformations of comparably low energy. This structural flexibility precludes the determination of the bioactive conformation in solution and, furthermore, may be responsible for the lack of receptor selectivity of many of the naturally occurring peptide hormones and neurotransmitters, since conformational adaptation to different receptor topographies takes place (1). In recent years, the introduction of conformational constraints into peptides either locally at a particular amino acid residue (N^{α}- or C^{α}-methylation, substitution of dehydro- or cyclic amino acids, etc.) or more globally (peptide cyclizations) emerged as a successful concept in the design of peptide analogs and peptidomimetics. In many cases, conformationally restricted peptide analogs showed high receptor selectivity and greatly improved conformational integrity.

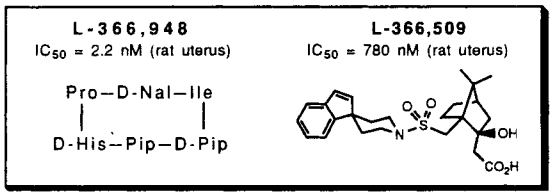
Efforts in the opioid peptide field led to a number of conformationally restricted analogs (agonists) with high preference for either μ or δ opioid receptors. Examples of cyclic analogs with high receptor selectivity include the μ-selective compounds H-Tyr-c[-D-A_2bu-Gly-Phe-Leu-] (1) and H-Tyr-D-Orn-Phe-Asp-NH_2 (2), and the δ-selective analogs H-Tyr-D-Pen-Gly-Phe-D(or L)-Pen-OH (3) and H-Tyr-D-Cys-Phe-D-Pen-OH (4). In the case of the μ-selective cyclic enkephalin analogs reversal of individual peptide bonds or their replacement by a thioamide moiety further improved receptor selectivity. Conformational analyses of the μ-selective cyclic analogs revealed that their peptide ring structures had limited structural flexibility but in some cases still showed conformational heterogeneity (e.g. cis/trans peptide bond isomerization). The observation that the exocyclic Tyr residue and the Phe side chain in these compounds still enjoyed extensive orientational freedom prompted the substitution of constrained (cyclic) analogs of these aromatic amino acids in the 1- and/or 3-position of the cyclic opioid peptide analog H-Tyr-D-Orn-Phe-Glu-NH_2. These substitutions drastically limited the available side chain conformational space, further reduced the conformational heterogeneity of the peptide backbone and in some cases improved μ selectivity. One of these compounds, H-Hat-D-Orn-Aic-Glu-NH_2 (Hat = 6-hydroxy-2-aminotetralin-2-carboxylic acid, Aic = 2-aminoindan-2-carboxylic acid), contains only two freely rotatable bonds and is the most rigid opioid peptide analog known.

Substitution of the conformationally restricted Phe analog tetrahydroisoquinoline-3-carboxylic acid (Tic) in position 2 of dermorphin/deltorphin-related tri-and tetrapeptides led to the discovery of a new class of δ opioid receptor antagonists without μ antagonist properties (5). The prototype antagonist, H-Tyr-Tic-Phe-Phe-OH (TIPP), showed high potency and unprecedented δ selectivity. The corresponding peptide amide, H-Tyr-Tic-Phe-Phe-NH_2 (TIPP-NH_2) was also a potent δ-selective δ antagonist and a moderately potent μ agonist, thus representing the first known compound with μ agonist/δ antagonist properties. The diastereoisomeric peptide, H-Tyr-D-Tic-Phe-Phe-NH_2, was a potent and highly μ-selective μ agonist, indicating that configurational inversion at the Tic^2 residue turned a δ-selective antagonist into a μ-selective agonist. Comparison of the opioid activity profiles of the Tic^2 peptides with those of the corresponding $NMePhe^2$-analogs (NMePhe = N^{α}-methylphenylalanine) revealed that imposition of conformational constraints in a peptide not only may alter receptor selectivity, but also may decrease, totally abolish or even enhance intrinsic activity ("efficacy").

1. P.W. Schiller and J. DiMaio, Nature (London) 297, 74 (1982)
2. P.W. Schiller et al., J. Med. Chem. 28, 1766 (1985)
3. H.I. Mosberg et al., Proc. Natl. Acad. Sci. USA 80, 5871 (1983)
4. H.I. Mosberg et al., Life Sci. 43, 1013 (1988)
5. P.W. Schiller et al., Proc. Natl. Acad. Sci. USA, in press

L 014 NON-PEPTIDE OXYTOCIN RECEPTOR LIGANDS, Peter D. Williams, Mark G. Bock, Bradley V. Clineschmidt[1], Chris Culberson, Ben E. Evans, Roger M. Freidinger, James L. Leighton, Joseph M. Pawluczyk, Debra S. Perlow, Douglas J. Pettibone[1], Daniel F. Veber, Departments of Medicinal Chemistry and [1]New Lead Pharmacology, Merck Research Laboratories, West Point, PA 19486.

Biologically active peptides play important roles in the physiology and pathophysiology of living systems and thus serve as important targets for drug design. Advances in molecular biology, spectroscopy, and chemical synthesis have made possible rapid progress in our understanding of the occurrence, structure, and function of these molecules and the way in which they interact with their receptors. Although synthetic peptides have proven to be particularly useful for determining critical structural information relating to biological activity, the development of peptides as therapeutic agents has often been thwarted in cases where a long duration of action and oral bioavailabilty are desired. Thus, a major goal in recent years has been the development of non-peptide ligands for peptide receptors. The design of non-peptide structures based on a knowledge of peptide structure/conformation activity relationships is an attractive and rapidly evolving area. Receptor-based screening of natural products and chemical sample collections, however, has proven to be a useful discovery method, with many examples of non-peptide ligands having been reported over the last few years. We have used this approach for the discovery of novel ligands for receptors of the neurohypophyseal hormones, oxytocin and vasopressin. For example, L-366,948 is a member of a novel class of selective oxytocin receptor ligands that was developed from a cyclic hexapeptide lead identified through receptor-based screening of natural products. L-366,948 is a potent antagonist of oxytocin-induced uterine contractions in the near-term pregnant rhesus monkey when given intravenously. The selectivity of L-366,948 for the oxytocin receptor versus the vasopressin V_1 and V_2 receptor subtypes, its reasonable duration of action, and its lack of agonist properties support the potential development of L-366,948 as a therapeutic agent for the treatment of preterm labor. Oral bioavailability in the cyclic hexapeptide class, however, is lacking. Our attention has now turned to a class of orally active, selective, non-peptide oxytocin receptor ligands related to L-366,509, the origin of which is based on a lead discovered through receptor-based screening of the chemical sample collection at Merck. Molecular modelling shows a tight spatial overlap of the indene and camphor moieties in L-366,509 with the critical naphthylalanine and isoleucine side chains in L-366,948, thus offering the view of L-366,509 as being a dipeptide mimetic and the potential for enhancing receptor affinity based on knowledge of structure-activity relationships in the cyclic hexapeptide class. Further developments in the camphor-based non-peptide class and the potential use of an oxytocin antagonist as a therapeutic agent for the treatment of preterm labor will be discussed.



Peptide Receptor Interactions

L 015 STRUCTURE-BASED DRUG DESIGN: INHIBITORS OF PURINE NUCLEOSIDE PHOSPHORYLASE, J. A. Montgomery,[1,2] S E. Ealick,[3] S. Babu,[1] C. E. Bugg,[4] M. D. Erion,[5] W. C. Guida,[6] and J. A. Secrist III,[2] [1]BioCryst Pharmaceuticals, Inc., Birmingham, AL 35244, [2]Southern Research Institute, Birmingham, AL 35255, [3]Cornell University, Ithaca, NY 14853, [4]University of Alabama at Birmingham Center for Macromolecular Crystallography, Birmingham, AL 35294, [5]Gensia Pharmaceuticals, Inc., San Diego, CA 92121, [6]CIBA-GEIGY Corp. Summit, NJ 07901.

Purine nucleoside phosphorylase catalyzes the reversible phosphorolysis of purine ribonucleosides and 2′-deoxyribonucleosides to the free base and ribose-1-phosphate or 2′-deoxyribose-1-phosphate as follows:

purine nucleoside + Pi = purine + (d)ribose-1-phosphate

The enzyme has been isolated from a variety of both eucaryotic and procaryotic organisms and functions in the purine salvage pathway. PNP isolated from human erythrocytes is specific for guanosine, inosine, and many of their analogs, although PNPs from other organisms show varying levels of specificity. The human enzyme is a trimer with identical subunits and a total molecular weight of about 97,000 daltons. Interest *in PNP arises from its* critical role in purine nucleoside metabolism and its role in T-cell function. *In* addition to protecting purine nucleoside analogs with chemotherapeutic potential -- such as 2′-deoxythioguanosine and 2′,3′-dideoxyinosine -- from destruction by PNP; inhibitors of this enzyme might be useful in the treatment of T-cell proliferative diseases, such as T-cell leukemias and lymphomas, in the suppression of the host-vs-graft response in organ transplant patients, and in the treatment of T-cell mediated autoimmune diseases such as rheumatoid arthritis and lupus. We determined the three-dimensional structure of human PNP using X-ray crystallography and have utilized these results in designing novel inhibitors of this key enzyme.[1]

Ideas for new compounds were considered by a drug design team consisting of crystallographers, molecular modelers, and organic chemists. Proposed targets were screened by modeling the enzyme/inhibitor complex using interactive computer graphics and energy minimization. The most promising compounds were then prioritized and synthesized. The resulting compounds were initially analyzed in two ways: (1) An *in vitro* assay was performed to determine the concentration of compound required to inhibit PNP activity to 50% of controls under standard conditions (IC_{50}), and (2) compounds were soaked into previously prepared crystals of PNP and subjected to X-ray diffraction analysis using difference Fourier maps. The results of these analyses frequently led to proposed modifications of existing compounds or to the identification of new target compounds. Further analyses, such as K_i determinations, and whole cell and *in vivo* assays, were performed on the most potent compounds. This iterative approach has allowed us to prepare several families of potent membrane-permeable inhibitors with IC_{50}'s in the 6-30 nM range. The inhibition is competitive with respect to inosine and noncompetitive with respect to phosphate. The structures of inhibitors and their interactions with PNP will be described. Selected inhibitors have been shown to effectively inhibit PNP in whole cells and in rats, and to reduce the catabolic cleavage of 2′,3′-dideoxyinosine (ddI) and other purine nucleosides. The half-life and AUC of ddI in rats are tripled in the presence of a PNP inhibitor.

[1]Ealick, S. E.; Babu, Y. S.; Bugg, C. E.; Erion, M. D.; Guida, W. C.; Montgomery, J. A.; Secrist III, J. A. *Proc. Natl. Acad. Sci. USA*, **1991**, *88*, 11540-11544.

L 016 DNA-PEPTIDE INTERACTIONS, Dusan Stanojevic, Kathleen C. Hayashibara, and Gregory L. Verdine, Department of Chemistry, Harvard University, Cambridge, MA 02138

Messenger RNA synthesis is regulated *in vivo* by proteins that bind specific DNA sequences and influence the rate of transcription initiation at nearby genes. An important objective in understanding gene regulation is to learn how such proteins bind their target DNA sequences so tightly and specifically. Efforts in this laboratory have led to the development of chemically based methods for studying molecular recognition in protein-DNA complexes. Using these methods, we have gained evidence that flexible peptides having non-repetitive secondary structure can make specific contacts that contribute significantly to the thermodynamic stability of a protein-DNA complex. These results have furnished insights into how small-molecule regulators of gene expression might be generated *de novo*. Efforts toward the development of such artificial gene regulators will be discussed.

Conformation and Biological Activity (Joint)

L 017 NEW HETERONUCLEAR NMR TECHNIQUES FOR THE DETERMINATION OF STRUCTURE AND DYNAMICS OF PEPTIDES AND PROTEINS - CONSEQUENCES FOR DRUG DESIGN, Horst Kessler, Jochen Balbach, Gerhard Müller, Dale F. Mierke, Peter Schmieder, and Stephan Seip, Organisch-Chemisches Institut, Technische Universität München, W-8046 Garching, Germany.

Surface interactions of peptides and proteins may strongly determine the molecular conformation and dynamics. This is especially important in small cyclic peptides. These effects can be studied by NMR using different solvents and performing restrained and free molecular dynamics with inclusion of the solvent. In this way the softness or rigidity within a peptide can be studied. More experimental access to conformational flexibility is obtained from homo- and heteronuclear coupling constants. For small to medium size peptides new techniques are developed to determine heteronuclear long range couplings $^3J_{CH}$, $^3J_{NH}$ with heteroatoms in natural abundance. For very large proteins, new heteronuclear techniques will be presented to determine $^3J_{HNC\alpha H}$, $^3J_{HNC\beta}$, $^3J_{NHC'}$ in uniformely labeled (^{15}N, ^{13}C) molecules. The usefulness of these techniques will be described on the 31 kDa homodimeric P13 domaine of mannose permease. For inclusion of coupling constants in MD calculations a energy penalty function is used directly based on the Karplus equations for the different coupling constants. This excludes problems arriving from the ambiguities from up to four bond angles for one coupling constants. In cases where the conformation cannot be described by one predominant conformer the method of time dependent J-restraints is used similar to the procedure previously developed for NOE derived distance restraints. Knowledge of softness and flexibility can be used to interprete selectivity and activity of cyclic RGD containing peptidic inhibitors of cell-cell recognition. In this case driven MD is used to explain the distinct differences in bioactivity and to prove the induced fit model.

L 018 TERNARY PEPTIDE COMPLEXES MIMIC CALCIUM SENSITIVE INTERACTIONS IN MUSCLE, Gary S. Shaw, Carolyn M. Slupsky, A. Patricia Campbell, Wendy A. Findlay, Stéphane M. Gagné, and Brian D. Sykes, Department of Biochemistry and MRC Group in Protein Structure and Function, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

In the thin filament of skeletal muscle, the binding of calcium to the calcium-binding protein troponin-C (TnC) induces a conformational change in the protein which alters its interaction with the inhibitory protein troponin-I (TnI) and ultimately leads to muscle contraction. We have focussed on several aspects of this calcium triggering including the structures of the calcium binding domains of TnC and of intact TnC, the nature of the conformational change that occurs in TnC upon calcium binding, and the structure and location of TnI binding to TnC. To accomplish this we have studied peptide fragments and domains of TnC and TnI along with the intact TnC, and binary and ternary complexes of them. These include SCIII and SCIV [34 residue peptides representing sites III and IV from the C-domain of TnC], TR_1C [a tryptic fragment (12-87) containing sites I and II from the N-domain of TnC], N-domain [a cloned fragment (1-90) containing the whole N-domain], and TnIp [the minimum inhibitory fragment (104-115) of TnI]. Both the N-domain and TnC have been cloned and expressed in *E. coli* so they have been ^{13}C and ^{15}N enriched.

Two dimensional ^1H NMR techniques have been used to show that the calcium bound forms of SCIII and SCIV each form symmetric two-site homodimers, while an equimolar mixture of SCIII and SCIV preferentially and stoichiometrically forms heterodimers. The structures of these dimers are similar to the C-domain of TnC. However, the SCIII/SCIV heterodimer is significantly more stable than the SCIII homodimer by approximately 6 kJ/mol and about 16 kJ/mol more stable than the SCIV homodimer. This is in agreement with calcium titration experiments which show that these dimers have very different calcium binding properties; SCIII/SCIV has two "high" affinity sites, the SCIII homodimer has one "high" and one "low" affinity site and the SCIV homodimer binds calcium with low affinity. It is also noteworthy that site IV is more negative overall than site III and this may influence calcium-binding to the second site. For the SCIII dimer, the binding of a second calcium may be inhibited due to a more rigid symmetric arrangement of hydrophobics at the dimer interface.

The structure of the apo TR_1C fragment of TnC has been determined using standard 2D 1H NMR techniques. The structure is similar to the N-domain in the X-ray structure and shows the lack of contacts of the C-helix with the A, B and D helices of the site I-II domain. This is the same helix that carries the extra negative charge in the III-IV heterodimer and may be crucial in metal ion binding.

The calcium saturated forms of the N-domain and intact TnC are being studied by triple resonance 3D experimentals of the type developed by Bax and co-workers. These structures will provide us with the structure of the Ca saturated state and information about domain-domain interactions.

The structure of the TnIp inhibitory peptide when bound to calcium-saturated TnC has been previously using the intramolecular transferred nOe approach [*J. Mol. Biol.* 222:405-421 (1991)]. We present evidence for the binding of this peptide to the hydrophobic pocket of the C-terminal domain of TnC, obtained from measurement of intermolecular transferred nOe's between TnC and TnIp, and observation of the perturbation of the chemical shifts of TnC residues upon TnIp binding. In particular we demonstrate that the interaction of TnIp with the C-terminal domain of TnC is identical with its interaction with the III-IV heterodimer.

Peptide Lead Generation From Screening

L 019 LEADS FROM RANDOM PEPTIDE LIBRARIES PREPARED BY SYNTHESIS, Shawn E. Ramer, Tai-Nang Huang, Wolin Huang, Ron C. West, Ming-Chu Hsu, and David L. Coffen, Roche Research Center, Hoffmann-La Roche, Nutley.

Modern advances in molecular biology and recombinant genetics have facilitated the identification, isolation, and production of proteins important in human disease. The availability of these "targets" has led to a two-pronged approach to drug discovery. The determination of protein function by site-directed mutagenesis and protein structure by physical methods assists drug design by enabling the identification of important binding elements in the target. The second approach involves the development of high-flux assays in which a large number of chemicals are screened for desired activity against the target. The sources of materials for such screening efforts have traditionally been natural (plant extracts and microbial fermentation broths) or collections of chemicals synthesized during the course of pharmaceutical development. We have initiated a project designed to provide a substantial collection of compounds for high-flux screening assays. Towards this end we are using solid phase peptide synthesis to prepare mixtures of short, soluble peptides which are tested in high-flux screens. The use of defined mixtures assists in the identification of single, active peptides. The initial validation of this approach was the identification of Arg-Gly-Asp-Ser-NH_2 (RGDS-NH_2) and Arg-Gly-Asp-Cys-NH_2 (RGDC-NH_2) as inhibitors of fibrinogen binding to glycoprotein IIb/IIIa. Subsequent use of these mixtures of peptides in a battery of existing screens has resulted in the identification of several novel inhibitors of biologically relevant interactions. The discovery of tyrosyl-prolyl-glycinamide (YPG-NH_2) as a micromolar inhibitor in a whole cell assay for inhibition of transcriptional transactivation by HIV Tat protein will be described as an example of the utility of this approach.

Special Problems in Peptide/Peptide Memetic Design and Distribution

L 020 RATIONAL DESIGN OF PEPTIDES WITH ENHANCED MEMBRANE PERMEABILITY, Ronald T. Borchardt, Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS 66045

Designing peptides having permeability through biological barriers (e.g., epithelial and endothelial cells) is a major challenge to pharmaceutical scientists. The two most significant biological barriers to drug delivery are the intestinal mucosa and the blood brain barrier. Both are cell monolayers with tight intercellular junctions which represent both anatomical and enzymatic barriers to the permeability of most polar molecules (e.g., peptides). In order to properly evaluate strategies for enhancing membrane permeability of peptides, our laboratory has developed *in vitro* models of these biological barriers using cell culture techniques. To simulate the intestinal mucosa, human adenocarcinoma cells (Caco-2) are grown onto microporous membranes, whereas the blood brain barrier model consists of primary cultures of bovine brain microvessel endothelial cells grown onto microporous membranes. These cell culture systems, as well as *in situ* intestinal perfusion and brain perfusion models, have been employed in our laboratory to evaluate strategies for enhancing membrane permeability of peptides. The strategies that will be discussed in this presentation include: (1) designing peptide conjugates targeted to endogenous transporter systems (e.g., bile acid, biotin) so as to enhance intestinal permeability of peptides; and (2) optimizing the lipophilicity, hydrogen-bonding potential and conformation of peptides so as to enhance their intestinal and blood brain barrier permeability by passive diffusion. (The author would like to acknowledge support for this research from The National Institutes of Health, The Upjohn Company, and Glaxo, Inc.)

L 021 BIODISTRIBUTION OF PEPTIDES STABILIZED AGAINST BIODEGRADATION, Thomas P. Davis[1], Steven J. Weber[1], Henry I. Yamamura[1], Frank Porreca[1], and Victor J. Hruby[2], [1] Department of Pharmacology and [2] Department of Chemistry, The University of Arizona College of Medicine, Tucson.

Our laboratory has been studying the central and peripheral biodegradation and metabolism of endogenous neuropeptides since 1981. Over the past few years we have applied our established in vitro and in vivo techniques to studies of unique, selective and biologically potent peptide analogues. In the present study we examined the effect of halogenation at the Phe[4] residue of the cyclic peptide [D-Pen[2], D-Pen[5]] enkephalin on biodistribution, stability and blood-brain barrier (BBB) penetration. Using intravenous administration in mice a significantly greater percentage of total [^{3}H] [p-Cl-Phe[4]] DPDPE reached the brain after 10, 20, and 40 minutes as compared to [^{3}H] DPDPE and both peptides were significantly displaced by pretreatment with naloxone or naltrindole. Using the in vitro BBB model of confluent, primary, bovine brain micro vessel endothelial cells (BMEC) grown on poly carbonate filters we studied the in vitro BBB penetration of both peptides. The BMEC permeability coefficient for DPDPE ($P = 0.00246 \pm 0.00014$ cm/min) was significantly less ($p < 0.01$) than that of[p-Cl-Phe[4]] DPDPE ($P = 0.00414 \pm 0.00017$ cm/min). Data from the in vivo BBB study shows the amount of intact [^{3}H] [p-Cl-Phe[4]] DPDPE crossing the BBB at 10, 20 and 40 min (0.166-0.188%) was significantly ($p < 0.01$) greater than that of [^{3}H] DPDPE (0.059-0.067%). Whole body distribution results revealed large amounts of both peptides were sequestered rapidly in the gall bladder and secreted into the small intestine intact. Hot plate antinociception tests after intravenous. administration also revealed [p-Cl-Phe[4]] DPDPE to elicit a much greater analgesic effect as compared to DPDPE. Stability of unlabeled and tritiated DPDPE and [p-Cl-Phe[4]] DPDPE to biodegradation was also determined both in vitro and in vivo. Both unlabeled and labeled peptides remained intact in all tissues studied. These results provide strong evidence that halogenation of DPDPE at the Phe[4] position increases BBB penetration, has a greater effect on antinociception threshold and retains excellent stability to biodegradation. (Supported by U.S.P.H.S.- N.I.H. grant DA06284).

Biomolecular Interactions/Complexes (Joint)

L 022 EXAMINING THE CONFORMATIONAL DIFFERENCES OF PEPTIDES IN SOLUTION AND IN PEPTIDE/PROTEIN COMPLEXES, Victor J. Hruby[1], Klaas Hallenga[2], Patricia Hill[1], N.R. Nirmala[2], G. Lippens[2], and K.C. Russell[1], [1]Department of Chemistry, University of Arizona, Tucson, AZ 85721, U.S.A. [2]University Libre de Bruxelles, Avenue Paul Heger, P2-CP 160, B-1050 Brussels, Belgium.

The binding of peptide ligands to biologically relevant proteins such as receptors, enzymes, antibodies, carrier proteins and other acceptors is of critical importance to many biological processes. It is believed that in general both the three dimensional structure of the peptide and of the receptor (acceptor) is changed as a results of this interaction. These changes often are important for transforming the protein into its "active" conformation. Thus an understanding of the nature of these interactions is central to any effort to understand these processes, and to develop a rational approach to peptide hormone, neurotransmitter, substrate, etc. ligand design. Oxytocin, H-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2, a peptide hormone originally found in the pituitary gland, has played a central role in the development of our current understanding of peptide-receptor interactions in biological systems. Using NMR and other biophysical methods, the solution conformation of oxytocin has been well studied and recently an X-ray structure of deamino-oxytocin has been determined. The results from these studies have been discussed in terms of the relationships of conformation and dynamics to biological activity. Most recently, using primarily NMR methods and distance geometry calculations, we have determined the conformation of oxytocin bound to its carrier protein in the brain, neurophysin. These studies demonstrate that oxytocin has a different conformation in each "environment." A comparison of the different conformations and their possible origins will be discussed. The implications of these findings to peptide ligand design will be given. Despite the complexities of the situation, methods are available for rational design of peptide ligands and the insights gained from these studies can aid in further developments. The work was supported by grants from the U.S. Public Health Service and the National Science Foundation.

L 023 SOLUTION CONFORMATION OF TUBOCURARINE, FREE AND BOUND TO A GENETICALLY ENGINEERED ACTIVE SITE PEPTIDE OF THE ACETYLCHOLINE RECEPTOR, Gil Navon[1], Yigal Fraenkel[1] and Jonathan M. Gershoni[2], [1]School of Chemistry, [2]Department of Cell Research and Immunology, Tel Aviv University, Ramat Aviv, Tel Aviv, Israel.

The nicotinic acetylcholine receptor (AChR) is a cation channel, which is activated by the neurotransmitter acetylcholine. A variety of drugs and toxins are active through their interaction with the AChR. For a rational drug design one has to determine their conformations in the bound state. The AChR is a very large, membrane bound, glycoprotein (M.W.~300,000). Thus direct NMR determination of the structure of the receptor-ligand complex seems presently highly improbable. The technique of transfer NOE can give the required information. Indeed, using this technique we have shown that acetylcholine undergoes a major conformation change upon binding to the native Torpedo AChR[1]. From the extended conformation in free solution it acquires a bent conformation.

We have now measured the conformations of acetylcholine and of its potent inhibitor d-tubocurarine, upon binding to a genetically engineered 37 kD construct protein which contains a 17 amino acid peptide of the binding site of the Torpedo AChR (Tα184-200). We have shown previously that this protein specifically binds acetylcholine, its agonists and antagonists[2]. The conformation of the acetylcholine bound to Tα184-200 was found to be identical to that bound to the native receptor. Measurements of the conformations of free and bound d-tubocurarine indicated a dramatic conformational change upon binding. Again, as in the case of acetylcholine, it is converted from an extended to a bent conformation. In this bent geometry hydrophobic and hydrophilic domains are created. The hydrophobic domain frontiers consist of the two aliphatic portions of the two tetrahydroisoquinolinic rings which have become closer. The hydrophilic domain contains the six oxygens. The major hydrophobic domains in ACh and d-tubocurarine are unique in that they consist of methyl groups which are connected to a positively charged nitrogen. This may be denoted as a '**positively charged hydrophobic domain**'. Recently, using t- NOE experiment we have identified in the Tα184-200 sequence a tryptophan residue that interacts with acetylcholine[3]. Thus, unlike the traditional thought that there must be a negatively charged residue such as aspartate or glutamate on the AChR to complement the ammonium group, we find that 'positively charged hydrophobic domains' are attracted to aromatic residues which combine electron rich π orbitals with hydrophobicity.

1. R.W. Behling , T. Yamane, G. Navon, L.W. Jelinski, PNAS **85**, 6721 (1988).
2. Y. Fraenkel, G. Navon, A. Aronheim, J.M. Gershoni,. Biochemistry **29**, 2617 (1990).
3. Y. Fraenkel, J.M. Gershoni, G. Navon, FEBS Letters, **291**, 225 (1991).

L 024 NMR STUDIES OF LIGAND/MACROMOLECULE INTERACTIONS, David Wemmer[1], Kalle Gehring[1], Philip Williams[2], Hiromi Morimoto[2], Devendra Jaiswal[2], Mark Kubinec[1], Stefan Highsmith[3], Richard Storrs[1], Bernhard Geierstanger[4], B.Volkman[1], [1]Dept. of Chemistry, Univ. of CA, Berkeley, and Structural Biology Division, Lawrence Berkeley Lab, [2]National Tritium Labelling Facility, Lawrence Berkeley Lab, [3]Dept. of Biochemistry, Univ. of the Pacific School of Dentistry, San Francisco, [4]Dept. of Biophysics, University of CA, Berkeley.

Characterization of ligands bound to macromolecules using NMR spectroscopy has developed dramatically over the past several years. When the macromolecule is not too large, it is possible to use incorporation of stable isotope labels (^{15}N and ^{13}C) to carry out filtering experiments to determine both the bound conformation and interactions with the macromolecule. However as the macromolecules grow in molecular weight, or the lines broaden due to chemical exchange, it becomes more difficult to study the binding. As an example of molecules in the lower molecular weight limit we will compare the binding of a small drug ligand, distamycin-A, to DNA with the binding of a peptide. The peptide had previously shown to bind preferentially to A-T rich DNAs (as does distamycin), and analogies were drawn between the peptide and the drug. However the NMR data obtained (albeit complicated by the effects of chemical exchange) show clearly that the binding mode does not fit the initial model. As examples of the binding of ligands to larger proteins we will present data using tritium NMR to selectively detect the resonances from bound ligand, even in cases where the broadening due to both high molecular weight and chemical exchange lead to rather large linewidths (100 Hz and greater). The binding of maltodextrins to the maltose binding protein from the periplasmic space of *E. coli* was analyzed. With maltose it was found that there is moderate anomeric specificity (the α form binding more tightly than the β). However with longer maltose oligomers the anomeric specificity was shown to be higher, and there were two distinct binding modes found for the β anomer, which were in rapid exchange with one another. A model was developed which explains these data qualitatively through differences in interaction of the terminal sugar with the binding pocket. In this example ^3H NMR was an ideal probe since resonance could be detected with essentially no background signal, and the very broad lines in the complexes could be followed under a variety of conditions. Another example using ^3H NMR in the ATP/myosin S1 system will be presented. In this case two bound forms of ADP could be detected when bound to the 105 kD myosin S1 fragment. The lines could be followed as a function of temperature, and the presence of vanadate. There is again evidence for chemical exchange. A final example of a tritiated hapten binding to an antibody Fab fragment will be presented. In this case a tritiated nitrophenylphosphate was used as a transition state analog to induce a catalytic antibody (the work of Prof. Peter Schultz, UCB Chemistry). The resonances of the bound NPP were easily detected at low concentration.

References: K.Gehring, P.G.Williams, J.G.Pelton, H.Morimoto, D.E.Wemmer Biochem. **30**, 5524 (1990).

Late Abstracts

USING PEPTIDES TO EXPLORE HOW PROTEINS ARE FOLDED AND SORTED IN VIVO. L. M. Gierasch, S. J. Landry, J. Maxwell, T. Scott, T. L. Triplett, N. Zheng, A. Bansal, and R. Kibbey. University of Texas Southwestern Medical Center, Dallas, Texas 75235-9041.

Proteins contain sequence motifs that direct them to various sorting pathways in cells, such as targeting sequences for the secretory pathway, for retention in the endoplasmic reticulum, for organellar import, and for receptor internalization via coated pits. Recognition of the sequence motifs often does not require a specific primary structure, but instead a pattern of residue types in a particular spatial arrangement. Recently a family of proteins, called molecular chaperones, has been found to interact with nascent chains to facilitate and modulate their folding, often concurrent with their localization. Chaperones also appear to be rather catholic in their binding, since many substrates can be bound by the same chaperone. We have used synthetic peptide models for various sequence motifs to explore the basis of their recognition. The results of these studies offer opportunities to design inhibitors for cellular processes and should also shed light on the general problem of how such sequence-*non*specific recognition takes place. For example, we find that representatives of the different classes of molecular chaperones recognize different features in their substrates: DnaK, which exemplifies the Hsp70 family, binds to peptides in extended conformations with both side chain and backbone interactions. GroEL, which exemplifies the Hsp60 family, binds to hydrophobic clusters of side chains that may be presented on an amphipathic α helix or other secondary structure. To function *in vivo* signal sequences require a hydrophobic core of ca. 10 residues preceded by a polar N-terminal region (usually positive) and followed by a more polar 6 to 8 residue pre-cleavage site region. We have found that this pattern of residues provides the ability to insert into a membrane and to fold as an α helix in such environments. We are now exploring how the targeting proteins from mammals and *E. coli*, SRP and SecA, respectively, exploit these sequence features in the absence of high sequence similarity. We have also examined peptides corresponding to sorting signals of receptors that are internalized via coated pits. Peptides corresponding to the sequence NPVY, which is a signal for internalization of the LDL receptor, adopts a β turn in solution. A known internalization-blocking mutation that causes familial hypercholesterolemia changes Y→C and blocks turn formation in the corresponding peptide. We are now looking at how this signal is recognized by proteins of the coated pit. In all of these studies, we are using the transferred nuclear Overhauser effect to determine the bound conformations of the peptides corresponding to sorting sequences.

RENIN INHIBITORS: A PARADIGM FOR PEPTIDE-BASED ORALLY ACTIVE COMPOUNDS, **Hollis D. Kleinert, William R. Baker, Saul H. Rosenberg, and Herman H. Stein, Division of Pharmaceutical Research and Development, Abbott Laboratories, Abbott Park, IL 60064**

Inhibition of the renin-angiotensin-aldosterone system, as demonstrated with angiotensin-converting enzyme inhibitors, has proven to be an ideal therapeutic target for the treatment of hypertension and congestive heart failure. Another approach to interfering with this system is through the inhibition of the enzyme, renin. Progress in discovering orally active renin inhibitors as been thwarted by the general problems associated with oral administration of peptide-based molecules. These obstacles include poor absorption of intact parent drug and/or rapid hepatic elimination resulting in low, variable oral bioavailability. We have discovered a potent, renin inhibitor with a dipeptide core, A-72517, which despite its substantial molecular mass of 706 daltons meets all of the criteria of an orally active drug. This compound has oral bioavailabilities of 8, 24, 32 and 53% in the monkey, rat, ferret and dog, respectively. As a result of this achievement reproducible, consistent pharmacological activity can be demonstrated. Dose-related reductions in blood pressure, plasma renin activity and plasma angiotensin II, paralleling increasing plasma drug concentrations were observed in conscious, salt-depleted dogs. The advancement that we describe here in attaining improved oral bioavailability of peptide-based compounds should serve as justification for continued research on this topic, the importance of which extends far beyond the discovery of oral renin inhibitors alone. Our research conveys a sense of what is possible and hope for the discovery of other important orally active enzyme inhibitors such as HIV protease inhibitors for the treatment of AIDS.

CONSTRUCTION AND USE IN SCREENING OF VERY LARGE SYNTHETIC PEPTIDE LIBRARIES: AN OVERVIEW, Kit S. Lam[1], Michel Lebl[2], Viktor Krchnak[2], Stephen Felder[2], Sydney E. Salmon[1], Victor J. Hruby[3], Shelly Wade[2], and Farid Abdul-Latif[2]. [1]Arizona Cancer Center and Department of Internal Medicine, University of Arizona, Tucson, [2]Selectide Corporation, Tucson, [3]Department of Chemistry, University of Arizona, Tucson.

We reported the synthesis of a very large synthetic peptide library (1-10 million) such that each resin bead expressed a specific peptide species -- the "one-bead one-peptide" concept [1]. Using an enzyme-linked immunoassay system, we were able to rapidly screen a peptide library and identify peptide ligands that bind specifically to a macromolecular target. Thus far, we have successfully applied this technique to identify peptide ligands that interact with streptavidin, avidin, anti-β-endorphin monoclonal antibody (MoAb), anti-insulin MoAb (specific against a discontinuous epitope), and several biological receptor molecules.

Since our peptide library is totally synthetic, we can easily select or omit specific amino acids in our library. In addition, D-amino acids, and unnatural amino acids such as glycosylated amino acids and N-methylated amino acids can easily be included in the synthesis of our libraries. Furthermore, secondary structure such as β-sheet, α-helix, or cyclic structure can be incorporated into the design of our libraries. Using a library that lacked histidine, we have discovered several non-HPQ sequences that are specific for streptavidin. In addition, we have also identified several cyclic and D-amino acid containing ligands that interact specifically with streptavidin. Although streptavidin and avidin both bind strongly to biotin (K_D = 10^{-14} to 10^{-15}M), we were able to identify peptide ligands that interact with avidin but not with streptavidin, or vice versa. In addition, we have also identified peptide ligands that bind to both.

In case of streptavidin and anti-β-endorphin MoAb where the peptide ligand motif is short, we may use multiple sequencing approaches to sequence multiple beads concurrently, and still be able to identify a specific peptide motif.

In addition to screening the peptide library with a binding assay as described above, we have recently developed a method using a sequential orthogonal approach to release peptides form the beads. The released peptides are then used for solution-phase testing. The bead-of-origin that tests positive can then be recovered and the residual peptides on the bead analyzed by a microsequencer.

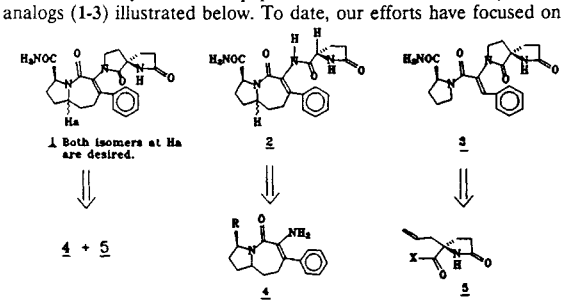
[1]Kit S. Lam, et al., Nature 354:82-84, 1991.

DETERMINATION OF LIGAND EXCHANGE RATES AND THEIR EFFECT ON TRANSFERRED NOE MEASUREMENTS, Robert E. London, Michael E. Perlman, and Donald G. Davis, Laboratory of Molecular Biophysics, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC. 27709

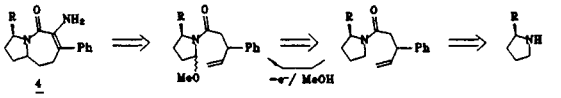
Bacterial purine nucleoside phosphorylase (PNP) is a 135 kD enzyme with six identical subunits (M.W. = 22.5 kD), which catalyzes the phosphorolytic cleavage of purine nucleosides and is used commercially for the preparation of nucleosides from the corresponding bases and ribose-1-phosphate. Information about the active site is currently fragmentary, and studies of the inhibitory potency of conformationally constrained nuclesides have suggested that the active site may differ substantially from that of the corresponding mammalian enzyme. The conformation of PNP-complexed tubercidin (7-deazaadenosine), an inhibitor of the enzyme with K_I = 18 μM, has been studied utilizing the transferred nuclear Overhauser enhancement approach. Transferred NOE data inconsistent with a single bound conformation led to more extensive modeling of the dependence of the TRNOE on exchange rate and on uncomplexed nucleoside parameters, and to models utilizing more than one bound conformation. Determination of the exchange rates based on: (1) analysis of the Swift & Connick relations for the observed shift and transverse relaxation rate as a function of the mole fraction of bound nucleoside; (2) CPMG studies of transverse relaxation rates as a function of pulse rate; and (3) rotating frame $T_{1\rho}$ relaxation as a function of the spin lock field provided consistent results for tubercidin exchange kinetics, and were used for analysis of the TRNOE data. Since relaxation matrix simulations indicated that direct and indirect dipolar relaxation pathways cannot always be distinguished via an observable lag of the NOE build-up curves, theoretical and experimental studies utilizing the transferred nuclear Overhauser effect in the rotating frame (TRROE) were carried out in order to determine whether the TRNOE and TRROE data collectively could provide a more useful basis for distinguishing between these possibilities. *Preliminary* studies support the feasibility of utilizing TRROE measurements in this way. The results support a model in which the predominant conformation of bound tubercidin has the base in a *syn* orientation, and the ribose in a 3'-exo conformation.

ANODIC AMIDE OXIDATIONS: A NOVEL METHOD FOR THE CONSTRUCTION OF LACTAM BASED PEPTIDE MIMETICS, Kevin D. Moeller, Yvette M. Fobian, Cathleen E. Hanau, Wenhao Li, and Lawrence D. Rutledge, Department of Chemistry, Washington University, St. Louis, MO 63130.

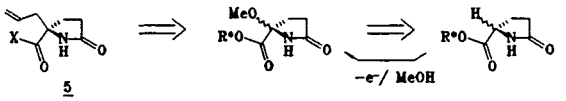
The anodic amide oxidation can be a powerful tool for functionalizing amines and amino acid derivatives, and can provide rapid entry into lactam containing polycylic ring skeletons. Recently, we have begun to employ this technique in the construction of conformationally restricted peptide mimetics like the thyroliberin analogs (**1-3**) illustrated below. To date, our efforts have focused on



the synthesis of key building blocks **4** and **5**. We have demonstrated that building blocks like **4** can be readily assembled from simple proline derivatives with the use of an anodic amide oxidation based procedure for annulating lactam rings onto amines. At the same time, an anodic



amide oxidation reaction has been used in the synthesis of **5**. In this example, the oxidation reaction was utilized to convert a chiral pyroglutamate ester into an *N*-acyliminium ion precursor that was then used to construct the central stereogenic atom.



This talk will highlight the key elements of both of these syntheses and then focus on the use of building blocks **4** and **5** for constructing conformationally restricted thyroliberin analogs.

TOPOLOGICAL TEMPLATES AS TOOL IN MOLECULAR RECOGNITION AND IN PROTEIN DESIGN, Manfred Mutter, Barbara Dörner, Christoph Sigel, Rainer Floegel, Catherina Servis, Gabriele Tuchscherer, Séction de Chimie, Université Lausanne, CH-1005 Lausanne, Switzerland.

Template molecules have been introduced as tertiary structure inducing device in protein de novo design (Template Assembled Synthetic Proteins, TASP)[1]. For molecular recognition studies, topological template molecules as structural motifs disposing functional groups R_i (e.g. interacting with an acceptor) in defined spatial positions are ideal candidates to mimic structural and conformational features of peptides and proteins (Fig.1)[2]. For selective and independent functionalization, orthogonal protection techniques are applied for temporary protection of the attachment sites. The synthesis of a topological template with spatially addressable sites includes the following operational steps: (i) chemical peptide synthesis applying orthogonal protection techniques, (ii) use of non natural building blocks and peptido-mimetics for inducing conformational constraints in the template molecule, (iii) selective cleavage of protecting groups and covalent attachment of the functional groups R_i. The independent attachment of groups R_i responsible for molecular interaction results for example in mimetics of (i) discontinous epitopes or binding sites of proteins; (ii) bioactive conformations of peptide ligands. By varying the structural parameters of the template, as well as the orientational flexibility and chemical nature of the functional groups, this new type of molecules can serve as lead in the rational search for biologically active compounds. Moreover, the preparation of TASK librairies according to strategies described for linear peptides appears to be of considerable practical interest.

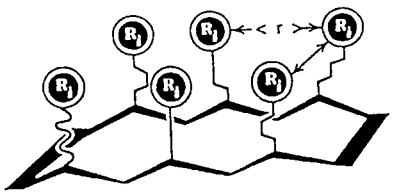


Fig.1: Topological template

1) M. Mutter (1988) Peptides: Chemistry and Biology, Proc. 10th Amer. Peptide Symp. (G.R. Marshall, ed.), Escom, Leiden, 349. 2) M. Mutter, S. Vuilleumier (1989), Angew. Engl. 28, 535.

EPITOPE DISCOVERY: VACCINES AS DRUGS

George P. Smith, University of Missouri-Columbia, Columbia, MO

Vaccine development can be considered a problem in drug discovery. The target receptors are protective antibodies displayed on the surface of resting B-cells. The natural ligands for these receptors--their "hormones," as it were--are disease-related antigens that engage the membrane-bound receptors and stimulate the B-cells to proliferate and secrete their receptors profusely in the form of humoral antibody. Our job as vaccine developers is to discover artificial receptor ligands that agonize their natural ligands: that is, that themselves can serve as protective immunogenic antigens.

As far as we know, the minimum requirement for a B-cell immunogen is that it bind specifically to the corresponding antibody. True, immunogens also have adjuvant components that serve to recruit the T-cell help required to stimulate B-cells effectively. But these adjuvant components do not direct the specificity of the elicited antibody: that's determined by specific docking of the epitope to the binding pocket of the antibody.

Since it is specific interaction of epitope with antibody that directs the specificity of the humoral immune response, protective antibodies themselves contain in principle all the information required to develop a protective antigen. Furthermore, protective antibodies are often much easier to come by than natural disease-related antigens; for instance, they are abundant in the blood of naturally or artificially immunized people or animals. If we had an easy way of finding artificial ligands for those antibodies, those ligands could serve as the epitope component of an entirely new type of vaccine component--one developed without ever having to discover the natural antigenic determinant the artificial epitope mimicks.

Phage display libraries provide a simple, rapid means of surveying billions of peptide structures for ligands that bind a given receptor. We are surveying a number of these libraries with model antibodies which (unlike the protective antibodies in a real vaccine program) are specific for known eliciting antigen. Do the artificial peptide ligands thus discovered serve as effective immunogens? That is, when they're injected into naive animals, do they elicit antibodies that cross-react with the antigen that elicited the model antibody in the first place? If the answer is "yes" a powerful new approach to vaccine development will have been discovered.

Peptide/Protein Design

L 100 A SMALL DESIGNED PROTEIN AS A POTENTIAL SOURCE OF SELECTABLE PEPTIDE SEQUENCES OF KNOWN STRUCTURE: THE MINIBODY, Elisabetta Bianchi, Antonello Pessi, Anna Tramontano and Maurizio Sollazzo, I.R.B.M.- P. Angeletti, 00040 Pomezia (Rome), Italy

An attempt was made to combine *rational* and *irrational* drug design strategies. Irrationality stems from the fact that, in order to find a ligand for a target molecule (receptor, antibody, etc.) we make use of selection from a large pool of randomized sequences. The selection process does not imply any previous knowledge of the structure of either the receptor or the ligand. Rationality is introduced by producing the random sequences as part of a protein, in which their conformational freedom is restricted by an appropriate framework, yielding a few, predictable structures. We chose immunoglobulins (Ig) as a model, because the hypervariable loops which bind the antigen, despite primary sequence variability, can assume only a limited set of conformations, known as canonical structures: for five out of six loops, these structures can be predicted directly from the sequence (C. Chothia *et al.*, Nature (1989) **342**, 877). Starting from a fragment of the variable heavy domain (V_H) of McPC603, we designed a sixty-one residue protein, named Minibody, which retains the regions corresponding to the two hypervariable loops H1 and H2. To help demonstrating that the Minibody loops cluster in space as predicted by the model, we engineered in the protein a metal binding site, formed by chelating residues provided by both loops. The Minibody, as well as a mutant in a critical chelating residue, was chemically synthesized by the Flow-polyamide method, purified to homogeneity and characterized by amino acid analysis, HPLC, SDS-PAGE and mass spectrometry. Preliminary analysis of the protein fold, obtained by Circular Dichroism and metal binding, is fully consistent with the model. A library of Minibodies with randomized loop sequences has been expressed on the surface of the pIII protein of f1 coliphage (M. Sollazzo *et al.*, in preparation).

L 101 CHARACTERIZATION OF MONOBIOTINYLATED DALDA AS A MU-SPECIFIC OPIOID SUITABLE FOR VECTOR MEDIATED BRAIN DELIVERY. Ulrich Bickel, Shizuo Yamada, and William M. Pardridge. Dept. of Medicine and Brain Research Inst., UCLA School of Medicine, Los Angeles, CA 90024, and Dept. of Biopharmacy, University of Shizuoka, Shizuoka 422, Japan.

The introduction of opioid peptides as useful drugs has been hampered by the the unfavorable pharmacokinetics of these hydrophilic substances, namely the poor penetration of the endothelial cells of brain microvessels, which form the blood-brain barrier (BBB) in vivo. Less toxic and possibly non addicting peptide based opioid drugs cannot be clinically evaluated unless brain drug delivery systems for these compounds are available. A promising approach is the recent introduction of a delivery system based on the combination of the chimeric peptide strategy and avidin-biotin chemistry as a versatile linker technique. The transport vector consists of a covalent conjugate of avidin and the anti-transferrin monoclonal antibody, OX26. Due to the abundance of transferrin receptors on brain microvascular endothelia, the complex undergoes receptor mediated transcytosis across the BBB and is able to deliver biotinylated peptide drugs in pharmacologically active quantities to the brain (U. Bickel et al., submitted). A biotinylated opioid peptide may be a potential ligand for the transport vector. Therefore, DALDA (Tyr-D-Arg-Phe-Lys-NH_2), a highly specific μ-receptor agonist (P.W. Schiller et al., J. Med. Chem. 1989, 32, 698) was biotinylated at the ϵ-amino group of Lys with the cleavable biotin linker, sulfosuccinimidyl 2(biotinamido)-ethyl-1,3 dithiopropionate (NHS-SS-biotin). The N-terminal α-amino group of Tyr, which is essential for bioactivity, was protected with fluorenylmethoxycarbonyl (FMOC), and deprotected following biotinylation to obtain biotinylated DALDA (bioDALDA). The biotin moiety can be removed by disulfide reductases or reducing agents like dithiotreitol to yield desbiotinylated DALDA (desbioDALDA). The DALDA derivatives were HPLC purified and identified by FAB-mass spectrometry. Receptor binding assays with rat brain membranes and ^{3}H-DAGO as a μ-selective ligand revealed K_i-values of 2.3 $\pm$ 0.4 nM, 6.5 $\pm$ 1.1 nM, and 4.0 $\pm$ 0.9 nM for DALDA, bioDALDA and desbioDALDA. Intracerebroventricular application of low doses (between 0.3 μg and 3 μg) of these peptides in rats resulted in profound and long lasting analgesia (up to 3 h), as measured by tail-flick latency. In conclusion, a monobiotinylated, μ-opioid peptide derivative has been synthesized which retains in vitro and in vivo potency, and may be systemically active following brain delivery.

L 102 A 4-HELIX BUNDLE PEPTIDE WITH POTENT HEPARIN NEUTRALIZATION CAPACITY, James G. Boyd, Anthony J. Hunt, Albert Profy, and Theodore Maione, Repligen Corporation, Department of Protein and Peptide Chemistry, One Kendall Square, Bldg. 700, Cambridge, MA 02139

Platelet Factor 4 (PF4) is a 70-amino acid protein which exists as a tetramer under physiological conditions. PF4 binds the anionic polysaccharide heparin and restores clotting activity to heparinized blood *in vivo*. The 12 C-terminal amino acids of PF4 reside in an amphiphillic α-helix with 4 lysine residues on the solvent exposed face. In the tetramer, these helices are arranged in a nearly antiparallel fashion. A 4-helix bundle (4HB) peptide, which incorporates the critical heparin binding residues of PF4 has been designed ad synthesized. Circular dichrosim spectra of the 4HB peptide feature absorption minima at 220 and 206 nm and concentration dependent helicity is observed. Molecular weight determination by gel filtration is consistent with formation of a tetrameric structure. The 4HB binds heparin with approximately 20-fold higher affinity than monomeric peptides derived from the PF4 C-terminal and with 5-fold less affinity than native PF4. In *in vitro* clotting assays, the 4HB efficiently neutralizes the ability of heparin to activate antithrombin III and restores clotting activity to heparinized human plasma. The relationship of heparin binding and tetrameric α-helical peptides is discussed

L 103 SCREENING OF A LEUCINE ZIPPER DIRECTED PEPTIDE ON PHAGE LIBRARY TO IDENTIFY FOS OR JUN LIGANDS ABLE TO INTERFERE WITH AP-1 MEDIATED SIGNAL TRANSDUCTION PATHWAYS, Laurent Bracco, Marie-Noelle Mary, Christine Pernelle, Christine Dureuil, Michel Janicot and Bruno Tocque, IBV / CRVA, Rhône-Poulenc Rorer, 13 Quai Jules Guesde, BP 14, 94403 Vitry-sur-Seine, France.

The peptide on phage library technology was applied to synthetic leucine zipper peptides. We first demonstrated that, as expected, phages bearing the Fos leucine zipper domain could specifically associate with beads derivatized with Jun but neither with a Jun (Leu to Pro) mutant nor with a Fos leucine zipper peptide. We next constructed a library with the following format : LXAXXEALXAXXEALXAXXEAL , in which positions critical for leucine zipper association and specificity were left random. This library was screened with respect to Fos and Jun leucine zipper sequences leading to the identification of several homologous ligand peptides. These results confirmed the importance of ionic interactions in the stabilization of the Fos/Jun complex and provided additional information on the leucine zipper recognition code. The activities of several peptides were further evaluated *in vitro* via a Scintillation Proximity Assay designed to monitor Fos/Jun association and *in vivo*, upon microinjection via their inhibitory effect on the insulin induced maturation of Xenopus oocytes.

L 104 CONFORMATIONAL CHANGES INDUCED BY CROSS-LINKING SIDE CHAINS INFLUENCE BIOLOGICAL ACTIVITY IN A HIGHLY CONSERVED REGION OF APOLIPOPROTEIN E

Demetrios T. Braddock, Peizhi Luo, Peter F. Davies, David G. Lynn, Stephen C. Meredith, The University of Chicago, Chicago, IL

*We have investigated a highly conserved anionic region of human apolipoprotein (apo) E, amino acids 41-60, as to its secondary structure and effect on binding of LDL and its receptor (LDL-R). We synthesized five peptides:

I. NH_2-GQTLSEQVQEELLSSQVTQELRAG-COOH

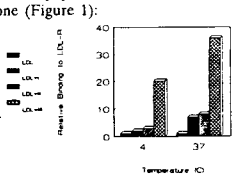
II. NH_2-GDTLSEKVKEELLESQVKQELLDA-COOH

III. NH_2-GDTLKEQVQEELLSEQVKDELKAG-COOH

IV. NH_2-EEKSGAKD^{15}GKEQEEDKK-COOH

V. NH_2-EEKSGADE^{15}GEKKEEDKK-COOH

I contains amino acids 41-60 of human apo E; peptides II and III are stabilized by two covalent crosslinks and one salt bridge each to favor two secondary structures, the π and α helix, respectively. Peptides IV and V are designed to have secondary structures similar to those of peptides II and III, respectively, but also contain isotopic labels and are highly soluble in water to facilitate structural determination by NMR. To study the effects of peptides on LDL binding to LDL-R, LDL were first incubated with peptides, and then with human skin fibroblasts. Pre-incubation of LDL with peptide resulted in increased specific binding over that of LDL alone (Figure 1): Ligand blotting of the LDL with or without peptide 2 shows a significant increase in binding of the LDL to the LDL receptor protein only in the presence of the putative α helix. More detailed binding isotherms indicated that III does not alter K_d of LDL for LDL-R, but increases the apparent number of LDL-R sites available for binding ligand. These results suggest a hitherto unknown function of this domain of apo E: modulation of LDL, and possibly other lipoprotein binding to LDL-R. Furthermore, this effect appears to be most pronounced when crosslinks constrain the peptide to fold into a putative α helical conformation. These studies illustrate the advantage of using crosslinked peptides to understand allosteric effects on bioactive peptides.



L 105 ASSEMBLY AND STABILIZATION OF PEPTIDE SUPERSECONDARY STRUCTURES, Jean A. Chmielewski, Pat Bishop, Ken Lewen, and Ray Lutgring, Department of Chemistry, Purdue University, West Lafayette, IN 47907

A knowledge of the way in which peptides and proteins assemble is essential for the design of catalysts with novel functions. We have designed a series of helical peptides with the ability to form aggregate structures in aqueous solution. Depending on the amino acid sequence of the peptide, specific helical bundles are obtained containing either two, four or six peptide units, which are held together via non-covalent forces. These helical bundles can be easily denatured thermally and with chemical denaturants. To increase the overall stability of the aggregated bundles, covalent linkages have been incorporated at the interfaces between individual peptides. In this way peptides with sixteen amino acid residues can be converted into stable protein structures with close to one hundred amino acids in one step. The utility of these methods in the design of novel enzyme structures will be discussed.

L 106 DESIGN AND EVALUATION OF NOVEL CALCITONIN LIGANDS TO STUDY THE CONTACT POINTS BETWEEN CALCITONIN AND ITS CLONED RECEPTOR, M. Chorev, M.P. Caulfield, C.T. Leu, J.L. Levy, M. Flannery, S.R. Goldring, and M. Rosenblatt, Division of Bone and Mineral Metabolism, Beth Israel Hospital, Boston, MA 02215

With the cloning of calcitonin receptor (CTR), approaches to studying the precise point-to-point contacts of amino acids in the calcitonin (CT) hormone molecule with amino acids in the receptor now can be devised. By generating a series of CT analogs, each containing a photoreactive crosslinking moiety at a single strategically-selected position in the CT molecule, we plan to produce a panel of CT -- CTR complexes. Following crosslinking, the covalently, stabilized complexes can be chemically or enzymatically cleaved into small fragments of CT -- CTR conjugates which can be isolated and microsequenced. Placement of the crosslinker at various positions along the CT sequence should yield differing CT -- CTR fragments which identify small regions of CT which interact with particular region of CTR. Subsequently, confirmation of specific amino acid interactions can be demonstrated by site-directed mutagenesis of CTR or by modification of CT. To this end, several aryl-ketone sCT photoaffinity ligands were designed and synthesized. Based on structure-activity studies and the presumed amphiphilic helical nature of CT, a series of benzophenone-substituted [Arg^{11,18}]sCT analogs spanning a major portion of the hormone were prepared. Lys^{11,18} were replaced with Arg^{11,18} to enable specific post-synthetic radiolabeling and introduction of photoactive moieties. Substitution of the amino terminus by *p*-benzoyl-benzoyl (pBz_2) or replacement of either Val^8, Leu^{16} or Leu^{19} by Lys(ϵ-pBz_2) yielded highly potent analogs. Binding affinities of these photoactivable sCT analogs revealed affinities in the low nM range (e.g. Kb = 4 nM for Nα-pBz_2[Arg^{11,18}]- and [Arg^{11,18},Lys^{19}(ϵ-pBz_2)]*sCT*). Preliminary studies indicate that at least one of these analogs identifies a major compatible band on SDS gels when crosslinked to cells expressing cloned CTR. This approach promises to reveal the nature of the CT hormone -- CTR complex which could be very useful in understanding signal transduction and designing novel agonists and antagonists.

L 107 EXPRESSION AND CHARACTERIZATION OF A TRUNCATED, SOLUBLE LOW DENSITY LIPOPROTEIN RECEPTOR, Kimberly A. Dirlam, Daniel G. Gretch, Douglas J. LaCount, Stephen L. Sturley, and Alan D. Attie, Department of Biochemistry, University of Wisconsin-Madison, Madison, WI 53706 The low density lipoprotein receptor (LDLR) mediates the clearance of LDL from the blood. The availability of a biologically active, soluble form of the LDLR would prove useful in the study of receptor-ligand interactions *in vitro* under both non-physiological and physiological conditions. We have employed a baculovirus system to express a truncated, soluble form of the LDLR containing the entire ligand binding domain. Homologous recombination was used to insert a 1.1 kb fragment of receptor coding sequence into the viral genome. PCR of the recombinant virus revealed an insert of appropriate size. The cloned DNA sequence encodes the first 354 aa of the human LDLR. The N-terminal 21 aa of the receptor serve as a signal peptide that should mediate the secretion of a protein containing 333 aa of the LDLR plus 2 foreign aa from a nonsense linker. The truncated receptor protein is secreted into the cell culture medium of infected *Spodoptera frugiperda* (Sf-21) cells. Following metabolic labeling with ^{35}S-methionine, the secreted receptor product was immunoprecipitated with a receptor-specific monoclonal antibody. Autoradiography of the reducing SDS-PAGE gel showed a single band with a molecular weight of 66 kDa. Two dimensional SDS-PAGE of ^{35}S-labeled secreted proteins indicated that the truncated receptor has a pI close to 4.0 as predicted. Non-reducing SDS-PAGE followed by immunoblot analysis resulted in a diffuse immunoreactive band above 100 kDa. In a competition assay, media containing the truncated receptor competed for the binding of ^{125}I-LDL to the human LDLR expressed on the surface of CHO cells. Upon purification of the truncated receptor, further studies will explore the thermodynamic and kinetic properties of LDL receptor-ligand interactions. Additionally, this product will be used for structural analysis of the receptor's ligand binding domain.

L 108 NUCLEAR MAGNETIC RESONANCE STUDIES OF A SOLUBLE CLASS II MHC/PEPTIDE COMPLEX.

Paul C. Driscoll, Department of Biochemistry, Oxford University, Oxford OX1 3QU, UK. Kazuyasu Sakaguchi, James G. Omischinski & Ettore Appella, Laboratory of Cell Biology, Building 37, NCI, NIH, Bethesda MD 20892. J. Jay Boniface, John Altman & Mark M. Davis, Department of Microbiology and Immunology, Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305.

Soluble glycosylated mouse I-E^k class II molecules have been produced in mammalian cell culture in quantities sufficient to perform a number of structural studies[1]. By the use of ^{13}C isotope labeling and two-dimensional heteronuclear NMR spectroscopy we have investigated the binding of a sixteen residue peptide from moth cytochrome c to this molecule. The total molecular mass of the complex is *ca.* 70 kDa. The peptide (A^{88}-N-E-R-A-D-L-I-A-Y-L-K-Q-A-T-K^{103}), labeled with NMR-active ^{13}C nuclei in the methyl positions of its four alanine residues, has been introduced into the MHC molecule in high yield. NMR experiments have been performed to investigate the ^1H-^{13}C correlation spectrum of the complex. The cross-peaks for two of the alanine methyl groups (A96 and A101) are significantly perturbed (in both linewidth and chemical shift, indicating that the C-terminal portion of the peptide is bound in the MHC groove in an environment rich in aromatic groups. Uniformly ^{13}C-labeled MCC(88-103) has been produced in a biosynthetic manner in *E. coli*. The spectra of the free and bound states of the labeled peptide have been investigated. The results are consistent with a single peptide binding in the groove of the class II MHC molecule in an extended conformation with different parts of the peptide exhibiting differential mobility. The five N-terminal residues of the peptide are relatively mobile and up to eleven residues at the C-terminal end of the peptide are sequestered in the binding groove. ^{13}C-editted NOESY experiments have been performed in order to investigate the detailed bound peptide conformation. The results of this study provide an insight at an atomic level into the nature of the binding of this peptide in the groove of the class II MHC molecule that has not hitherto been possible using other biophysical methods. The pattern of signals observed is generally consistent with a picture of the bound peptide that has been derived from an extensive biological investigation[2] and provides a basis for modelling the class II MHC/peptide interaction.

1. Wettstein *et al.*, J. Exp. Med. (1991) 174, pp. 219-228.
2. Jorgensen *et al.*, Ann. Rev. Immunol. (1992) 10, pp. 835-873.

L 109 PEPTIDE MODELS OF PROTEIN FOLDING: DESIGN AND INITIAL STRUCTURAL CHARACTERIZATION OF A HELIX–TURN-HELIX HAIRPIN.

Youcef Fezoui†°, David Weaver† and John J. Osterhout°, †Department of Physics, Tufts University, Medford, MA 02155 and °The Rowland Institute for Science, 100 Cambridge Parkway, Cambridge, MA 02142

The goal of this work is to produce a peptide model which can be used to study interacting secondary structural elements. Secondary structures have been postulated to be early intermediates in protein folding and peptides have been employed extensively as models for isolated secondary structural units, particularly beta-turns and alpha-helices. The interaction of secondary structural elements is believed to be important in folding kinetics. The development of a helix-turn-helix peptide would provide a model system to study both the stability and kinetics of association of connected helices. To this end we have designed a peptide which contains two helical microdomains connected by a short turn. The designed peptide has two helical regions (17 amino acids per helix), each of which contains a pattern of hydrophobic amino acids based primarily on that of coiled coil peptides. The turn region connecting the helices has of four amino acids which were chosen for their turn-promoting propensities. Preliminary structural characterization of the peptide has been accomplished with CD and two-dimensional NMR. These experiments indicate the presence of helix at room temperature. The concentration dependence of the peptide has been studied by CD and NMR. No evidence for concentration dependent behavior was detected over a 200 fold range of concentration.

L 110 ANALYSIS OF MHC CLASS II PEPTIDE INTERACTION WITH M13 PEPTIDE DISPLAY LIBRARIES,

Juergen Hammer, David Bolin and Francesco Sinigaglia, Department of Inflammation and Autoimmune Diseases, F. Hoffmann-La Roche Ltd., Nutley, NJ 07110-1199, USA, and Roche Milano Ricerche, via Olgettina 58, I-20132 Milan, Italy

The major histocompatibility complex (MHC) class II molecules are highly polymorphic membrane glycoproteins that bind peptide fragments of proteins and display these peptides for recognition by CD4+ T cells. Most of the amino acid differences that distinguish MHC allelic variants lie in the peptide binding cleft, and different allelic forms of MHC molecules bind distinct peptides.

We have recently demonstrated that affinity purified MHC class II molecules can specifically bind to peptide sequences displayed by modified protein III on the surface of M13 phages. This allowed us to use M13 peptide libraries to screen for phages that bind to MHC class II HLA-DR1 alleles. The comparision of the peptide pool binding to DR1 with the original peptide pool of the M13 library revealed new insights in peptide-DR1 interaction (Hammer, J. et al., J. Exp. Med., Oct. 1992).

To understand the principles of allele specificity for HLA-DR binding peptides we extended our analysis to HLA-DR4Dw4 and DRw11 class II alleles. We have isolated large M13 peptide pools selected by those alleles. Sequence analysis of the peptide encoding region of the selected phages led to the identification of general and allele specific anchor residues. The results provide a simple biophysical basis for the promiscuity and the specificity of peptide recognition by HLA-DR molecules. Furthermore, the knowledge of these anchor positions should prove useful for the design of MHC-specific antagonists.

L 111 NMR STUDIES ON THE INTERACTON OF ACYL-ACP AND BLOCH DEHYDRASE

Blake Hill*, John M. Schwab#, and James H. Prestegard*, Dept. of Chemistry*, Yale University, New Haven, CT 06520 and Dept. of Medicinal Chemistry#, Purdue University, West Lafayette, IN 47907

Acyl carrier proteins (ACPs) of *E. coli* and related organisms belong to a class of small, monomeric proteins that shuttle a nascent fatty acid chain between the enzymes of type II fatty acid synthetase systems. As such, the system presents numerous opportunities for control of synthesis through protein-protein interaction. A particularly interesting stage of fatty acid biosynthesis in *E. coli* under anaerobic conditions is reached when the growing fatty acid chain is ten carbons long and the acylated ACP (acyl-ACP) interacts with 3-hydroxydecanoylthioester dehydrase (Bloch dehydrase): This is the point where unsaturated and saturated fatty acid syntheses diverge. We have been exploring protein-protein interactions in this system using heteronuclear NMR techniques.

We have prepared (R)-4-fluoro- and (S)-4-fluoro-decanoyl-ACPs (~9 kDa) along with uniformally ^{15}N-labeled acyl-ACP. Our ongoing investigations include ^{19}F-NMR titration and heteronuclear NOE studies with Bloch dehydrase (~37 kDa). The spectra from these studies indicate a specific interaction between the acyl chain fluorine and the active site of dehydrase. Based on the solution structure of ACP previously generated from our lab, we have begun assigning the ^{15}N-labeled acyl-ACP with the long term goal of defining the key ACP residues involved in the interaction.

L 112 SYNTHETIC PEPTIDE COMBINATORIAL LIBRARIES: TOOLS FOR BASIC RESEARCH AND DRUG DISCOVERY, Richard A. Houghten, Clemencia Pinilla, Jon R. Appel, Colette T. Dooley, Philippe Blanc*, and Ronald R. Tuttle*, Torrey Pines Institute for Molecular Studies, San Diego, CA 92121, *Houghten Pharmaceuticals, San Diego, CA 92121

Synthetic peptide combinatorial libraries (SPCLs) [*Nature* 354: 84-86, 1991] can be used in virtually any existing *in vitro* assay system to determine highly effective peptide sequences. Examples of the studies carried out in this laboratory utilizing SPCLs include: the identification of antigenic determinants and the study of antibody/antigen interactions using ELISA; radio-receptor assays to determine novel, opioid peptides unrelated to the naturally occurring enkephalins; enzyme inhibition assays; microdilution assays to determine potent antibacterial and antifungal peptide sequences; and plaque assays to determine short peptide sequences that are effective at the submicrogram per ml level for the inhibition of plaque formation by both Herpes Simplex Virus Type 1 and Type 2.

The above results will be described, as will the rapid determination of high affinity peptide interactions with positional scanning SPCLs, and the use of SPCLs directly in *in vivo* assays to determine highly active peptide sequences that lower blood pressure.

L 113 SELECTION OF CALMODULIN-BINDING PEPTIDES FROM A BACTERIOPHAGE RANDOM PEPTIDE LIBRARY, Gordon A. Jamieson, Jr., Marcia. A. Kaetzel, and John R. Dedman, University of Cincinnati, Cincinnati, OH 45267

Molecular recognition in biological systems is commonly implemented through the interaction of small peptide domains. Localized perturbations in structure, initiated via these interactions, can induce profound alterations of biological function. A strategy to identify peptides which bind to a specific site on a protein is to use random peptide libraries of large diversity. This approach eliminates the necessity of screening catalogues of natural and synthetic compounds. In the current study we used a bacteriophage random peptide library (Devlin et al. 249:404, 1990) to identify peptides which bind calmodulin (CaM) in a Ca-dependent manner. Bacteriophage were isolated by Ca-dependent affinity chromatography on CaM-Sepharose. The sequence of the random peptide insert of 68 independent bacteriophage isolates was determined. Twenty-eight unique sequences were obtained. Analysis of the peptide sequences revealed several remarkable features. All of the peptides contain a tryptophan (TRP); all possess a net positive charge. Three classes of peptides were identified. Eleven have TRP in position 3 of the inserted sequence followed by a proline (PRO), a helix breaker (Group I). The TRP-PRO motif is found in 6 additional isolates, but not in positions 3 and 4 (Group II). In Group III the TRP varies between positions 4 and 13 and is not followed immediately by a PRO. Selected peptides were synthesized. Peptides containing the TRP-PRO motif competed with melittin-Sepharose for binding of radiolabelled-CaM with relative affinities similar to the high-affinity CaM-binding wasp venom peptide, mastoparan. These data, and Chou-Fasman analyses which predict five of the sequences to form a strong beta-sheet structure, argue that TRP does not need to be located within an alpha-helical structure in order for peptides to interact with CaM in a Ca-dependent manner. These results demonstrate that peptides binding to independent sites on CaM can be identified via a direct approach through the use of a random peptide library and affinity chromatography.

L 114 FUNCTIONAL ANALYSIS OF A CATHEPSIN B-LIKE *SCHISTOSOMA MANSONI* PROTEIN Sm31, Mo Klinkert, §Elliott Shaw, Livia Pica-Mattoccia and Donato Cioli, Institute of Cell Biology, Consiglio Nazionale delle Ricerche, Rome, Italy and §Friedrich-Miescher Institute, Basel, Switzerland

Human schistosomiasis is a parasitic disease caused by the trematode *Schistosoma*, affecting over 200 million people in tropical countries. One of the key features of schistosome metabolism is the ingestion of red blood cells and the subsequent digestion of haemoglobin as a source of nutrients for the parasite. A cysteine proteinase resembling cathepsin B has been identified as a major metabolic enzyme responsible for haemoglobin degradation in the gut of the schistosome. The enzyme elicits a strong immune response in infected humans. Using human infection sera to screen a cDNA library prepared from adult *S. mansoni* RNA, a full-length cDNA clone encoding a 31 kDa protein (Sm31) has been isolated. Nucleotide and deduced amino acid sequences confirmed Sm31 as the schistosome homologue of mammalian lysosomal cathepsin B. By incubating a radiolabelled cysteine proteinase active site-directed synthetic inhibitor with total *S. mansoni* proteins, the target of inhibition was Sm31, as confirmed by antiserum raised specifically to the protein. Enzyme assays showed that specific peptidyl diazomethylketone inhibitors are very effective against the recombinant protease. In order to evaluate the cathepsin B-like enzyme as a possible chemotherapeutic target, we have examined the effects of diazomethylketones on in vitro cultures of *S. mansoni* adults. Preliminary results show that Cbz-Tyr-Ala-CHN_2 kills parasites, suggesting that the enzyme may be a promising antischistosomal target. In order to generate more definitive structural data, experiments to express and purify the protein in reagent quantities are underway.

L 115 **FREE ENERGY ANALYSIS OF HIRUDIN ILE^{59} SIDE CHAIN IN BINDING TO THE THROMBIN EXO SITE** Yasuo Konishi, Shi-Yi Yue, Zbigniew Szewczuk, Sean Taylor and Lynne LeSauteur, Biotechnology Research Institute, National Research Council Canada, Montreal, Quebec, Canada H4P 2R2

The thrombin inhibitors based on the hirudin sequence have a critical residue, Ile^{59}, for the binding to the fibrinogen recognition exo site of thrombin [Yue et al., Protein Engineering **5**, 75-83 (1992)]. The roles of CH, CH_2 or CH_3 group in the Ile^{59} side chain were studied by a series of analogs in which Ile$_{59}$ was substituted with Gly, Ala, aminobutyric acid, Nva, or Val experimentally and theoretically. For example, the role of the δ-CH_3 group in the Ile^{59} side chain was estimated as the free energy difference (1.0 Kcal/mol) between an inhibitor, (dF)PRP-Aha-Abu-DFEEIPEEYLQ (K_i = 0.82 ± 0.02 nM), and its Val analog, (dF)PRP-Aha-Abu-DFEEVPEEYLQ (K_i = 4.7 ± 0.2 nM). Similarly, the contributions of β-CH, γ-CH_3, γ-CH_2 were estimated as 1.4, 1.6 and 1.2 Kcal/mol, respectively. This suggests that any part of the Ile^{59} side chain contributes more or less equally to the potency of the inhibitor. For the theoretical estimation of the Ile^{59} side chain contribution, the free energy perturbation calculation was carried out for the hirudin$^{55\text{-}65}$ fragment using X-ray coordinates of hirudin peptide-thrombin complex. The contributions of γ-CH_3 and δ-CH_3 were estimated as 1.8 and 2.0 Kcal/mol, respectively, from the free energy perturbation calculation of Val^{59}-hirudin$^{55\text{-}65}$ and Nva^{59}-hirudin$^{55\text{-}65}$. These are in good agreement with the corresponding experimental results (1.6 and 1.0 Kcal/mol, respectively). Under the same simulation conditions, the dynamic trajectory of Val^{59} and Nva^{59} in these analogs showed larger conformational fluctuations than that of Ile^{59}. This may be interpreted as the γ-CH_3 and δ-CH_3 of Ile^{59} contributing to the potency of the inhibitor not only through the intermolecular interactions but also by stabilizing the active conformation intramolecularly.

L 116 STRUCTURE-ACTIVITY RELATIONSHIP STUDIES OF P-SELECTIN (23-30)-NH_2 FRAGMENT, Marian Kruszynski, Leon Epps, Miljenko Mervic, Margaret Falcone, Douglas Riexinger, Rodger P. McEver* and George A. Heavner, Centocor, Inc., Malvern, PA 19355; *Oklahoma Medical Research Foundation, University of Oklahoma, Oklahoma City, OK 73104

The selectins are three structurally related membrane glycoproteins that bind to oligosaccharide ligands on target cells during early inflammation. An acute inflammatory reaction involves recruitment of neutrophils, their migration into tissues and accumulation at sites of injury or infection. P-selectin is a receptor for neutrophils and monocytes that is rapidly translocated from secretory granule membranes to the plasma membranes of activated platelets and endothelial cells. We have identified several non-contiguous peptide sequences within the lectin domains that inhibit both P- and E-selectin-dependent adhesion.

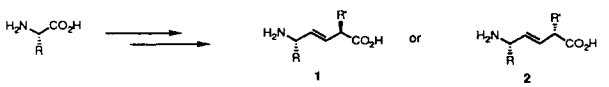
In order to increase the potency of these peptides, we have investigated the structural requirements for activity. Here we present the results of studies on the peptide corresponding to the 23-30 sequence of P-selectin (Tyr-Thr-Asp-Leu-Val-Ala-Ile-Gln-NH_2). Single substitutions have determined that the C-terminal hydrophobic region is important for inhibitory activity. Several substitutions within the N-terminal segment increase the IC_{50} from 0.28 mM to 18μM. The ability to significantly increase the activity by rational substitutions makes these peptides attractive candidates for treatment of inflammatory disorders by inhibiting selectin-mediated neutrophil adhesion.

L 117 **Mutations in T7 RNA Polymerase which Support the Proposal for a Common Polymerase Active Site Structure,** Gary Bonner, Eileen M. Lafer, and Rui Sousa, A502 Langley Hall, Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, USA

The nucleic acid polymerases are a focus of interest in biotechnology research as both therapeutic targets and reagents for research. However, the lack of structural information has limited engineering approaches to these enzymes. It has been proposed that most nucleotide polymerases share a common active site structure and folding topology, a 'polymerase fold'. This proposal was based on the identification of limited sequence conservation in 3 motifs--dubbed A, B, and C--found in most nucleic acid polymerase sequences (Delarue *et al.*, **J. Protein Engineering**, 1990). In order to test this proposal we have generated 22 mutations of residues within motifs A, B, and C of T7 RNA polymerase (Bonner et al., **EMBO J.** 10, 1992). If the Delarue *et al.*, alignment is valid, then some of these mutations would 'correspond' to mutations previously characterized in DNA polymerase I (Polesky *et al.*, **J. Biol. Chem.**, 1990), so that a direct comparison of putatively corresponding mutant phenotypes is possible. Characterization of these T7 RNAP mutants showed the following: 1. Most of the mutations resulted in moderate to drastic reductions in T7 RNAP transcriptional activity supporting the idea that motifs A, B, and C identify part of the polymerase active site. 2. The degree of conservation of an amino acid within these motifs correlated with the degree to which mutation of that amino acid reduced transcriptional activity supporting the predictive ability of the Delarue *et al.*, alignment in identifying the most functionally critical residues. 3. A comparison of DNAP I and T7 RNAP mutants revealed similarities between 'corresponding' mutant phenotypes. 4. The KF structure is shown to provide a reasonable basis for interpretation of the differential effects of mutating different amino acids within motifs A, B, and C in T7 RNAP. These observation support the proposal that these polymerase active sites have similar 3-dimensional structures and, by extension, imply that the KF and T7 RNAP structures can be used to model other polymerases for the design and interpertation of structure-function studies and protein engineering experiments.

L 118 A GENERAL ASYMMETRIC SYNTHESIS OF *TRANS*-ALKENE DIPEPTIDE ISOSTERES, Mark Lipton and Michael Koscho, Department of Chemistry, Purdue University, West Lafayette, IN 47907

Trans-dipeptide isosteres represent an important class of peptide mimetics due to their close structural resemblance to dipeptides and their stability toward hydrolysis. A flexible synthesis of enantiomerically pure *trans*-alkene dipeptide isosteres from α-amino acids has been devised which permits the construction of a wide variety of molecules with the generic structures **1** or **2**. The synthesis employs a chelation-controlled addition to a protected α-amino aldehyde followed by a modified [2,3]-Wittig rearrangement. The synthetic results, as well as the discrimination between products **1** and **2** will be discussed.



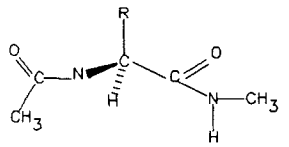
1 **2**

L 119 DIFFERENCES IN IMMUNOLOGICAL RESPONSE BETWEEN PROTEINS DEPENDENT ON RECEPTOR BINDING; A COMPARISON OF VACCINE THERAPY WITH HIV-1 GP160 OR GP120 IN INFECTED PATIENT VOLUNTEERS, Lawrence Loomis*, Felecia Mann*, Felix Lopez*, Robert Redfield+, and Deborah Birx+, *HIV Laboratory, H.M. Jackson Foundation, and +Department of Retroviral Research, Walter Reed Army Institute of Research, Rockville, MD 20850

The HIV-1 envelope protein (LAI strain) has been expressed in two different forms: gp120, the processed form (Chinese Hamster Ovary, Genentech) is capable of binding the CD4 receptor protein whereas gp160, the pro-protein, (*Baculovirus*, MicroGeneSys) does not. We are conducting clinical trials in which early stage HIV-1-infected patient volunteers receive multiple immunizations with either gp120 or gp160. We are examining changes in antibody reactivity to the entire HIV-1 envelope protein, and fine-mapping the response to various sub-regions and specific epitopes. SPECTROTYPE: to measure changes to conformationally intact antigen, antibodies from patient sera prior to and post vaccine therapy were separated using isoelectric focusing, then blotted to nitrocellulose and reacted with labeled whole gp120 from either of two strains, or with peptides. PEPSCAN: to measure antibody changes to specific linear epitopes, the entire sequences of three strains of HIV-1 were constructed in 12-mer peptides overlapping by eight. Changes to individual epitopes were measured by ELISA against these peptides. RESULTS: Patient volunteers immunized with gp160(LAI) had strong new responses to conformationally intact antigens, as well as antibody responses to linear epitopes throughout the envelope sequence. Patients immunized with gp120(LAI) had responses which were equally strong, but more closely matched the response observed in natural infection. The differences in response may be attributable to the binding of gp120 to the CD4 receptor and subsequent immune presentation differing from gp160, which does not bind the cellular receptor.

L 120 SOLVATED STRUCTURE ANALYSIS BY THE AMSOL METHOD OF CONFORMATIONALLY RESTRICTED AMINO ACID ANALOGUES IN A DIPEPTIDE MODEL, Sandor Lovas, D. David Smith and Richard F. Murphy, Department of Biomedical Sciences, School of Medicine, Creighton University, Omaha, NE 68178

Conformationally restricted amino acid analogues are commonly used in structure-activity studies of regulatory peptides. Determination of structure is based on NMR spectroscopy combined with Molecular Dynamics simulation but it is frequently impossible to determine the structure in water solution because many peptides are hydrophobic. The computational task of Molecular Dynamics simulation with explicit water molecules is tedious and molecular force field with precise parameters are not available for simulating aqueous or other solutions. AMSOL is a recently developed semi-empirical quantum chemical method that is capable of evaluating free energy of solvation within experimental error so that it is possible to predict solute structure and rotational barriers. The following amino acid derivatives, L- and D-Tic, L- and D-β-Me-phenylalanine, L- and D-N-Me-phenylalanine and L- and D-3-carboxy-1,2,3,4-tetrahydro-2-carboline, were placed in a model dipeptide:



Following full geometry optimization, conformation searches, with rigid bond length, were made and the resulting structures were analyzed with emphasis on side chain topology. Definition of the topology of peptides is of crucial importance in understand structural requirements for biological activity of peptides.

L 121 THE MINIMUM LENGTH OF A COILED COIL
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Whitehead Institute for Biomedical Research
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Nine Cambridge Center, Cambridge, MA 02142

The coiled coil motif is a particularly attractive system for both peptide design and studies of macromolecular (protein-protein) recognition. The coiled coil is formed by two α-helices that wrap around one another in a left handed supercoil. The helices are comprised of "heptads" of seven amino acid residues characterized by a 4, 3 hydrophobic repeat which forms the dimerization interface.

One important determinant of coiled coil formation and stability is the number of heptads. This has been addressed previously using designed peptides; it was concluded that a minimum of 29 residues, or 4 heptads, were required for the formation of a coiled coil (Lau, Taneja and Hodges, *J. Biol. Chem.*, **259**, 13253, 1984). This result has important implications for the design of coiled coils and for their incorporation into de novo proteins.

An alternative approach is to delete residues from the sequence of a naturally occurring coiled coil. Residues have been deleted from the sequence of the coiled coil from GCN4, and the effect of these truncations on coiled coil formation have been assessed using circular dichroism, sedimentation equilibrium and NMR spectroscopy.

Removal of residues from the N terminus of the sequence of the coiled coil from GCN4 (which contains 35 residues, or 4.5 heptads) indicates that coiled coils can be formed from sequences which contain less than four heptads.

L 122 ANALYSIS OF THE SEQUENCE SPECIFICITY OF HIV GP41 HOMO-OLIGOMERIC INTERACTIONS BY MUTAGENESIS OF A CHIMERIC CONSTRUCT, Kevin R. MacKenzie and Donald M. Engelman, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven CT 06511.

The two-stage model of protein folding, which hypothesizes that specific side-by-side association of stable transmembrane domains follows a separate process of insertion of these domains into lipid bilayers, has been recently supported by a number of experimental systems. The demonstration of such specific interactions reveals a new type of target for alteration of biologic activity by disruption of protein-protein interactions. We are interested in identifying systems where lateral association of transbilayer structures has biological relevance, and in subsequently working towards an understanding of the nature of these interactions.

The sequence of the transmembrane domain of HIV gp41 is highly conserved between isolates, suggesting some conserved function. We have fused the transmembrane sequence of the HIV envelope protein, gp41, to the C-terminus of staphylococcal nuclease and have begun to characterize the behavior of both the resultant chimera and the isolated hydrophobic peptide obtained by proteolysis of the chimeric protein. Our data demonstrate that the transmembrane domain of gp41 causes oligomerization of the chimera in detergent environments.

The facile manipulation of the DNA sequence by PCR methods has permitted an exploration of the sequence dependence of oligomerization of the HIV gp41 membrane anchoring domain. We present the results of this mutagenesis and studies of the isolated peptide using CD to characterize its secondary structure and FRET (fluorescence resonance energy transfer) to determine the oligomeric state of the peptide in different detergent and lipid environments. The results are correlated with *in vivo* data from mammalian cells on the maturation and function of full length HIV gp41 bearing mutations in the transmembrane region.

L 123 CONFORMATIONAL SUBSTATES AND HELICITY QUANTITATION OF A STRUCTURED PEPTIDE UNDER VARIOUS CONDITIONS USING NMR AND CD Gene Merutka, Dimitrios Morikis, Rafael Brüschweiler, and Peter E. Wright, Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037

NMR and CD methods are used to examine structure and helicity of the peptide Ac-WEAQAREALAKEAAARA-NH_2. This peptide is a modification of a seventeen residue alanine-based peptide examined previously and found to be nearly completely helical by circular dichroism [Merutka et al., Biochemistry (1991) 30, 4245-4248]. Substitutions in the sequence were made to reduce anticipated overlap in the NMR spectra while still preserving the helical nature of the peptide. Fast 2D-TOCSY experiments providing the assignments of all resonances under different conditions (variable temperature and H_2O/TFE concentrations) were used for chemical shift analysis and for the study of exchange behavior of labile protons. Secondary structure connectivity diagrams as well as long-range side-chain side-chain contacts are established on the basis of NOESY/ROESY data. These data, in conjunction with CD experiments, allow the characterization of a conformational ensemble exhibiting a high degree of helicity.

L 124 SYNTHETIC PEPTIDES AND ANTIPEPTIDE ANTIBODIES AS PROBES TO STUDY INTERDOMAIN INTERACTIONS INVOLVED IN VIRUS ASSEMBLY AND VIRUS-CELL RECEPTOR BINDING, A. Robert Neurath, Nathan Strick and Shibo Jiang, New York Blood Center, New York, NY 10021

Synthetic peptides and antipeptide antibodies have been widely used as probes to map antibody-binding sites and T-cell epitopes on proteins. However, such probes also have the potential to delineate contact sites involved generally in protein-protein interactions, in the association of domains within a protein and in virus-cell receptor binding. We applied such probes to define: (1) regions on the human immunodeficiency virus type 1 (HIV-1) surface glycoproteins (gp) gp120 and gp41 involved in inter-subunit association; (2) sites on gp120 essential for attachment of HIV-1 to the CD4 cell receptor; (3) definition of binding sites for cell receptors on the surface of the hepatitis B virus (HBV) envelope protein and (4) the cell surface component involved in attachment of HBV to liver (human hepatoma) cells and on to other cells susceptible to HBV infection. Results of these studies revealed the following: (1) two segments on HIV-1 gp120, nonadjacent in their primary amino acid sequence, contribute to the binding site for the CD4 cell receptor; (2) portions of immunodominant antibody binding sites on gp120 and gp41 appear to be involved in gp120-gp41 association, suggesting the partial cryptic nature of these epitopes on assembled virus particles; (3) an overlap between gp41 and CD4 binding sites on gp120; (4) the cryptic nature of sites involved in fusion of HIV-1 with cells; (5) location of binding sites for the cell receptor for HBV on the pre-S1 region of the envelope protein; (6) identification of recognition sites for the HBV env protein on interleukin 6 and (7) delineation of regions on IL-6 involved in the HBV-IL-6 interaction.

L 125 IDENTIFICATION AND CHARACTERIZATION OF STRUCTURAL MUTANTS OF STRP G, Karyn T. O'Neil, Ronald H. Hoess, Daniel P. Raleigh and William F. DeGrado, DuPont-Merck Pharmaceutical Co. Wilmington, DE 19880-0328 and Dept of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA 19104-6059.

The NMR structure of the IgG binding domain from group G streptococcus (Strp G) has recently been described in detail by several groups. We are attempting to identify mutations that alter the way in which Strp G folds. A synthetic gene encoding Strp G has been fused to the minor coat protein gIII of bacteriophage M13. This results in Strp G being displayed on the surface of the phage, and allows for the rapid selection of mutants that alter Strp G folding. Once mutations are identified, the mutant Strp G is transferred to a plasmid for overexpression of protein in quantities that allow us to do biophysical studies. We have identified a segment of StrpG, near the begining of the α helix that tolerates several substitutions without grossly altering the overall fold. However the melting temperature as measured by CD becomes biphasic when the mutation is introduced. NMR studies are underway to identify specific structural details of the mutant proteins.

L 126 STRUCTURE AND ACTIVITY OF A HYBRID SEQUENCE, DISULFIDE STABILIZED FORM OF THE MLCK M13 PEPTIDE, Mark D. Pagel, Donald K. Blumenthal, and David E. Wemmer, Department of Chemistry, University of California, Berkeley, Berkeley, CA, 06510, and Deparment of Pharmacology & Toxicology, University of Utah, Salt Lake City, UT, 84112.

A peptide has been synthesized that has a hybrid sequence, partially derived from the bee venom apamin and patially from the M13 peptide of Myosin Light Chain Kinase. The structure of the folded peptide was shown by NMR to consist of the same disulfides and basic three dimensional structure as native apamin, containing a β-turn and α-helix. The propagation of the helix was studied without the influence of helix nucleation. The helix was shown to abruptly end at residue 20, and did not terminate gradually over several residues as expected. The hybrid peptide bound tightly to Calmodulin in a Ca2+-dependent fashion, as measured by circular dichroism and fluorescence spectroscopy. Comparison of the hybrid peptide with the solution structure of the M13-Calmodulin complex revealed that only minor changes in Calmodulin's M13-bound conformation were necessary to accommodate the presence of the apamin sequence in the hybrid peptide. In general, this hybrid sequence peptide has demonstrated that peptide-protein functional interactions can be examined with small peptides of stable, predictable structure.

L 127 RECONSTRUCTION OF PROTEIN CONFORMATIONS FROM ESTIMATED C-ALPHA COORDINATES, Philip W. Payne, Protein Design Labs, 2375 Garcia Avenue, Mountain View, CA 94043

Protein C_α coordinates are used to accurately reconstruct complete protein backbones and side chain directions. This work employs potentials of mean force to align semi-rigid peptide groups around the axes that connect successive C_α atoms. The algorithm works well for all residue types and secondary structure classes and is stable for imprecise C_α coordinates . Tests on known protein structures show that root mean square errors in predicted main chain and C_β coordinates are usually less than 0.3 Å. These results are significantly more accurate than can be obtained from competing approaches, such as modeling of backbone conformations from structurally homologous fragments.

L 128 EXOGENOUS INHIBITION OF THE CONFORMATIONAL SWITCH IN THE *env* GLYCOPROTEIN gp120 OF HIV1

Jennifer Reed & Volker Kinzel, Dept. of Pathochemistry, German Cancer Research Center, Heidelberg, Germany

The ability to undergo an abrupt conformational switch from ß-sheet to α-helix on moving from a polar to a less polar environment has been shown to be conserved within the CD4-binding of the *env* glycoprotein gp 120 from human immunodeficiency virus type 1 despite considerable variability in the primary structure. This 'switch' process appears to be essential for binding to the T-cell receptor, amino acid substitutions that disrupt the switch simultaneously destroying CD4-binding activity and vice versa[1]. It therefore represents a potential strain-independent target for therapy. Endogenous inhibition such as that achieved by amino acid substitution is therapeutically impractical, however. We present here the initial results of a search for exogenous substances capable of preventing CD4 binding through the mechanism of switch inhibition. Two classes of modified peptides were found to be active as switch inhibitors. On the basis of shared chemical properties a non-proteinaceous switch inhibitor was found which was able to mimic their activity. A sensitivity to certain types of side chain modifications suggests that a high degree of specificity may be obtained. The chemical similarities among these compounds lay the basis for quantitative structure/activity analysis for the design of an optimally effective inhibitor.

[1]Reed, J. & Kinzel, V. 1991 Biochemistry 30:4521

L 129 STRUCTURAL EVIDENCE FOR INDUCED FIT BINDING TO AN ANTI-PEPTIDE ANTIBODY. Ursula Schulze-Gahmen, James M. Rini and Ian A. Wilson, Research Institut of Scripps Clinic, 10666 North Torrey Pines Rd., La Jolla, CA 92007.

The anti-peptide antibody 17/9 recognizes a six amino acid residue epitope in a synthetic peptide from influenza virus hemagglutinin HA1 (residues 101-106, DVPDYA). The three-dimensional structure of the unliganded Fab-fragment and its complex with two peptides of different length in three different crystal forms were determined by x-ray crystallographic methods. The analysis of structural differences between the free and bound Fab and between the various complexes allows the segregation of crystal packing and peptide binding as causes of structural changes. Peptide binding to the Fab causes a fundamental conformational change in the structure of the H3 hypervariable loop. Seven out of eleven residues adopt very different conformations in Φ, Ψ space causing r.m.s.differences in the main chain of up to 4.0 Å and in the side chains of up to 9.0 Å. At the same time the hydrogen bonding pattern of the H3-loop changes drastically.

The conformation of the bound peptide is the same in all three complex crystal forms. Residues 100 to 103 are bound in extended conformation, while residues 104 to 107 form a type I β-turn. The antigen antibody interactions have been characterized extensively by inhibition assays with peptide analogs. The results of these studies correlate well with the structural data. Those positions in the peptide epitope, for which analogs show very weak activity, have many and close interactions with the Fab. Peptide residue Tyr 105 seems to be most important for peptide binding and for inducing the conformational change in the H3-loop. This residue could not be accommodated in the binding pocket of the free Fab without bad sterical clashes but has a very close complementary fit with the Fab in the complex structure.

This well studied system provides an example for the structural basis of high affinity peptide protein interactions and their correlation with functional data, which can be applied to other protein ligand systems.

L 130 MEMBRANE ASSOCIATION - A PREREQUISITE FOR NEUROPEPTIDE Y RECEPTOR BINDING, T.W.Schwartz[1], J.Fuhlendorff[1], A.G.Beck-Sickinger[2], A.Holm[3], M.Barfoed[4], K.Borch[4], H.Lund[4], B.Lundt[5], N.L.Johansen[5], and H.Thøgersen[5], [1]Laboratory for Molecular Endocrinology, University Hospital 6321, Blegdamsvej 9, DK-2100 Copenhagen Denmark; [2]Department of Organic Chemistry, University of Tübingen, Germany; [3] Department of Chemistry, Royal Danish Veterinary University, Copenhagen; [4]Department of Biophysics and [5]Division of Pharmaceuticals Research, Novo Nordisk A/S, Copenhagen, Denmark.

Neuropeptide Y is a 36 residue peptide belonging to the PP-fold family which are characterized by X-ray and ^{1}H-NMR (in aqueous solution), to have a well defined 3D structure composed of a long amphiphilic alpha-helix which is stabilized by hydrophobic interactions with an antiparallel poly-proline helix. The PP-fold is instrumental in presenting the two ends of the peptide to the receptors. For the Y2 type of NPY receptor it is known that the side chains of only the C-terminal four residues are directly involved in receptor binding. These residues are, however without any receptor activity unless they are placed either on a long amphiphilic, C-terminal fragment of the peptide or in a mini-NPY (a bridged, PP-fold-truncated construct lacking residues 5-24). The whole, stabilized PP-fold motif of NPY displays a clear, hydrophobic patch along the axis of the fold. We have substituted residues 17,21,24, and 28 in this hydrophobic patch in NPY with hydrophilic residues, Ala's, or hydrophobic residues, either singly or together. Both the biological activity of the peptides, as Y1 and Y2 receptor binding and activation, and the surface chemical properties of the NPY analogs in terms of phi (surface pressure)-A(area)-isotherms and by rate and extent of adsorption at an air-water interface, were determined. A surprisingly good correlation was found between the surface activity of these analogs and their receptor activity. Certain analogs, for example [E^{17},E^{21},N^{24},N^{28}]NPY, do not display any surface activity at all, and these analogs do not react with the NPY receptors at all, despite the fact that they are full length NPY analogs having all the C-terminal sidechains required for receptor recognition. It is concluded that in NPY analogs, surface activity appear to be a prerequisite for receptor binding. It is suggested that surface activity/hydrophobicity should be taken into account when designing peptide mimetics at least for NPY peptides, but probably also for many other peptides.

L 131 FAB-PEPTIDE INTERACTIONS AS DETERMINED BY X-RAY CRYSTALLOGRAPHY, Robyn L. Stanfield, Jayant B. Ghiara, Arnold C. Satterthwait, and Ian A. Wilson, Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA, 92037

Monoclonal antibodies have been raised against a 40-amino acid, disulfide linked loop corresponding to the principal neutralizing determinant (PND) of GP-120 from the HIV-1 virus (MN isolate). These antibodies neutralize virus, apparently by preventing the cell-fusion step in HIV-1 virus mediated cell death. The antibodies recognize an epitope near the highly conserved sequence GPGRAF. Fab fragments from three of the antibodies have been co-crystallized with linear and cyclic peptides and their structures have been determined by x-ray crystallography. Fab 50.1, which is specific for the MN-type sequence, recognizes a 9-amino acid epitope (CKRIHIGPG). The peptide binds in an extended conformation with a turn starting at residues GPG. The Fab-peptide interactions are dominated by hydrophobic pockets on the surface of the Fab, into which the peptide Ile residues fit tightly. The structure of Fab 50.1 has also been solved in its peptide-free form. The comparison of the free and bound Fab structures graphically demonstrates that antibodies can undergo large tertiary and quaternary conformational changes in order to optimally bind antigens. Fab 59.1 is a broadly neutralizing antibody, having neutralization or inhibition activity against the MN, IIIb, WMJ2, and SF2 isolates. The structure of 59.1 has been solved in complex with a 24-amino acid peptide. Fab 58.2 is derived from another broadly neutralizing antibody. This Fab has been co-crystallized with two linear peptides and two cyclic peptides designed to mimic β-hairpin turns. The conformations of the peptides in complex with Fabs 59.1 and 58.2 will be presented and the peptide-Fab interactions will be described. Study of the conformations of various PND peptides in complex with these three neutralizing Fabs should be very useful for future HIV-1 vaccine and drug design.

L 132 RESIDUE HELICAL PREFERENCE VALUES OBTAINED FROM MODEL MONOMERIC PEPTIDES OF DEFINED SEQUENCE, Earle Stellwagen and Soon-Ho Park, Department of Biochemistry, University of Iowa, Iowa City, IA 52242

The circular dichroic spectra of peptides having the sequence acetylYEAAAKEAXAKEAAAKAamide in aqeuous solutions at zero degrees and neutral pH form a nested set which is amenable to two-state helix/coil analysis. The mean residue ellipticity of each peptide was measured at 222 nm and zero degrees as a function of pH and [KCl]. These measurements are expressed as the ΔG for helix formation. At any pH, addition of KCl above 1.5 M decreases helicity, giving a ΔG of 110 ± 20 cal/mole per M salt. This likely represents the effect of KCl acting as a lyotrophic reagent on the helix/coil equilibrium. Extrapolation of this limiting relationship to the absence of KCl at neutral pH should indicate the relative ΔG for each peptide residue devoid of electrostatic effects. These ΔG values in general compare very favorably with the $\Delta\Delta G$ values obtained from the sequence dependence of the formation of dimeric helical bundles (O'Neil and DeGrado, Science 250, 646-651). Extrapolation of the limiting relationship observed for the charged and uncharged forms of an ionic residue indicates that the uncharged form preferentially stabilizes the helix. Comparisons of the observed and extrapolated ΔG in the absence of salt, indicate that the electrostatic interactions stabilize the helix at neutral pH by only 190 ± 40 cal/mole. The modest stabilization is reflected in the apparent pK values for the glutamate and lysine residues in the absence of salt, 4.3 ± 0.2 and 11.7 ± 0.1, respectively. Comparsion at acidic pH indicate that the electrostatic interactions at acidic pH destabilize the helix by 190 ± 60 cal/mole. This most likely reflects the unpaired partial charge in the N-terminal frayed end. In addition, location of a His, Lys or Arg at position X further destabilizes the helix at acidic pH by 150 ± 10 cal/mole likely due to i,i+3 antagonistic interactions.

L 133 CROSS-LINKING OF A SYNTHETIC PEPTIDE FROM THE α_2-ADRENERGIC RECEPTOR TO G-PROTEINS.

J.M. Taylor, G. Jackobs, R.G. Lawton and R.R. Neubig. Depts. of Pharmacology and Chemistry, University of Michigan, Ann Arbor, MI 48109.

The interaction between purified G protein (G_o/G_i) and the α_2-adrenergic receptor (α_2-AR) was examined using a 14 aa peptide (Q) synthesized from the distal third cytoplasmic loop of the receptor. Peptide Q (aa 362-373 with a c-terminal cys) inhibits agonist binding to the platelet α_2-AR and directly activates GTPase activity of G_o/G_i (Dalman and Neubig 1991 *J. Biol. Chem.* 266(17):11025-9 and Dalman *et. al.* 1991 *FASEB J.* 5:A1594). These data suggest that peptide Q acts by directly stimulating the G-protein. In this study, a photoreactive peptide Q derivative was designed and used to label purified bovine brain G_o/G_i. Peptide Q was conjugated to the heterobifunctional diazopyruvoyl cross-linking agent (Goodfellow *et. al.* 1989 *Biochem.* 28:6346-60) N-bromoacetyl-N'-(3-diazopyruvoyl)-*m*-phenylene diamine (Br-DAP). Spectral data, amino acid composition and mass spectrometry indicated that DAP was incorporated into peptide Q at cys 14. Following photolysis of Q-DAP with G-protein both the α and β subunits of G_o/G_i showed band shifts of G protein subunits on SDS-PAGE. This indicates that peptide Q-DAP bound covalently to both the α and β subunits of purified G_o/G_i. Western blot analysis using monoclonal antibodies directed against the β subunit (MS/1) and α_o confirmed cross-linking to both the α and β subunits of G_o. Cross-linking to both subunits was blocked by unlabeled peptide Q. These observations suggest that specific interactions of this α_2-AR peptide (and probably the α_2-AR itself) with G protein involve both the α and β subunits. Supported by HL/GM 46417.

L 134 A C-TERMINAL PEPTIDE FRAGMENT OF THE ROTAVIRUS INTRACELLULAR RECEPTOR BINDS TO VIRUS PARTICLES: PROSPECTS FOR THE DEVELOPMENT OF AN ANTI-VIRAL DRUG. John A. Taylor, Judy A. O'Brien, Janice C. Meyer and A. Richard Bellamy. Department of Cellular and Molecular Biology, University of Auckland, Private Bag 92019, Auckland, New Zealand.

Many viruses that mature at membrane surfaces, assemble by a process involving the interaction of a nucleoprotein particle with the cytoplasmic "tail" of a virally encoded integral membrane protein which acts as a receptor. For rotaviruses, the principle agents of infant gastroenteritis, this interaction takes place at the cytoplasmic face of the ER membrane. The viral single-shelled particle (SSP) binds to the transmembrane glycoprotein NS28, an interaction which precedes budding into the ER lumen where mature virions are assembled.
We have localised the region of the receptor which forms the SSP-binding domain to a short region at the extreme carboxy-terminus of NS28 by fusing portions of the receptor to a carrier protein. The resulting fusion protein can be purified from recombinant *E.coli* in large quantities. Subsequent analysis of the purified receptor domain has shown that soluble oligomeric forms can bind to SSP's and cause their aggregation. A C-terminal fragment of as little as 21 amino acids can bind virus particles when fused to a solid support *via* the carrier protein. Mutational analysis has shown that retention of a methionine residue at the extreme C-terminus of the recepror is essential for virus binding..
The interaction between these virus-encoded proteins represents a key step in the assembly of infectious virus particles: it therefore presents a potential target for the action of an anti-viral drug. Structural analysis of the receptor binding domain should therefore provide information on which the rational design of an anti-rotavirus drug could be based.

L 135 METAL ION-BINDING PEPTIDES: EFFECT OF CROSS-LINKING ON LANTHANIDE AFFINITY, William F. Trompeter, Curt M. Breneman, Jeffrey A. Bell, Department of Chemistry, Rensselaer Polytechnic Institute, Troy, NY 12180, and Margaret A. Lindorfer, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

Can a small peptide be designed to function as effectively as a larger protein? Within the context of the enormous design challenge presented by this question, we have focused our attention on a series of ion-binding peptide analogues of the "EF-hand" loop found in calcium binding proteins. The series is comprised of a control and several closely-related peptides with the capacity to form intramolecular cross-links. Using this series we have set out to address the following two questions. 1. Can the affinity of a peptide for metal ions be so enhanced through the introduction of specifically positioned cross-links that the association strength approaches the level exhibited by large proteins? 2. Will a metal ion act as an effective template for a cross-linkable peptide and thereby promote, either kinetically or thermodynamically, the formation of properly constrained monomers over other products? Answers to the above questions are being pursued using a variety of techniques including sensitized terbium luminescence and NMR titrations with lanthanum, to determine binding affinities, and HPLC to follow the results of the templating experiments. From our preliminary binding studies we have been able to demonstrate a 100-fold enhancement in binding through the introduction of a simple disulfide bond. Information derived from this initial work should lead to better designed peptides with improved ion binding performance.

L 136 A SYNTHETIC PEPTIDE INHIBITOR OF HUMAN IMMUNODEFICIENCY VIRUS REPLICATION: CORRELATION BETWEEN SOLUTION STRUCTURE AND VIRAL INHIBITION, Carl Wild, Terrence Oas*, Charlene McDanal, Dani Bolognesi, Thomas Matthews, Departments of Surgery and of *Biochemistry, Duke University Medical Center, Durham, NC 27710

A peptide designated DP-107 was synthesized containing amino acid residues 558-595 of the envelope glycoprotein gp160 of human immunodeficiency virus type 1 strain LAI (HIV-1_{LAI}). Algorithms for secondary structure have predicted that this region of the envelope transmembrane protein should form an extended α-helix. Consistent with this prediction, analysis by circular dichroism (CD) indicated that, under physiological conditions, DP-107 is ≈85% helical. The high degree of stable secondary structure in a synthetic peptide of this size suggests self-association typical of a coiled coil or leucine zipper. In biological assays, the peptide efficiently blocked virus-mediated cell-cell fusion processes as well as infection of peripheral blood mononuclear cells by both prototypic and primary isolates of HIV-1. A single amino acid substitution in the peptide greatly destabilized its solution structure as measured by CD and abrogated its antiviral activity. An analogue containing a terminal cysteine was oxidized to form a dimer, and this modification lowered the dose required for antiviral effect from 5 to about 1μg/ml. These results suggest that both oligomerization and ordered structure are necessary for biological activity. They provide insights also into the role of this region in HIV infection and the potential for development of a new class of antiviral agents.

L 137 SIDE CHAIN HELIX PROMOTING EFFECTS AT THE C-CAP POSITION IN ISOLATED PEPTIDE HELICES,

Hongxing X. Zhou[1], Pingchiang Lyu[2], David E. Wemmer[2], and Neville R. Kallenbach[1]. Department of Chemistry,[1]New York University, New York, NY 10003 and [2] University of California, Berkeley, CA 94720

The stability of alpha helical structure is determined by the contribution of side chains in the middle of a sequence, and by position dependent interactions (Presta and Rose, *Science*1988, **240**,1632) of side chains near or at the N and C ends. The N terminal residue that is partially helical is denoted as Ncap, that at the C terminus as Ccap. Statistically, Asn, Ser, Asp, Thr occur in helices of known proteins with high frequencies at the Ncap position, while Gly, Asn are excessively represented at the C cap (Richardson and Richardson, *Science*1988, **240**, 1648). An experimental test of the role of capping side-chains at the Ncap reveals that the side chains that occur with high frequency at Ncap stabilize isolated helices by specific H-bonding interactions between polar side chains and the main chain (Lyu et al, *JACS* 1992, **114**, 6560-6562; Lyu et al, *Biochem.*, submitted). We present here evidence for a strong helical promoting effect by Asn residues at the Ccap position in the same system of model peptides, Lyutides. The underlying stabilizing interactions are being investigated using high resolution NMR. We tentatively propose that this effect involves hydrogen bonding between the Asn side chain with the helix backbone, as in the Ncap case.

Computational Methods in Peptide Drug Design

L 200 IMPORTANCE OF AGONIST-CALCIUM INTERACTION IN CONFORMATION-ACTIVITY RELATIONS OF PEPTIDE HORMONES, Vettai S. Ananthanarayanan, Department of Biochemistry, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5

Many agonists, including peptide hormones, cause alterations in intracellular Ca^{2+} levels as part of their signalling mechanism. The role, if any, of extracellular Ca^{2+} in governing agonists' action is, however, less understood. We had proposed (V.S.A. (1991) Biochem. Cell Biol. **69**, 93-95) that agonists may bind extracellular Ca^{2+} in the lipid milieu and that the Ca^{2+}-bound state of the agonist may be its biologically active conformation. To verify this proposal, we have studied the interaction of several peptide hormones with Ca^{2+} in a nonpolar environment. Ca^{2+} binding caused significant CD and fluorescence spectral changes in bombesin, glucagon, LHRH and substance P in trifluoroethanol. Glucagon, LHRH and substance P showed two, while bombesin showed one, binding sites for Ca^{2+}. All of them also bound Mg^{2+} but with lesser affinities than those for Ca^{2+} which were in the 10^4 M^{-1} range. Two-dimensional ^{1}H-NOESY data on substance P and bombesin suggested that Ca^{2+} binding rigidifies the otherwise flexible conformations of these hormones. Glucagon and its 19-29 fragment, insulin and its B-chain, substance P and its 7-11 fragment, and bombesin (in 1-100 μM range) translocated Ca^{2+} across the lipid bilayer of unilamellar vesicles of dimyristoyl lecithin that contained a Ca^{2+}-sensitive dye (fura-2 or arsenazo III) entrapped within. In bombesin, glucagon and substance P, the C-terminal regions which display significant bioactivities were also the main Ca^{2+} binding and transporting moieties. Studies on LHRH and substance P analogues showed that conversion of C-terminal amide into acid causes major alterations in hormone-Ca^{2+} interaction. Our studies provide a basis for biochemical data that indicate a role for extracellular Ca^{2+} in the actions of the above hormones. They point to the possibility of the Ca^{2+}-bound forms of these hormones interacting with the respective receptors in the lipid milieu. These observations should help us understand the conformational aspects of signal transduction and the design of the potent hormone analogues. Supported by the Medical Research Council of Canada.

L 201 DESIGN OF LOCAL VERSUS GLOBAL CONFORMATIONAL CONSTRAINTS IN THE SYNTHESIS OF HIGHLY POTENT AND SELECTIVE DYNORPHIN A ANALOGUES. Nathan Collins*, Jean - Philippe Meyer*, Victor J. Hruby*, Terasa Zalewska#, Henry I. Yamamura#, Peg Davis#, Frank Porreca#. Dept of Chemistry* and Dept of Pharmacology#, University of Arizona, Tucson AZ 85721.

We have previously shown that disulfide - containing peptide analogues of dynorphin A_{1-11} (Dyn A_{1-11}) cyclized between positions 5 and 11 exhibit unexpected selectivity for κ opioid receptors in the central vs peripheral nervous systems. [$Cys^{5,11}$]Dyn A_{1-11} (**1**) and [$Cys^{5,11}$,D-Ala^8]Dyn A_{1-11} (2) are respectively 3,800 and 11,000 fold more potent in the brain compared to the ileum. We have modeled low energy ring conformations of [$Cys^{5,11}$,X^8]Dyn A_{5-11} where X=Ala, D-Ala, N-Me Ala (trans), N-Me Ala (cis), which were examined for the ability to stabilize reverse turns about the 8 position. For X=Ala (cf **1**) a marked β - turn population about Arg^7-Ala^8 relative to the rest of the ring was seen. This effect was increased for X=D-Ala (cf **2**) and was maximized when X=N-Me Ala where both isomers of the tertiary amide showed >80% population of a reverse turn about this region. Assuming that this is an important conformational feature determining selectivity in the globally constrained cyclic peptides **1** and **2**, [N-Me Nle^8]Dyn A_{1-11} (**3**) was synthesized as a "locally" constrained linear analogue which strongly favors a reverse turn about Arg^7-N-Me Nle^8. As a control [$Cys^{5,11}$,N-Me Nle^8]Dyn A_{1-11} (**4**) was also prepared. Both 3 and 4 were highly potent in the guinea pig (GP) brain (kappa IC_{50} vs [^{3}H]U69,593: **3** - 0.07nM; **4** - 1.01nM)but while **4** displayed weak agonist activity in the GP ileum, **3** was highly potent (IC_{50}=1.3nM). Our studies using local vs global conformational constraints thus indicate that a reverse turn about position 7 - 8 is generally important for κ selectivity and potency, but is not a key determining factor in selectivity between κ receptors in the GP brain and ileum.

L 202 **MOLECULAR DYNAMICS SIMULATIONS OF PROTEIN UNFOLDING,** Valerie Daggett and Michael Levitt, Department of Cell Biology, Stanford University School of Medicine, Stanford, CA 94305

It is generally accepted that a protein's primary sequence determines its three-dimensional structure. It has proved difficult, however, to obtain detailed structural information about the actual protein folding process and intermediate states. We present the results of molecular dynamics simulations of the unfolding of reduced bovine pancreatic trypsin inhibitor. The resulting partially "denatured" state was compact but expanded relative to the native state (11-25%); the expansion was not caused by an influx of water molecules. The structures were mobile, with overall secondary structure contents comparable to those of the native protein. The protein experienced relatively local unfolding, with the largest changes in the structure occurring in the loop regions. A hydrophobic core was maintained although packing of the side chains was compromised. The properties displayed in the simulation are consistent with unfolding to a molten globule state. There were two distinct stages of unfolding to the molten globule state: (1) the protein expanded quickly and the internal packing was disrupted while the secondary structure remained intact; and (2) there was further loss of tertiary interactions, some loss of secondary structure and an increase in the population of β-turns. Our simulations provide an in depth view of the molten globule state, the protein unfolding process, and the details of water-protein interactions that cannot yet be obtained experimentally.

L 203 DEVELOPMENT OF RECEPTOR SUBTYPE-SELECTIVE ANALOGS OF CARDIAC NATRIURETIC PEPTIDES. [1]De Léan A., [1]Mimeault M. and [2]Fournier A. [1]Department of Pharmacology, Université de Montréal, Montréal, Qué. H3C 3J7; [2]INRS-Santé, Pointe-Claire, Qué. H9R 1G6, Canada.

Atrial natriuretic factor (ANF), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP) induce vasodilation, inhibition of the renin-angiotensin system as well as of kidney function by interacting with two broad classes of specific receptors. The class ANF-R_1 is directly coupled to cyclic GMP production and is subdivided into at least two subtypes with differential specificity: ANF-R_{1A} as high affinity for ANF and BNP but low affinity for CNP while ANF-R_{1C} has high affinity only for CNP. In contrast the class ANF-R_2 is apparently coupled to inhibition of adenylate cyclase activity and displays similar affinity for all three natriuretic peptides. We have recently provided evidence for another receptor subtype ANF-R_{1B} which displays high affinity only for ANF. In order to better understand differences between ANP-R_{1A} and ANF-R_{1B} subtypes, we have devised chimeric peptides of rat ANF(99-126) which binds with equal affinity to both subtypes and of porcine BNP(72-103) which discriminates both subtypes and studied their receptor binding and guanylate cyclase activating properties in rat kidney papillary membranes which contain both receptor subtypes. Analogs containing the ring portion of porcine BNP but the N- and C-terminal segments of ANF possess higher affinity for ANF-R_{1A} and display a selectivity ratio over 600-fold. In contrast, analogs with the ring portion of ANF but the N- and C-terminal end of BNP had lower affinity for ANF-R_{1A} and were less selective. These results provide insight about the distinctive structural elements of cardiac natriuretic peptides involved in discriminating ANF-R_{1A} and ANF-R_{1B} subtypes. These analogs can be used to further study the pharmacological properties of these receptor subtypes and their function in kidney physiology. (Supported by MRC program grant on molecular pharmacology and genetics of cardiac natriuretic peptides.)

L 204 DIST, A COMPUTATIONAL TOOL FOR GEOMETRIC MATCHING AND ITS APPLICATION TOWARD EPITOPE GRAFTING, David P. Hearst and Fred E. Cohen, Department of Pharmaceutical Chemistry, University of California San Francisco, San Francisco, CA, 94143

DIST, a geometry matching algorithm based on distance matrices is an effective tool for screening protein scaffolds in search of sites for grafting of catalytic or binding motifs. We have applied DIST toward the identification of scaffold proteins onto which the antibody-binding epitopes of lysozyme can be grafted. Such a graft would be performed by site directed mutagenesis of key residues in the scaffold so that the identity and geometry of the new residues correspond to those in the desired epitope. Once prepared, the new, grafted epitope can be evaluated using ELISA to determine whether the new protein binds effectively to antibodies generated by the original antigen. The resulting protein ideally will retain the antigenic properties of the original epitope, while gaining the physical properties (e.g. thermal stability, ease of expression and purification, and potential for drug delivery) of the scaffold protein.

L 205 A LOW RESOLUTION MODEL FOR PROTEIN STRUCTURE PREDICTION, David A. Hinds and Michael Levitt, Department of Cell Biology, Stanford University, Stanford, CA 94305

We have developed an exceptionally simple model of protein structure for which we can exhaustively analyze all possible folds for small proteins. This model avoids the conformational complexity problem by only capturing the large-scale features of a protein fold, and avoids the problem of local energy minima because it is feasible to estimate the conformational energies of all possible structures. We find that we can reliably separate native-like structures from the vast majority of non-native folds using only simple structural and energetic constraints. Our method has significant generality and predictive power, and does not require foreknowledge of any native structural details.

We represent a polypeptide chain as a self-avoiding path of connected vertices on a bounded tetrahedral lattice. A model structure contains half as many vertices as there are residues in the sequence. We do not enforce a fixed mapping between lattice points and residues. Instead, we optimize the alignment of a particular sequence to each lattice structure to form the most favorable combination of tertiary interactions. The effective interaction energies for all pairs of amino acids are estimated from observed contact frequencies in well-refined X-ray structures.

We tested the feasibility of using this model for structure prediction by seeing how far we could go towards predicting the native structures of various small proteins using a combination of structural and energetic criteria. We chose the selection criteria to yield the greatest net enrichment of native folds. While our model is not sufficiently realistic to make a unique prediction feasible, we found that we could reduce populations of 10^7 bounded paths to on the order of 1000 possibilities, with corresponding increases in the proportion of native-like structures.

This method may be useful for the determination of energetically favorable conformations of peptides binding to proteins of known structure. The bounding volume for the conformational search can be designed to conform to the surface of a target molecule. The lattice can also be pre-loaded with a known structure, allowing estimation of interaction energies between this template and all possible peptide conformers.

L 206 A COMPUTATIONAL METHOD FOR *DE NOVO* DESIGN OF ORGANIC PEPTIDOMIMETIC LIGANDS,

W. Jeffrey Howe and Joseph B. Moon, Computational Chemistry, Upjohn Laboratories, 301 Henrietta St., Kalamazoo, MI 49001.

Automated *de novo* molecular design refers to the computer-based design of potential ligands to proteins of therapeutic interest. The ligand model is constructed in a target protein's receptor site by algorithmic connection of small molecular fragments. We have previously described a method (called GROW) for automated *de novo* construction of peptides and peptide-like ligands, and have provided several examples of its design of active compounds [1]. In this poster we describe recent work in extending the method to the design of organic (non-peptidic) ligands. Two variations of the method are discussed: (a) the use of a customized, target site-dependent fragment library in cases where the binding site is in complex with a known ligand, and (b) the use of a more generic fragment library where fragment analogy to a known ligand is either not possible (no protein/ligand complex structure is available) or not desired. Examples are provided which illustrate GROW's simulated annealing-based growth, evaluation, and optimization procedure.

1. a) Moon, J. B. and Howe, W. J., Proteins: Struct. Funct. Genet. 11(1991)314; b) Moon, J. B. and Howe, W. J., Tetrahedron Comput. Meth. (1992, in press); c) Blinn, J. R., Chou, K.-C., Howe. W. J., Maggiora, G. M., Mao, B., and Moon, J. B., In Bertran, J. (Ed.) Molecular Aspects of Biotechnology: Computational Models and Theories, Kluwer Acad. Publ., Netherlands, 1992, pp. 17-38.

L 207 PEPTIDE BINDING SITES ON PROTEINS, R. L. Jernigan, D. G. Covell and L. Young, National Institutes of Health, National Cancer Institute, Laboratory of Mathematical Biology, 9000 Rockville Pike, Building 10, Room 4B56, Bethesda, MD 20892

New methods of calculation to locate the most probable binding sites for peptides on the surfaces of proteins of known structure have been developed and tested. The methods are simpler than usual molecular calculations in order to permit a rapid screening of molecular surfaces for sites that would interact strongly with any hypothetical peptide. The approach has been calibrated and tested on 23 available peptide-protein X-ray structures. The basis for the evaluation of conformers is the independently derived potential functions for residue-residue interactions, reported by Miyazawa and Jernigan, which resemble a pair-wise hydrophobicity scale. Clusters of favorable binding points exterior to the surface of the target protein are derived. With one exception, the observed sites always fall among the best (top 0.6%) of the predicted sites. The favored clusters of points delineate small sets of well defined loci for inhibitor design.

L 208 CONFORMATIONAL STUDIES ON NOVEL CAAX INHIBITORS OF P21*RAS* PROTEIN FARNESYL TRANSFERASE Toni Kline, Katerina Leftheris, Michael Porubcan, Marcia DeVirgilio, and Alicia D. Kahle , Division of Chemistry, Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 4000 Princeton, New Jersey 08543.

The *ras* proto oncogenes encode a family of GTP-binding proteins involved in cell growth. GTP hydrolysis to the GDP-ligated state functions as the terminating switch in the normal cell cycle; in transformed (oncogenic) *ras* proteins this regulation is lost. *Ras* function requires the protein to be membrane-anchored, a process facilitated by a sequence of post-translational modifications that render the protein more lipophilic. In the first step of this sequence, cysteine^{186} is farnesylated. The enzyme responsible, farnesyl transferease, recognizes and alkylates the N-terminal residue of most CA_1A_2X peptides (Brown and Goldstein, 1990). The outstanding exception to this is the inhibitory activity when A_2 is phenylalanine. Unlike tetrapeptide substrate inhibitors bearing an aliphatic A_2 residue (CVIM, CVVM), CVFM is not farnesylated and therefore is a true inhibitor (Brown and Goldstein, 1991,1992). Substrates and inhibitors may bind to the same enzyme in different modes: In order to investigate the relevance of this phenomenon to farnesyl transferase, conformational studies of several tetrapeptides were undertaken. CAAX analogues were synthesized to induce specific conformational preferences and/or intramolecular associations: We evaluated the *in vitro* effects of α-alkylation at A_1 as well as N-alkylation at each of the four possible nitrogens. NMR studies on modified CAAX sequences were conducted to determine if secondary structure could be observed in solution and predictive of inhibitory-vs-substrate activity.

L 209 **MOLECULAR SHAPE COMPARISON OF ANGIOTENSIN II RECEPTOR ANTAGONISTS: QUANTITATIVE VOLUME MATCHING OF FLEXIBLE MOLECULES. Brian B. Masek*, Arshad Merchant, and James B. Matthew , Department of Medicinal Chemistry, ICI Pharmaceuticals Group , A business unit of ICI Americas Inc. Wilmington, Delaware 19897-2500 USA.**

Molecular shape comparison (MSC) seeks to find the spatial properties common to two or more molecules. A new and powerful analytical method for comparing molecular shapes by optimizing the overlap of molecular volumes has been developed. This shape comparison method provides both a quantitative measure of the shape similarity and a means to align molecules such that shape similarity is maximized. Our MSC method has been enhanced with an option to allow discrimination between groups with different chemical properties. Atoms or groups of atoms may be assigned to different classes based on specific properties such as electrostatic potential, hydrogen bonding ability, or hydrophobicity. This enables matches based on criteria such as alignment of hydrophobic groups or hydrogen bond acceptor groups, etc. In this study, we report shape comparisions of angiotension II (AII) receptor antagonists from two structural classes, 4-(biphenyl)methoxyquinoline derivatives such as ICI D8731 and N-(biphenylmethyl)imidazole derivatives, such as DuP 753. Starting with a list of low-energy, conformations for the two molecules, each conformation of the first molecule is paired with each of the conformations of the second molecule. For each of these conformational pairs, an MSC comparison, which generates multiple MSC maxima, is initiated. Seven high scoring conformational pairings were found with shape matching based on the intersection of the total molecular volume, while nine high-scoring pairs were identified with matching by atom type. MSC identifies conformational pairs with high shape similarity, as measured by the intersection volume, and thus generates and prioritizes several alternative models for the AII antagonist pharmacophore.

L 210 MOLECULAR SURFACE RECOGNITION BY A COMPUTER VISION BASED TECHNIQUE, Ruth Nussinov, Raquel Norel, Daniel Fischer and Haim J. Wolfson, School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel and Laboratory of Mathematical Biology, PRI/Dynacorp, NCI/FCRDC, Frederick, MD 21702-1201

Correct docking of a ligand onto a receptor surface is a complex problem, involving geometry and chemistry. Geometrically acceptable solutions require a good match between corresponding patches of surfaces of the receptor and of the ligand, and no overlap between the van der Waals spheres of the remainder of the receptor and ligand atoms. In the quest for favorable chemical interactions, the next step involves minimization of the energy between the docked molecules. Our work addresses the geometrical aspect of the problem. It is assumed that we have the atomic coordinates of each of the molecules. In principle, since optimally matching surfaces are sought, the entire conformational space needs to be considered. As the number of atoms residing on molecular surfaces can be several hundreds, sampling of all rotations and translations of every patch of surface of one molecule with respect to the other can reach immense proportions. The problem we are faced with here is reminiscent of object recognition problems in computer vision. Using an indexing approach based on a transformation invariant representation, the algorithm scans efficiently groups of surface dots (or atoms) and detects optimally matched surfaces. Potential solutions displaying receptor - ligand atomic overlaps are discarded. Our technique has been applied successfully to seven cases involving docking of small molecules, where the structures of the receptor - ligand complexes are available in the crystallographic database, and to three cases where the receptors and ligands have been crystallized separately. In two of these three latter tests, the correct transformations have been obtained. Our approach is very efficient, with worse case complexity n^3, where n is the number of surface atoms considered. In practice it is better than n^2.

L 211 THE MOLECULAR STRUCTURE OF DENDROTOXIN HOMOLOGUES DETERMINED BY MOLECULAR MODELLING METHODS AND THEIR COMPARISON WITH THE CRYSTAL AND THE 2D-NMR STRUCTURES, Albert Opalko[1],Timothy Clarridge[3], Roger Crossley[1] Andrew E. Derome[3] and Panachandam Swaminathan[2], [1]Wyeth Research UK Ltd,.Huntercombe Lane South, Taplow, Maidenhead, SL6 0PH, U.K. [2]Wyeth-Ayerst Research, CN-8000, Princeton, NJ 08543 USA and [3]Dyson Perrins Laboratory, University of Oxford, South Parks Rd., Oxford, OX1 3QY, U.K.

The dendrotoxins are a family of homologous snake toxins which are present in the venom of the eastern green mamba (dendroaspis angusticeps) and the black mamba (dendroaspis polylepis polylepis) snakes. These toxins are small proteins and vary in size from 57 to 60 amino acid residues and some are very potent blockers of voltage-gated potassium channels. All the pharmacologically active and inactive toxins have a high degree of homology. Each of these toxins have six cysteine residues and a comparison of their sequences indicate that this feature, which is capable of forming three disulphide bridges, is common to all the proteins. These proteins also have a high degree of homology with bovine pancreatic trypsin inhibitor (BPTI), a serine protease inhibitor. As there is NMR evidence to indicate that there are structural similarities with BPTI and some of the dendrotoxin homologues, the crystal structure of BPTI was used as a starting point for preparing the 3D structures of the dendrotoxins. The minimisations of these modelled structures along with the molecular dynamics calculations are discussed. The crystal structure of α-dendrotoxin and the 3-D structure of Toxin I (a dendrotoxin homologue) determined by NMR is the subject of a comparative analysis of structural features .

L 212 SYSTEMATIC SEARCH + MONTE CARLO MINIMIZATION (SSMCM) – AN EFFICIENT CONFORMATIONAL SEARCH METHOD FOR CONSTRAINED SYSTEMS, Enrico O. Purisima and Hervé Hogues, Biotechnology Research Institute, National Research Council Canada, Montreal, Quebec, H4P 2R2, CANADA.

Monte Carlo Minimization is a powerful method for overcoming the multiple-minima problem in conformational search. Its ability to escape from local minima is due in part from its capacity to make global conformational changes through rotations about selected bonds. This is in contrast to molecular dynamics methods where small step sizes must be taken in order to carry out the integration of the equations of motion. A major difficulty that we have encountered in carrying out Monte Carlo minimization on constrained systems such as rings, loops in proteins or small ligands docked in a protein cleft is that dihedral angle changes must be carried out in a concerted fashion in order to satisfy closure requirements or to avoid steric clashes with surrounding groups. Otherwise, the relief of the steric strain becomes the overriding factor in the energy-minimization and results in the loss of the partially optimized structural features present in the previously accepted conformation. The trajectory of the conformational search then reduces to that of random search. In order to address this problem, we have combined the global sampling power of Monte Carlo minimization with the exhaustive nature of systematic search. We initially carry out a systematic search over a chosen set of torsional angles to obtain a set of sterically feasible conformations. This, in general, yields a large number of conformations – too many for individual energy minimization. At this point, we then carry out a Monte Carlo minimization search in which the starting conformation for each minimization is obtained from the set of sterically feasible conformations. In this way, closure and steric constraints are automatically satisfied. Examples will be given from small cyclic peptides, protein loops and ligand docking problems.

L 213 MAPPING THE SPECIFICITY OF PROTEASES USING MULTIPLE SUBSTRATE KINETICS, Volker Schellenberger, Chris W. Turck, Ronald A Siegel and William J. Rutter, Hormone Research Institute, Box 0534 and Departments of Medicine and Pharmacy, University of California, San Francisco, CA 94143

The use of substrate mixtures to characterize the specificity of enzymes has so far been limited to compounds with similar kinetic parameters, because experimental data were analyzed by applying the kinetics of two competing substrates. In this study we introduce a statistical method for the analysis of reactions with many competing substrates which makes use of the specific features of multiple substrate kinetics. Computer simulations demonstrate that the precision in the kinetic parameters obtained from multiple substrate reactions increases with the number of substrates in the mixture. The method was applied to serine protease-catalyzed acyl transfer reactions. Mixtures of peptide nucleophiles were incubated with various enzymes and an excess of a specific ester substrate. The decrease in nucleophile concentrations was monitored by HPLC analysis of the dansylated mixture. The efficiency of 21 nucleophiles of the general structure H-Xaa-Ala-Ala-Ala-Ala-NH_2, where Xaa represents D-alanine, citrulline, and all natural amino acids except cysteine, was determined. Relative rate constants were obtained with high precision in the range of 10^3. The order of preference in chymotrypsin-catalyzed acyl transfer reactions is: positively charged > aliphatic > aromatic >> negatively charged, D-Ala, Pro P'_1 side chain. Trypsin prefers hydrophobic residues, but like chymotrypsin aliphatic residues are better than aromatic residues in P'_1 position. P'_1 side chains can interact with two loops of the enzymes, residues 35-39 and 59-64. These loops differ both in the number and character of amino acid residues for chymotrypsin and trypsin. These structural differences explain the observed differences in the S'_1 specificities of both enzymes.

L 214 THE CUTICULAR PEROXIDASE OF FILARIAL PARASITES: A POTENTIAL TARGET FOR DRUG DESIGN, Murray E. Selkirk[1], Marketa J. J. M. Zvelebil[2], Liang Tang[1], Edith Cookson[1] and Janet M. Thornton[2]
[1]Department of Biochemistry, Imperial College of Science, Technology & Medicine, London SW7 2AY, UK, and [2]Department of Biochemistry and Molecular Biology, University College London, London WC1E 6BT

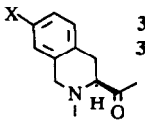
Human lymphatic filariasis is a chronic debilitating disease which affects some 90 million people worldwide. It is caused by a nematode parasite which resides in the lymphatic system of infected individuals. Chemotherapy is problematic, and new drugs are being sought to combat infection. The parasites are enclosed by a thick cuticular matrix composed primarily of collagenous proteins. We have recently determined that the major soluble protein in this matrix is a homologue of glutathione peroxidase, normally a cytosolic detoxification enzyme. This may function to neutralise products of the oxidative burst of host granulocytes, and the accessibility of the protein suggests that it may be a valid target of directed drug design. We have therefore modelled the structure of the protein and the substrate (glutathione) binding site based on the previously-determined three-dimensional structure of bovine glutathione peroxidase. Active enzyme has been produced via expression of the parasite gene in insect cells, and a panel of substrate analogues are being screened for inhibitory properties in order to provide a start-point for the refined design of inhibitors.

L 215 CONFORMATIONAL ANALYSIS OF CYCLO(2,9)-Ac-QCRSVEGSCG-OH FROM THE C-TERMINAL LOOP OF HUMAN GROWTH HORMONE, Teruna J. Siahaan, Matthew W. Conrad, Soma Chakrabarti, Dave Vander Velde, and George S. Wilson, Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, Kansas 66045.

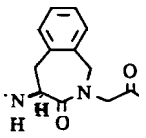
Human growth hormone (hGH) is a protein (192 amino acids) that functions in humans as a regulator and stimulator of growth. There are several antibodies that react with hGH, but the mechanisms of recognition between these antibodies and the hGH domains are not well understood. We are trying to determine if and how these interactions involve the three dimensional structure of the peptide domain. Our work entails the synthesis and conformational analysis of cyclo(2,9)-Ac-Gln-Cys-Arg-Ser-Val-Glu-Gly-Ser-Cys-Gly-OH (**1**) from the C-terminal of hGH . This compound has disulfide linkage between Cys-2 and Cys-9. This region is chosen for its cyclic nature with conformational restriction. In hGH, this domain has binding activity to a monoclonal antibody Mab-12. Conformational analysis of **1** was done using nuclear magnetic resonance (NMR) in various solvents and molecular dynamics/energy minimizations. Homonuclear Hartman-Hahn (HOHAHA) spectroscopy was performed to assign the chemical shift of all the protons, and nuclear Overhauser spectroscopy (NOESY) was used to determine through space interactions between protons in peptide **1**. The interproton distances from NOESY were used as NOE-constraints in the molecular dynamics/energy minimization simulations. From these simulations, we found several low energy conformations which agree with the experimental data. In the future, we will study the bound conformation of **1** to Mab-12.

L 216 CONFORMATIONAL RESTRICTION OF PHE AND TYR SIDECHAINS IN OPIOID PEPTIDES. THE INFLUENCE OF THE SIDE CHAIN TOPOLOGY ON RECEPTOR AFFINITY AND SELECTIVITY, D. Tourwé[a], K. Verschueren[a], P. Davis[b], F. Porreca[b], V.J. Hruby[c], G. Toth[c,d] and G. Van Binst[a],[a]Eenheid Organische Chemie, Vrije Universiteit Brussel, Pleinlaan 2, B 1050 Brussel, Departments of Pharmacology[b] and Chemistry[c], University of Arizona, Tucson, AZ, and [d]Biological Research Center, Szeged, Hungary.

The linear opioid peptides dermorphin : H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2 **1** and deltorphin II : H-Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2 **2** have but opposite receptor selectivities (μ for **1**, δ for **2**). Extensive conformational studies, both experimental and theoretical have emphasized the importance of the conformation of the N-terminal "message" part for potency and selectivity . A critical element is the relative orientation of the Tyr^1 and Phe^3 sidechains. Due to the flexibility of peptide sidechains, the proper topography during receptor interaction can only be studied by appropriate constraining or fixing of the sidechain conformers. Considering the staggered conformations around C^{α}-C^{β} for Phe and Tyr, the gauche(-) (χ_1 = -60°) or gauche(+) (χ_1 = 60°) can be fixed by using a 1,2,3,4-tetrahydro isoquinoline-3-carboxylic acid structure **3a,b** (Tic, Figure 1). The trans (χ_1 = 180°) conformer can be fixed by cyclizing the aromatic ring onto the nitrogen of the succeeding amino acid, thus leading to a 4-amino-tetrahydro-2-benzazepine-3-one structure **3c** (tPhe-Gly). We now report the results of this type of topology control in linear dermorphin and deltorphin II analogues and in the δ-selective cyclic analogue DPDPE **4**. Substitution of Tyr^1 by Htc or Phe^3 by Tic results in large drops in receptor affinities or in GPI and MVD activities. The fixation of the Phe^3 sidechain in dermorphin into the trans rotameric position results in a compound which retains good affinity for the μ-receptor, but as a result of a large increase in affinity for the δ-receptor, becomes δ-selective. The NMR parameters of the residue-2 signals can be correlated with the orientation of the adjacent sidechains. The use of the trans constraint for the design of δ-selective peptides will be discussed.



3a : X = H Tic
3b : X = OH Htc



3c : t-Phe - Gly

L 217 DESIGN OF AN ANALOGUE OF THE GLYCOPHORIN A TRANSMEMBRANE REGION BY MOLECULAR MODELING AND SITE-DIRECTED MUTAGENESIS, H.R Treutlein[1,2], M.A. Lemmon[2], D.M. Engelman[2] and A.T. Brünger[2]. [1]Ludwig Institute for Cancer Research, P.O. Royal Melbourne Hospital, Victoria 3050, Australia. [2]Department of Molecular Biophysics and Biochemistry, Yale University, P.O.Box 6666, New Haven, CT 06511, USA.

Recent studies suggest specific roles for transmembrane helix association in a range of biologically important functions, but understanding of the conformation and energetics of the association process has been elusive. We have investigated the stability, structure and energetics of a large number of conformations of pairs of helical proteins. We employed molecular dynamics simulations to study the association of two helices in vacuum using an automated procedure that can be applied to any amino-acid. Experimental information has been gained through mutational analysis of a chimeric protein to investigate the dimerization of a transmembrane region. The transmembrane domain in question was fused to the C-terminus of staphylococcal nuclease. Both theoretical and experimental techniques have been applied to the transmembrane region of the glycophorin A (GpA), a protein which is known to dimerize inside membranes of human red blood cells and SDS. Dimerization of GpA has been shown to be spontaneous and highly specific [M.A. Lemmon et al., *J.Biol.Chem* 267(11), 7683 (1992)]. The results of the molecular modeling work of the transmembrane region of the GpA dimer agrees very well with the results of the mutational studies [Treutlein, Lemmon, Engelman and Brünger, in *Proceedings of the 26th Hawaii International Conference on System Sciences*, (IEEE Computer Society Press, Los Alamitos, CA, 1993), in press] and can be used to predict those residues that can disrupt dimerization. On the bases of our model we designed an amino-acid sequence consisting of leucine residues and 7 residues that are found to be important for dimerization according to our model. Mutational and the modeling studies show that both the GpA wild-type transmembrane domain and the newly designed protein region indeed behave similar. In particular, the newly designed transmembrane domain still dimerizes.

L 218 THE BIS-PHENYLALANINE MOIETY OF SUBSTANCE P: EVALUATION OF SYNTHETIC ANALOGUES FOR THE MODULATION OF EPITHELIAL CELL GROWTH.

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and
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Substance P is a ubiquitous neuropeptide purported to serve as a transmitter of the primary sensory neuron. Recently published findings from our laboratory have supported the notion that this undecapeptide plays a primary role in various cell growth associated functions such as wound healing and tissue development. More recent studies using actively dividing murine epithelial cells have shown that substance P stimulates cell growth at remarkably low (picomolar) concentrations, thus providing a highly sensitive assay system for structure-activity relationship studies of substance P analogues. The apparent importance of the two adjacent phenylalanine residues at positions 7 and 8 in substance P to its biological activity, and the coincidental existence of geminal phenyl groups in the potent substance P antagonists CP96345 and RP67580, have prompted us to investigate the biorelevant molecular topological characteristics of substance P's Phe7-Phe8 moiety using a combination of computer-assisted molecular modelling (of both substance P and its receptor), synthetic organic chemistry, and the murine epithelial cell growth assay. Results from these studies, particularly those which utilize conformationally restricted amino acid residues incorporated into substance P, will be discussed.

L 219 INVESTIGATION OF *E.Coli* HEAT-STABLE (STa) ENTEROTOXIN PEPTIDE CONFORMATIONS. 1. STAGES TOWARDS THE DESIGN OF AN ANTAGONIST, David J. Ward, Matthew J. Saderholm, Clark Wells[1], Tom L. Hayden[1], William Glunt[1], Nathan S. Zingg and Judith G. Shelling, Department of Biochemistry and [1]Department of Mathematics, University of Kentucky, Lexington, KY 40536

The 18-19 residue heat-stable (STa) enterotoxin peptides STp and STh, produced by porcine and human strains of *E.Coli* respectively, cause diarrheal diseases. They have a highly homologous 13 residue section whose bioactive conformation is stabilized by three disulfide bonds. These features allow us to compare the crystal structure of an STp analog with conformational aspects of an STh analog determined in solution by Nuclear Magnetic Resonance (NMR) spectroscopy.

We have performed distance geometry calculations with two programs, incorporating 0, 2 and 10 NOE distance constraints, followed by restrained energy minimization and molecular dynamics. We have also energy minimized the crystal structure, plus hydrogens, in conditions as per the other simulations (providing structures for RMSD comparisons), and carried out simulated annealing calculations with and without constraints.

We have sought to describe changes in ST protein-like secondary structure on going from crystal to solution conditions, and to relate this, via peptide dynamics, to the small number of NOEs observed for ST peptides. Also, to interpret the role(s) and structural aspects of the disulfide bonds, and to define protocols for peptide simulations incorporating distance geometry calculations.

L 220 CONFORMATIONAL PREFERENCE OF CYCLIC THROMBIN INHIBITORS: MONTE CARLO CONFORMATIONAL SEARCH Shi-Yi Yue, Zbigniew Szewczuk, Bernard F. Gibbs and Yasuo Konishi, Biotechnology Research Institute, National Research Council Canada, Montreal, Quebec, Canada H4P 2R2

Cyclization of the hirudin C-terminal fragment (55-65), Ac-DFEEIPEEYLQ-OH, which blocks the fibrinogen recognition exo site of thrombin, drastically improved its proteolytic stability with an additional improvement in potency of up to 2-fold. Three cyclic analogs with ring sizes of 18, 17 and 16 atoms showed inhibitory potencies of IC_{50} 0.57 ± 0.01, 1.6 ± 0.1 and 9.7 ± 0.7 μM, respectively, using the clotting assay. Energy minimization with the constraints to the bound conformation indicated no energy distortion in the complex of these inhibitors with thrombin. However, a Monte Carlo conformational search of these inhibitors indicated two distinctive conformers. One is similar to the active conformation with a main chain RMS deviation of the cyclic part less than 0.8Å, while the other (inactive) has different configurations particularly with Pro-60. The population of these active and inactive conformers varied depending on the ring size. The inhibitor with an 18 atom ring favoured the active conformation (65%). This was decreased to 35% in the inhibitor with a 17 atom ring. The inhibitor with a 16 atom ring showed a strong preference for the inactive conformation (93%). The good correlation of the conformational preferences of the cyclic inhibitors based on the Monte Carlo conformational search with the potency of the inhibitors opens up a new theoretical approach in analyzing biologically active compounds.

L 221 ROLE OF THE N- AND C TERMINAL REGIONS AND CHARGE IN THE MITOGENIC ACTION OF THE OSTEOGENIC GROWTH PEPTIDE (OGP), Itai Bab, Zvi Greenberg, Andras Muhlrad, Arie Shteyer, Malka Namdar and Michael Chorev, Faculties of Dental Medicine and Medicine, The Hebrew University of Jerusalem, Jerusalem 91010, ISRAEL.

We have recently reported the discovery of a 14-amino acid osteogenic growth peptide (OGP) (Bab et al, EMBO. J. 11:1867, 1992). In vivo OGP increases bone formation and trabecular bone density. It occurs physiologically in a complex with an OGP binding protein (OGPBP). In vitro OGP has a biphasic effect on osteoblastic and fibroblastic cell proliferation; at low concentrations it is highly stimulatory with an inhibition at higher doses. To explore the mechanism of this activity the effect of synthetic OGP analogs (i) extended at the N- or C- terminus with Cys or Cys(NEM) or (ii) where Tyr^{10} is substituted with by 3-I(Tyr) were tested in MC 3T3 E1 osteoblasts and NIH 3T3 fibroblasts. N- terminal modified analogs and Cys^{15} (OGP) shared the proliferative activity of OGP. The N- terminal derivatives extended with Cys or Cys(NEM) failed to react with the OGPBP. Cys^{15} (OGP)NH_2 and [3-I(Tyr^{10})]OGP, that form a complex with the OGPBP, show only a dose dependent suppression of cell proliferation. This suppression is further shared by a number of other positively and negatively charged peptides which are unrelated to OGP. Non-charged peptides had no effect on cell proliferation. These data suggest a role for the OGP free N- terminal and C- terminal region in binding to the OGPBP and putative OGP receptor, respectively. It appears that the OGP proliferative activity represents the net effect of a specific stimulation and nonspecific inhibition related to the peptide's high positive charge.

Peptide Mimetics

L 300 SYNTHETIC PEPTIDE ANALOGUE STUDIES OF SINGLE-STRANDED NUCLEIC ACID BINDING PROTEINS, Zehan Abdul-Manan, Janet Crawford, Yousif Shamoo, Ann Patten, Lynne Regan, Kenneth R. Williams, Dept. of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, CT 06510.

Previously, we have used synthetic peptides to study the nucleic acid binding properties of a conserved 90-residue RNA-binding domain (RBD) of the yeast poly(A)-binding protein (YPAB) (Nadler et al., J. Biol. Chem. 267, p. 3757, 1992). The 44-residue synthetic peptide corresponding to the carboxy half of the RBD retained 50% of the overall free energy of binding to nucleic acids as the entire 577-residue YPAB protein. Surprisingly, shuffling of its sequence had very little effect on binding. To further explore the relationship between the higher order structure and nucleic acid binding affinity of synthetic peptides, similar studies are now being carried out on the NH_2-terminal RBD (residues 1-92) in the A1 heterogeneous nuclear ribonucleoprotein (hnRNP). In this instance, the higher order structure and binding properties of the intact, 92-residue domain are being compared to that of two synthetic peptide analogues corresponding to residues 4-49 and 50-93, respectively. Despite the fact that circular dichroism studies indicate that these two synthetic peptide analogues retain little of the native structure that is present in the 1-92 fragment, the 50-93 analogue appears to bind more tightly than the 1-92 fragment does to RNA. Taken together, these studies suggest that in the absence of higher order structure, the amino acid composition of synthetic peptide analogues may be a more important determinant of binding affinity than the amino acid sequence. In light of this finding, considerable care needs to be exercised in interpreting such studies. As a corollary, we have designed conformationally constrained "designer" synthetic peptides to further explore the relationship between higher order structure and nucleic acid binding affinity in synthetic peptide analogues.

L 301 THE USE OF RANDOM PEPTIDE LIBRARIES DISPLAYED ON M13 BACTERIOPHAGE TO MAP ANTIBODY EPITOPES, Nils B. Adey and Brian K. Kay, Department of Biology, University of North Carolina, Chapel Hill, NC 27599

M13 bacteriophage display is a powerful method to assay binding of a vast array of different peptides. Our lab has constructed two M13 libraries that display 36 or 22 random amino acids at the amino-terminus of the PIII coat protein, then isolated phage that were bound by a number of different antibodies. The peptides displayed by phage that were bound by a goat antibody raised against the Fc fragment of mouse IgG antibody share the consensus motif RTISKP which matches a region within the mouse immunoglobin gamma-3 heavy chain protein. Peptides bound by an antibody raised against human prostate tumors share the consensus motif MYXXLH; we are currently investigating if this motif can be used to identify the tumor antigen. The majority of peptides bound by mouse anti-Sm autoantibodies do not share a motif but contain a significant enrichment in the number of prolines and (H, K, or R)P, VP, SP, PS, NXRVP, DGVP, and possibly PG peptides. Interestingly, the Sm B protein is proline rich and contains the peptides VP, RVP, GVP, RP, PG, and PS. Currently, we are constructing new random peptide libraries which will be used to isolate peptides bound by anti-tumor antibodies and antibodies involved in auto immunity. The results could be useful in designing vaccines to regulate the immune response.

L 302 INTESTINAL EPITHELIAL CELL PEPTIDE TRANSPORT: STRUCTURE TRANSPORT AND IMPROVING ORAL PEPTIDE DELIVERY, Gordon L. Amidon, Shiyin Yee and Mandana Asgharnejad, College of Pharmacy, The University of Michigan, Ann Arbor, Michigan 48109-1065

Improving the oral systemic availability of peptides is a major barrier in the development of peptide type theraputic agents. Efforts to improve the systemic availability must focus on the mechanisms of permeation and metabolism in the gastrointestinal tract and liver. We have studied the permeation and metabolism of peptides up to 8 amino acids to date. For small polar di- and tripeptides and analogues, we have studied the di-/tripeptide transporter (DTPT) utilized by the β-lactam antibiotics and recent results indicate that many of the ACE inhibitors utilize this mucosal cell pathway. Given the importance of this class of drugs, it is important to establish the structure transport relationships that govern efficient transport via this membrane transporter. Recent results on the oral absorption of a variety of ACE inhibitors will be presented and discussed with a particular orientation towards structure transport. The transport and oral availability of the ACE inhibitors will be compared with other peptide type drugs in order to present a general picture of oral delivery of peptide type drugs. Finally, recent prodrug approaches to utilizing the peptide transporter will be presented and it will be shown that the systemic availability of a poorly absorbed amino acid, α-methyldopa, can be improved to nearly 100% by implementing a strategy based on the peptide transporter.

L 303 FUNCTIONAL AND STRUCTURAL STUDIES OF PHOSPHOLAMBAN AS A MODEL ION CHANNEL PROTEIN. Shy Arkin[#], Edward G. Moczydlowski[*], Saburo Aimoto[‡] Steven O. Smith[§] and Donald M. Engelman[§] Departments of Cell Biology[#], Pharmacology[*], and Molecular Biophysics and Biochemistry[§], Yale University School of Medicine, New Haven CT 06510. Institute for Protein Research, Osaka 565, Japan[‡].

Phospholamban is a non-covalent homopentameric membrane protein that is believed to function in the regulation of the Ca^{2+} pump in cardiac SR. We have begun to explore the potential usefulness of phospholamban as a model for molecular mechanisms of ion permeation, since its small size (52 residues) makes it amenable to spectroscopic methods of structure determination.

We report electrophysiological results obtained with a highly purified synthetic peptide corresponding to the transmembrane domain of phospholamban. This peptide readily incorporates into planar lipid bilayers and exhibits reproducible single-channel fluctuations. The channel is cation specific and selective towards Ca^{2+} (127 pS) over K^+ (106 pS) and Na^+ (62 pS). The phospholamban channel under the conditions studied is predominantly in an open state and exhibits several sub-conducting states. The channel forming property of phospholamban may represent the underlying regulatory mechanism acting on the Ca^{2+} pump.

In addition, we have overexpressed phospholamban in a chimeric construct in *E. coli*, and found that the chimera associates in SDS gels as a pentamer similar to the native molecule. Using PCR mutagenesis we have shown that conservative changes in several residues in the transmembrane domain abolish pentameric association (e.g. when Leu 40 is changed to Ile the protein is no longer a pentamer).

Taken together the conductance measurements and mutagenesis data provide an approach for correlating structure and function of this cardiac ion channel.

L 304 PEPTIDE MIMETICS: TURN INDUCTION IN HYDRAZINO PEPTIDES, A. Aubry[a], J. P. Mangeot[a], J. Vidal[b], A. Collet[b], S. Zerkout[c], A. Lecoq[c] and M. Marraud[c], [a] CNRS-URA-809, University of Nancy I, BP 239, 54506 Vandoeuvre, France, [b] CNRS-UMR-117, ENS de Lyon, 69364 Lyon 07, France, [c] CNRS-URA-494, ENSIC-INPL, BP 451, 54001 Nancy, France

Among the peptidomimetic groups which have been investigated so far, the hydrazide group (CO-NH-NH) has received little attention,[1] and very little is known about the conformational influence of this modification on a peptide chain. Recently, new procedures have been proposed for obtaining chiral hydrazino acids in good yields,[2] and for regioselective coupling of the hydrazine nitrogens.[3] We have prepared four hydrazino peptides: MeOCO-*h*Pro-NHiPr, *1*, MeCO-*h*Pro-Gly-NHiPr, *2*, tBuCO-Pro-*h*Gly-NHiPr, *3*, and tBuCO-Pro-*h*Ala-NHiPr , *4* deriving from the hydrazino analog, denoted by *h*, of proline, glycine and alanine. Their structural and conformational features have been studied by the combined use of ^{1}H-NMR, IR spectroscopy, X-ray diffraction and molecular dynamics (SYBYL program). In these derivatives, a local structure typical of hydrazino residues appears in solution with a very high occurrence, and in the crystal structure of *1*, *4* and the Nα-benzyl derivative of *3*. It is characterized by an intramolecular hydrogen bond between the hydrazide carbonyl and the following N-H bond, thus closing an 8-membered cycle. Furthermore, the N-H bond points at the lone pair of the non-acylated sp^3 hydrazide nitrogen accommodating the absolute R configuration in the (L)-α-hydrazino acid residues. This double interaction enhances the stability of this "expanded" γ-like structure with reference to the classical γ-turn in peptides. It is characterized by the average torsional angles C'-N-N-Cα (ν) = 120°, N-N-Cα-C' (φ) = -100°, N-Cα-C'-N (ψ) = 0° with an average N···O hydrogen bond distance of 3.0 Å. If the α-hydrazino residue is assigned to the (i+2)th position in the chain, the average Cα(i+1)···Cα(i+3) distance is 5.5 Å, and the Cβ(i+2) atom occupies in the "expanded" γ-like structure the same orientation as in the βII'-turn. Thus, the α-hydrazino residues appear as a tool for inducing a folded structure in peptide analogues.

1) Niedrich, H. and Köller, G., *J. Prakt. Chem.*, 1974, *18*, 751-758
2) Vidal, J., Drouin, J. and Collet, A., *J. Chem. Soc., Chem. Commun.*, 1991, 435-437
3) Lecoq, A., Marraud, M. and Aubry, A., *Tetrahedron Letters*, 1991, *32*, 2765-2768

L 305 INTERACTION OF HUMAN HISTOCOMPATIBILITY ANTIGEN HLA-A2 WITH SYNTHETIC PEPTIDES, Maria A.Bednarek, Kenneth C. Parker*, Barry R. Cunningham, Hans J. Zweerink, John E.Coligan* and James P.Springer, Merck Research Laboratories, Rahway, NJ 07065 and *Biol.Res.Br., NIAID, NIH, Bethesda, MD 20892

Class I proteins of the major histocompatibility complex bind intracellulary generated peptides in the lumen of the endoplasmic reticulum. Although the three-dimensional structure of HLA-A2, one of the most intensively studied class I proteins, has been determined by X-ray crystallography, structural requirements for peptide binding are still not clear. It has been previously shown that most peptides bound by HLA-A2 are 8 or 9 amino acids long with two "anchor" residues: Leu at p2 and Val or Leu at p9. We studied the importance of every amino acid residue of the influenza A virus matrix peptide 58-66 (GILGFVFTL; M1) for binding to HLA-A2. In a direct reconstitution assay (K.C.Parker et al., J.Biol.Chem. 267: 5451, 1992) we tested single-substituted analogs of M1 peptide with Ala, Lys, Glu and D-amino acid of the original sequence, substituted in turn for each amino acid of M1. Further studies included single and multi-substituted analogs of M1 and the HLA-A2 restricted HTLV-1 tax 11-19 peptide (LLFGYPVYV) with other natural or unusual amino acids. Replacement of "anchor" residues at p2 and p9 with charged residues or their D-isomers was detrimental to binding and confirmed a prime role of the p2 and p9 positions for binding to HLA-A2. The data indicated significant additional contributions to the stability of the complexes from residues at the p3 and p5-7 positions. The M1 analog with Leu at p2 formed an unusually stable HLA-A2 complex with a $t_{1/2}$ for β_2m dissociation of 250 h.

L 306 INTERACTION OF A GPIIb–IIIa DERIVED PEPTIDE WITH CALCIUM AND WITH PLATELET BINDING PEPTIDES, Michael Blumenstein, Department of Chemistry, Hunter College, 695 Park Ave., New York, NY 10021.

Structural studies of an eleven residue peptide derived from gpIIb–IIIa, and shown to bind fibrinogen (D'Souza et al., (1991) Nature, 350, 66–68) have been undertaken using NMR spectroscopy. Calcium binding has been demonstrated, and ^{1}H and ^{13}C NMR experiments have been employed to delineate the nature of the calcium binding site, and to compare it to the sites found in EF hand proteins. One and two dimensional NMR has also been utilized to study the complex of the peptide with peptides derived from fibrinogen γ–chain, as well as with RGD peptides. This approach offers a possible means of determining the "active" conformation of RGD peptides, and could prove useful in inhibitor design.

L 307 AZAPROLINE : A β-TURN INDUCING RESIDUE OPPOSED TO PROLINE, G. Boussard[a], A. Lecoq[a], A.Aubry[b] and M. Marraud[a], [a] CNRS-URA-494, ENSIC-INPL, BP 451, 54001 Nancy, France, [b] CNRS-URA-809, University of Nancy I, BP 239, 54506 Vandoeuvre, France

Many efforts have been spent for the design of bioactive peptide analogues with constrained structure aiming at the selection of the putative "bioactive conformation". In an attempt to modulate the conformational properties of peptide analogues, we have considered the possibilities offered to us by the substitution of a nitrogen atom for the CHα group, which gives rise to an α-aza-amino acid residue (NH-NαR-CO). Azapeptides have been encountered rather rarely and no in depth conformational study has appeared yet. On the other hand, proline, which is unique among the coded amino acids because of its nearly planar pyrrolidine ring, is recognized as being of special significance in its effect on chain conformations and on the process of protein and peptide folding.[1] More particularly, it often allows cis / trans isomerization of the preceding tertiary amide bond and initiates β-folding of the Pro-X sequences. We have considered the aza-analogue of proline (AzaPro) and carried out the conformational study in solution (^{1}H-NMR and IR spectroscopy) and in the solid state (X-ray diffraction) of the AzaPro-containing analogues of two model peptides with the RCO-Pro-Ala-NHR' and RCO-Ala-Pro-NHR' sequences well-known to adopt opposed conformations: the former essentially fits the β-turn structure and the latter an open conformation.[2] AzaPro inverts the conformational properties of the peptide sequence and induces β-folding of the RCO-Ala-AzaPro-NHR' sequence whereas RCO-AzaPro-Ala-NHR' shapes an open structure. Both crystal conformations show that, due to severe sterical hindrances, both AzaPro nitrogens are practically of the sp^3 type, with three bond angles of about 110°. This reduces the electronic conjugation in the C'O-N bonds, and the C'-N bond length is about 1.40 Å instead of 1.32 Å observed for a standard peptide bond. Thus Aza-proline exhibits interesting structural and conformational features for the design of bioactive peptide analogues and inhibitors of enzymes involving proline-containing substrates.

1. Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein Chem.* 1985, *37*, 1-109.
2. Boussard, G.; Marraud, M.; Aubry, A. *Biopolymers*, 1979, *18*, 1297-1331.

L 308 **PEPTIDE MIMETICS OF THE FIBRINOGEN RGDX SEQUENCE AS ANTITHROMBOTIC.**

Philippe R. Bovy, Robert Garland, Foe S. Tjoeng, Mark E. Zupec, Jeffery Zablocki, Joseph G. Rico, Thomas E. Rogers, R. J. Lindmark, Susan G. Panzer-Knodle+, Nancy S. Nicholson+, Beatrice B. Taite+, Masateru Miyano+, Larry P. Feigen+, Steven P. Adams. Medicinal & Structural Chemistry Dept., Monsanto Company, 700 Chesterfield Village Pkwy., St. Louis, MO 63198 & +G. D. Searle Co., 4901 Searle Pkwy., Skokie, IL 60077

We have applied an iterative peptidomimetic approach to the RGD sequence resulting in compounds that block fibrinogen (Fg) binding to its $\alpha_{IIb}\beta_3$ integrin receptor, inhibit platelet aggregation in vitro and in vivo and prevent platelet mediated thrombosis.

Iterative structure modifications of the RGDF tetrapeptide led to incremental improvement in activity and resulted in design and synthesis of small peptidomimic of RGDF which contain no α-aminoacid and yet bind to the fibrinogen receptor with several hundred-fold improved affinity over RGDF itself. Final optimization of the structure gave low molecular weight $\alpha_{IIb}\beta_3$ antagonists (MW < 400) more potent than *Echistatin* (low nanomolar range in dog PRP/collagen). The new compounds typically incorporate benzamidine as bioisosteric replacement for Arg-Gly and feature new surrogates for the Asp-Phe dipeptide.

Oral bioavailability and in vivo activity in dogs have been demonstrated for some of these compounds (in 30-day studies, 2-5 mg/kg/day, oral delivery, blocked platelet aggregation for 12-24 hours).

L 309 AN ELECTROSTATIC MECHANISM OF SUBSTRATE RECOGNITION BY ACETYLCHOLINESTERASE

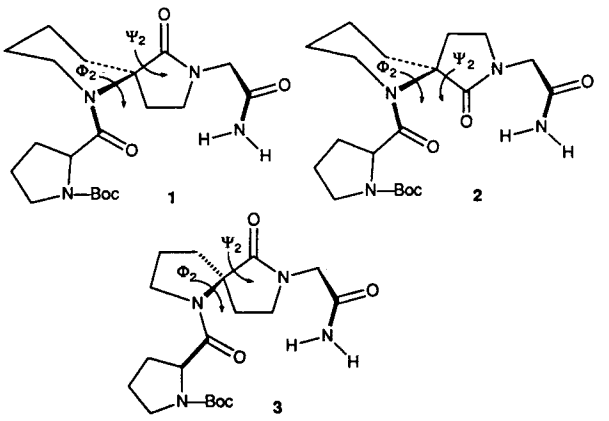
Carlos H. Faerman‡, Daniel R. Ripoll‡, Israel Silman§ and Joel Sussman§ ‡Molecular Biology Biotechnology Research Institute, National Research Council of Canada 6100 Royalmount Ave., Montreal, Quebec H4P 2R2 , Canada and §Department of Structural Chemistry The Weizmann Institute of Science Rehovot 76100, Israel

Acetylcholinesterase (acetylcholine hydrolase, E.C. 3.1.7; AChE) is a serine hydrolase whose principal role is termination of signal transmission at cholinergic synapses by rapid hydrolysis of the endogenous neurotransmitter, acetylcholine, in the synaptic gap. In accordance with its biological role, AChE is a very rapid enzyme, its turnover number being on the order of 100 microseconds. Recently the X-ray structure of AChE from Torpedo Californica has been solved (Sussman et al, 1991). Consequently, a detailed analysis of its properties in relation to its three dimensional structure is now possible. One of the most remarkable features of AChE is a deep and narrow gorge that contains, near its bottom, a catalytic triad consisting of serine, histidine and glutamate. A large part of the lining of the gorge, circa 40% of its total surface area, is contributed by the rings of 14 highly conserved aromatic amino acids. In contrast only a small number of negatively charged amino acids are found in the gorge. In the present study we re-examine the forces governing substrate guidance taking into account the electrostatic properties of the AChE molecule. Using the program DELPHI, we have calculated the electrostatic potential and field of AChE on a grid of points lying both inside and outside the gorge. The results presented here provide a new frame to study further AChE unique catalytic properties and will help characterize key residues and areas of AChE that may play an important role in interactions with ligands. This is of great importance for the design of new inhibitors of AChE.

L 310 X-RAY CRYSTALLOGRAPHIC EVIDENCE FOR A β-TURN CONFORMATION IN SPIROLACTAM-BASED PEPTIDOMIMETIC ANALOGS OF Boc-Pro-Leu-Gly-NH_2,

William B. Gleason[1,2], Rodney L. Johnson[1], Michael J. Genin[1] and William H. Ojala[2], Department of Medicinal Chemistry[1] and Biomedical Engineering Center[2], Department of Laboratory Medicine & Pathology, University of Minnesota, Minneapolis, MN 55455

In the course of investigating conformationally restricted analogs of Pro-Leu-Gly-NH_2, three spirolactam derivatives have been synthesized and characterized by X-ray crystallography.



The torsion angles Φ_2 and Ψ_2 are rigidly fixed by the spirolactam system and are appropriate for designing β-turn mimics. We have determined experimentally that compounds **1** and **3** are type II β-turn mimics and that compound **2** mimics the type II' β-turn.

L 311 PRINCIPLES OF SPATIAL DISPOSITION AND CHARACTER OF RESIDUES OF CONSTRAINED PEPTIDES AND PEPTIDOMIMETICS.

Mark I Greene and H. Uri Saragovi. Department of Pathology and Laboratory Medicine. University of Pennsylvania, Philadelphia, PA 19104-6082.

We have developed constrained peptides and peptidomimetics which mimic complementarity determining region (CDR) loop projections. CDR loops typify the attachment sites of members of the immunoglobulin gene family. We have defined certain of the principles that determine effective binding of the compounds to other proteins.

Through the study of antibody combining sites at high resolution we have developed insight into the spatial disposition of aromatic and hydrophilic residues and their contribution and role in ligand binding. In addition we have determined a critical role for water molecules in this interaction. The precise distribution of aromatics will affect the association and affinity of interaction of ligands with either constrained petides or mimetics. The basic principles will be described.

L 312 INCORPORATION OF DIPEPTIDOMIMETICS INTO SUBSTANCE P. SYNTHESIS AND BIOLOGICAL ACTIVITY.

Uli Hacksell,[a] Susanna Borg,[a] Charlotta Elfström,[a] Annika Jenmalm,[a] Kristina Luthman,[a] Gunnar Lindeberg,[b] Fred Nyberg,[c] and Lars Terenius[d]

[a]Dept. of Organic Pharmaceutical Chemistry, Box 574, [b]Dept. of Immunology, Box 582, [c]Dept. of Pharmacology, Box 591, Uppsala Biomedical Centre, Uppsala University, S-751 23 Uppsala, [d]Dept. of Drug Abuse Research, Karolinska Institute, Box 60500, S-104 01 Stockholm, Sweden.

Several dipeptidomimetics were incorporated into Substance P as replacements for Phe-Phe or Phe-Gly. The compounds were either derivatives of the vinyl isosteres of Phe-Phe or Phe-Gly or contained a heterocyclic ring as replacement for the amide bond.

The syntheses of the modified Substance P-analogues were performed using the solid-phase method with Boc/Bzl chemistry. The products were purified by reversed phase HPLC and characterized by NMR-spectroscopy and mass spectrometry.

The Substance P-analogues were evaluated as inhibitors of a specific Substance P cleaving enzyme (Substance P endopeptidase) and in NK_1-receptor binding assays.

L 313 G PROTEIN-BOUND CONFORMATION OF MASTOPARAN-X, A RECEPTOR-MIMETIC PEPTIDE, Tsutomu Higashijima and Muppalla Sukumar, Department of Pharmacology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75235-9041

A family of GTP-binding regulatory proteins (G proteins) convey signals from cellular surface receptors to intracellular effector proteins. Over 1000 receptors for diverse hormones, neurotransmitters and drugs are coupled to G proteins, and a cell expresses up to 10 G protein-coupled receptors, 3 or more G proteins, and several effectors. Therefore the precise interaction of specific receptors and G proteins is central to accurate cellular information processing.

We have found that mastoparan, a wasp venom toxin whose primary structure is Ile-Asn-Leu-Lys-Ala-Leu-Ala-Ala-Leu-Ala-Lys-Lys-Ile-Leu-NH_2, directly activates G protein by mimicking agonist-bound receptors functionally and structurally. Thus, mastoparan is an excellent low-molecular-weight receptor analog to study the mechanism and structural basis of activation of G proteins by receptors. We used *mastoparan-X* (Ile-Asn-Trp-Lys-Gly-Ile-Ala-Ala-Met-Ala-Lys-Lys-Leu-Leu-amide), another wasp venom toxin with similar activity as mastoparan, for the elucidation of G protein-bound (biologically active) conformation of this peptide by ^{1}H-NMR, using transferred nuclear Overhauser effects (TRNOE). TRNOE allows transfer of information concerning cross-relaxation between two nuclei on the bound ligand to the free ligand resonances via chemical exchange, and the cross-relaxation rate is a sensitive function of the interproton distance. The TRNOE data provide an approximate set of interproton distances which can be used as distance constraints in restrained molecular dynamics simulations, to obtain conformations consistent with NMR data.

The Gi (a G protein that mediates inhibition of adenylyl cyclase) and Go (an abundant G protein in brain)-bound conformation of mastoparan-X are very similar with the exception of minor differences at the termini, and, in both cases, a major part of the molecule adopts an amphiphilic alpha-helical conformation. The lysine residues are known to be crucial for activity and it is thus likely that at least the polar face of the amphiphilic helix is in contact with the G proteins. The G protein-bound conformation of mastoparan-X could explain the structure-activity relationships of mastoparan analogs. The G protein-bound conformation of mastoparan-X will be useful in the design of potent and selective analogs and in the development of models for receptor-G protein interaction at a molecular level.

L 314 FROM FUNCTION TO STRUCTURE: HIGH RESOLUTION FUNCTIONAL ANALYSIS OF ANTIBODY-ANTIGEN INTERACTIONS, Lei Jin, Brian M. Fendly, Fred E. Cohen and James A. Wells, Department of Protein Engineering, Genentech Inc. and Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143

A comprehensive mutational analysis was used to analyze the side-chains on human growth hormone(hGH) important for binding 21 different anti-hGH mouse monoclonal antibodies(MAbs) whose equivalent concentrations for 50% binding(EC_{50}) ranged from 10^7 to 3×10^{10} M^{-1}. A combination of homolog- and alanine-scanning mutagenesis coupled with a robot-aided ELISA were used to create high resolution "functional epitopes" for each MAb. Every functional epitope mapped to at least two polypeptide segments of hGH that were close together in the folded protein to form a patch. Although these patches sometimes overlapped, each was different indicating no two MAbs bound identically to hGH. The MAbs bound to determinants in loops and helices that were generally most accessible to a 9Å radius probe. Only a few side-chains dominated each functional epitopes and these tended to be Arg> Pro > Glu ~Asp ~ Phe~Ile(Ala, Cys, and Trp were not tested). Our studies indicate that most of the accessible surface of hGH is potentially antigenic in the mouse and suggest that functional epitopes are dominated by fewer side chains than may be in the contact epitope. In addition, we found that based on the structural constraints we derived from these functional data and information from secondary structure prediction, we can correctly predict the folding pattern of hGH.

L 315 CONFORMATIONAL ANALYSIS OF PEPTIDE TURN MIMETICS: APPLICATIONS IN CONFORMATIONAL MIMICRY AND RECEPTOR ANALYSIS, Michael E. Johnson, Liang Xue and Zhao Lan Lin, Center for Pharmaceutical Biotechnology and Department of Medicinal Chemistry & Pharmacognosy, University of Illinois at Chicago, P.O. Box 6998, Chicago, IL 60680

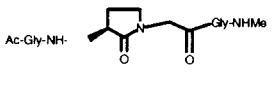
In recent work, we have shown that small cyclic organic structures, derivatized at the appropriate locations with pharmacophores equivalent to amino acid side chains, provide excellent analogs of peptide β-turns, with mimetics of a turn structure in fibrinopeptide A showing excellent activity as substrates or inhibitors of thrombin [Nakanishi et al. (1992) *Proc. Nat'l. Acad. Sci. USA* **89**, 1705-9], with mimetics of a critical turn region in the CD4 protein exhibiting micromolar activity in inhibiting binding by the HIV gp120 protein [Chen et al. (1992) *Proc. Nat'l. Acad. Sci. USA* **89**, 5872-6], and with other mimetics exhibiting activity as rigid analogs of small, flexible peptides. In this presentation, we have developed a detailed conformational analysis of two generations of peptide β-turn mimetics and two alternate γ-turn mimetics through molecular modeling and NMR spectroscopy. We show that two bicyclic ring structures can provide good positioning of the side chain substituents, but that the ring structures are much more rigid than the native peptide turn structure, leading to potential loss of activity where induced fitting plays a role in binding. In contrast, suitably derivatized 11- and 10-member mono-cyclic ring structures can exhibit excellellent conformational mimicry of type I and II β-turns, and exhibit conformational flexibilities similar to those of cyclized peptides. Application of these results to such mimetic systems as enkephalin, bradykinin and hypertrehalosemic hormone will be discussed.

(Supported in part by grants from the NIH and the American Heart Association of Metropolitan Chicago.)

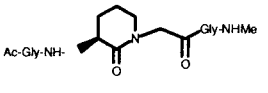
L 316 THEORETICAL INVESTIGATION OF β-TURN MIMETICS, Michael Krug and Günter Hölzemann, Drug Design Group and Medicinal Chemistry Department, E. MERCK, Frankfurter Straße 250, D-6100 Darmstadt, Germany.

Conformation restricting entities which mimic a β turn are widely used as building blocks for the investigation of biologically active compounds. Although conformational analysis of these mimetics were performed experimentally and theoretically it is difficult to compare them due to the different methods used.

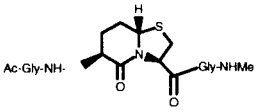
We have investigated the turn forming and turn retaining properties of four compounds (**I-IV**) described in the literature:



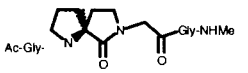
I



II



III



IV

Molecular dynamics simulation and various analysis procedures were successfully used to differentiate these properties.

To be independent from the usual classification of turns using the peptide angles φ and ϕ, a new parameter set consisting of two distances and one torsional angle was derived. These results will be compared to the analysis using the φ/ϕ notation and the root-mean-square deviation.

This method will allow the characterisation of the β turn properties of new compounds prior to synthesis.

L 317 EXPLORING MINIMAL STRUCTURAL REQUIREMENTS FOR PEPTIDES BINDING TO MHC CLASS II, Angela F. Liu, C. Mark Hill, Keith W. Marshall, John P. Mayer, and Jonathan B. Rothbard, ImmuLogic Pharmaceutical Co. 855 California Ave. Palo Alto, CA 94304

Understanding the minimal structural features for peptide binding to MHC class II proteins is a necessary requirement for the development of peptide mimetics and potential drug design. A significant advance in simplifying peptide ligands was the demonstration of a peptide containing a single tyrosine and lysine in a polyalanine backbone bound HLA-DR1Dw1 with high affinity. By assaying a variety of analogs of this simplified peptide the following conclusions were established ; (i) either an aromatic amino acid (Y, F, W) or an aliphatic (V, I, L, M) in the amino terminal portion of the peptide was essential for binding to DR1Dw1 and DR4Dw4, (ii) the optimal location for this residue was at the third residue of a thirteen amino acid peptide (iii) the lysine was important for solubility, but it had minimum contribution in binding to the class II molecule. A separate set of peptides containing either N-methyl amino acids or reduced peptide (ψCH_2NH) bonds was synthesized to study the role of the peptide backbone in binding. Peptides containing N-methyl amino acids bound DR1 and DR4 with equal or better affinity than the parent sequence, except the peptides with N-methyl substitution at 3rd or the 12th position. In contrast, most of the analogs with reduced peptide bonds were shown to bind these alleles poorly. Collectively the data was consistent with the notion that the majority of the binding energy of a peptide is came from a single sidechain contact and the peptide backbone hydrogen bonding to the MHC protein.

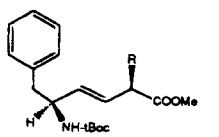
1. T. S.Jardetzky, J.C. Gorga, R. Busch, J. Rothbard, J.L. Strominger, and D.C. Wiley, EMBO J., 9: 1797-1803, 1990.

L 318 SYNTHESIS OF SOME NOVEL PHE-GLY AND PHE-PHE MIMETICS

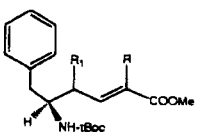
Kristina Luthman, Annika Jenmalm, Susanna Borg, Charlotta Elfström and Uli Hacksell

Dept. of Organic Pharmaceutical Chemistry, Uppsala Biomedical Centre, Uppsala University, Box 574, S-751 23 Uppsala, Sweden.

We have synthesized Phe-Gly and Phe-Phe mimetics containing a stable amide bond replacement using efficient and stereoselective reactions. The compounds were designed to mimic the amide bond both geometrically and electrostatically. Some compounds were derived from the vinyl isosteres of Phe-Phe (1) and Phe-Gly (2) and were of type **3** and **4**.



1 R = CH_2Ph
2 R = H



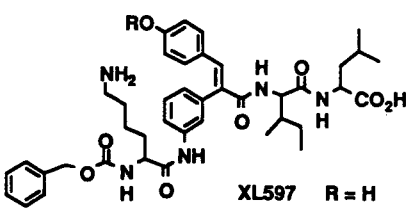
3 R = CH_2Ph
4 R = H

In some dipeptidomimetics the amid bond was replaced by a heterocyclic ring system with similar electronic properties as the amide bond.

The novel mimetics were evaluated as inhibitors of a specific Substance P cleaving enzyme (Substance P endopeptidase) and in NK_1-receptor binding assays.

L 319 THE DESIGN AND SYNTHESIS OF A PEPTIDOMIMETIC FOR NEUROTENSIN, Thomas P. Maduskuie, Jr., William K. Schmidt, Leo S. Bleicher, Joseph Cacciola, Walter Cheatham, John M. Fevig, Alexander L. Johnson, Susan A. McComb, David A. Nugiel, David A. Spellmeyer, S. William Tam, Matthew E. Voss, and Regina M. Wagner, The Du Pont Merck Pharmaceutical Company, Inc., Chemical Sciences Dept., Wilmington, DE 19880-0353

Many endogenous proteins and polyamino acids serve to activate receptors, inhibit or regulate biological systems. But these endogenous compounds can not be used as drugs, due to their in vivo instability and size. If we could dissect these compounds to determine the vital components for activity and combine this with conformational information from molecular modeling, we may be able to design and synthesize a simple nonpeptide analogue to mimic endogenous proteins. Neurotensin is a 13 amino acid peptide that has been shown to have a variety of pharmacological effects in analgesia, psychosis, cardiovascular and cognitive areas. We have designed and synthesized a peptidomimetic for neurotensin 9-13, the minimum fragment required for activity. XL597 and XK896 have been shown to have high affinity binding to the neurotensin receptor (Ki's = 27 nM and 25 nM respectively), and to have potent activity in analgesic and antipsychotic models in mice.



XL597 R = H

XK896 R = CH_3

L 320 MOLECULAR SHAPE COMPARISON OF ELASTASE INHIBITORS: QUANTITATIVE SURFACE SEARCHING OF THE TURKEY OVOMUCOID INHIBITOR WITH A TRIPEPTIDE INHIBITOR, James B. Matthew and Brian B. Masek Medicinal Chemistry Dept., ICI Pharmaceuticals Group, A business unit of ICI Americas Inc., Wilmington, Delaware 19897

Molecular shape comparison (MSC) seeks to find the spatial properties common to two or more molecules. A new and powerful analytical method for comparing molecular shapes by optimizing the overlap of molecular "Skins" has been developed. This method provides a quantitative measure of the shape similarity by maximizing the intersection volume of molecular Skins; a molecular surface of finite thickness. We report shape matching of a small tripeptide inhibitor of elastase class proteins with the 56 residue turkey ovomucoid inhibitor (TOMI). Skin based comparisions using either the binding loop of TOMI alone (residues 15-20) or the entire TOMI protein successfully found the alignment expected from comparison of their crystallographic complexes (i.e. HLE/TOMI complex and PPE/tripeptide complex). To match a large elastase inhibitor such as TOMI with a small inhibitor or drug we found that it is important to use a skin match rather than a volume match since the skin of the small inhibitor is matched only to the exposed skin of the larger molecule. In the MSC of the tripeptide with the entire TOMI protein, random or blind searching for skin matchs involved optimization of the shape match from 172 starting positions. The tripeptide center of mass was placed at points randomly selected from a set of 500 points on the TOMI van der waals surface (within 10 Å of the Leu 18 on the TOMI binding loop (1 point/Å^2)). Its orientation was randomized before optimization was initiated. The best match, 86.4 Å^3, was found three times and corresponds to the experimental alignment. The next best match was 78.1 Å^3 giving a discrimination factor in this case of 10%. Our MSC method has been enhanced with an option to allow discrimination between atoms or groups of atoms based on specific properties such as electrostatic potential, hydrogen bonding ability, hydrophobicity, or simply mainchain vs. sidechain. This enables matches based on criteria such as alignment of hydrophobic groups or hydrogen bond acceptor groups, etc.

L 321 ^{99m}Tc- and ^{111}In-LAMININ PEPTIDE FRAGMENTS FOR MALIGNANCY DIAGNOSIS, Michael Mokotoff,* Sastry S. Jonalagadda,* Dennis P. Swanson,* Manuel L. Brown,† and Michael W. Epperly,† Schools of Pharmacy* and Medicine,† University of Pittsburgh, Pittsburgh, PA 15261

In order for a tumor cell to metastasize it must be able to exit its tissue of origin, enter the blood or lymphatic circulation, and exit the respective circulation to localize at a distant, preferential organ site. This process requires that the tumor cell be capable of penetrating the basement membrane of the extracellular matrix and of blood and lymph vessel walls. Laminin is a major glycoprotein constituent of basement membrane and its binding to laminin receptors on tumor cells promotes the metastatic activity of the tumor cells. CDPGYIGSR-NH_2 and YIGSR-NH_2, synthetic peptides from the B1 chain of laminin, have been identified as sites for cell binding. High affinity receptors for laminin have been identified on the surface of both tumor and normal cells. However, malignant tumor cells frequently express greater levels of the laminin receptor than their normal or dysplastic counterparts. Therefore, we have begun the development of radiolabeled derivatives of laminin-like peptide fragments which may permit the non-invasive diagnosis of malignant tumors. The first approach we took involved the incorporation of a bifunctional chelator, DTPA, into two laminin fragments, thus allowing the chelation of ^{111}In to the peptides. Using solid phase peptide synthesis methods, with 4-methylbenzhydrylamine (MBHA) as the anchoring resin, we prepared G-Y(2BrZ)-I-G-S(Bzl)-R(Tos)-MBHA and C(Meb)-D(Bzl)-P-G-Y(2BrZ)-I-G-S(Bzl)-R(Tos)-MBHA. These protected peptides, still bound to the resin, were mono-acylated by reaction of the free N-terminus with the HOBt active ester of DTPA. After HF cleavage and HPLC, pure DTPA-GYIGSR-NH_2 and DTPA-CDPGYIGSR-NH_2 were obtained. Both DTPA conjugated peptides tested negative in an in vitro cell attachment assay (courtesy of Dr. Hynda Kleinman, NIH), suggesting that the DTPA interferes with laminin receptor binding. Both peptides were radiolabeled with ^{111}In and used for imaging mice containing either B16F10 or B16BL6 melanoma tumors. In separate experiments, both protected peptides bound to MBHA were acylated with Boc-nicotinyl hydrazine (NH), cleaved with HF and pure NH-GYIGSR-NH_2 and NH-CDPGYIGSR-NH_2 obtained. These derivatives tested strongly positive in the in vitro cell attachment assay, and were radiolabeled with ^{99m}Tc-glucoheptonate and used for imaging mice containing either B16F10 or B16BL6 melanoma tumors. The results of the imaging will be presented.

L 322 MULTIPLE ACTION OF INCORPORATED SUGARS ON THE PEPTIDE BACKBONE: SYNTHETIC, CONFORMATIONAL AND IMMUNOLOGICAL ASPECTS, Laszlo Otvos, Jr., David C. Jackson and Laszlo Urge, The Wistar Institute, Philadelphia, PA 19104, and Department of Microbiology, The University of Melbourne, Parkville, Victoria, Australia

The effect of glycosylation on the chemical and biological properties of the peptide backbone was investigated from several aspects.

1. Reducing carbohydrates containing 1-10 sugar moieties can be converted to their 1-amino derivatives and coupled to Fmoc-Asp-O^{t}Bu. The Fmoc-Asn (sugar)-OH reagents are good synthons for the solid-phase synthesis of glycopeptides. No considerable solubility, acid lability or mass spectral analytical problems were found for even a maltodekaose conjugated derivative of a tridekapeptide.
2. Incorporation of monosaccharides results in the stabilization of reverse-turns regardless of whether the sugar is placed inside (attached to functional asparagine, serine or threonine) or outside (carried by an extra asparagine residue) of the turn. A series of conformational variants can be obtained by walking N-acetyl-glucosamine and galactosamine through the peptide molecules. The shorter the peptide, the more pronounced the turn-stabilizing effect. The length of the saccharide does not seem to play a crucial role.
3. The size of the sugar greatly influences, however, the processing and/or recognition of the peptide backbone. Nearby epitopes are masked and the formation of disulfide bonds are delayed as a function of the length of proximal carbohydrates. Since post-translationally modified peptides are believed to bind to MHC, incorporation of sugar moieties may be useful to diminish unwanted immune responses.

L 323 PEPTIDE LIGANDS FOR THE IMMUNOGLOBULIN RECEPTOR (IG) OF A B-CELL LYMPHOMA

Renschler, M.F.[1], Bhatt, R., Dower, W., Levy, R.[2],Division of Oncology[1,2], Stanford University, Stanford CA 94305, and Affymax Research Institute, Palo Alto, CA 94304

The IG of B-cell lymphomas is a clonal receptor unique to each patient's lymphoma. It is ideally suited for targeting with a peptide for therapeutic purposes. The purified IG of a lymphoma cell line was screened with three phage libraries displaying random 8-mer and 12-mer peptides. After four rounds of panning, the peptide sequences of the phage binding to the IG were analyzed.

The IG of the human B-cell lymphoma cell line SUP-B8[1] was purified from supernatants of a fusion heterohybridoma with the mouse myeloma line K6H6/B5[2]. The IG was immobilized on microtiter plates. Phage libraries displaying approximately 5x10^8 random peptides at the N-terminus of protein III of the filamentous bacteriophage fd-tet were used for screening[3]. Approximately 10^{11} phage were applied to each of six wells. After extensive washing, phage were eluted with glycine HCl, pH 2.2. Recovered phage were amplified in *E.Coli*, precipitated and titered. After 3 and 4 rounds of panning, individual phage clones were screened for binding to the IG. The following enhancement (ratio of recoveries from the tumor IG vs. an irrelevant IG), representative of the enhancement with all three libraries, was seen: 1. round 1.0, 2. round: 3.3, 3. round: 52, 4. round: 350. The peptide sequence of the phage were determined by DNA sequencing. The peptides recovered from 3 different libraries independently show sequences falling into the following categories: KPWWxxR, KPWYxxR, xWYxxxW, xxWYRWxx, where K=Lysine, P=Proline, W=Tryptophan, Y=Tyrosine, R=Arginine, and x= any amino acid. The binding of these phage to the IG is specific and can be inhibited with monoclonal antibodies directed against the variable region (idiotype) of the IG, but not with antibodies directed against the constant region of the IG.

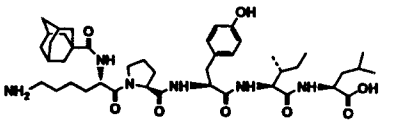
References:
1. Carroll, WL et. al. *Blood*, **71**:1068-1073,1988.
2. Carroll, WL et. al. *J.Immunol. Methods* **89**:61-72,1986.
3. Cwirla, SE et. al. *Proc.Natl.Acad.Sci.USA* **87**:6378-6382, 1990.

L 324 **CONFORMATIONAL ANALYSIS OF A HIGHLY CONSTRAINED GnRH ANTAGONIST BY NMR AND COMPUTATIONAL TECHNIQUES.** Josep Rizo[1], Bryan Sutton[1], Joshua Breslau[1], Steven C. Koerber[2], Jean E. Rivier[2], Arnold T. Hagler[3], and Lila M. Gierasch[1]; [1]Dept. of Pharmacology, Univ. of Texas Southwestern Med. Ctr., 5323 Harry Hines Blvd., Dallas, TX 75235-9041; [2] The Clayton Found. Lab. for Peptide Biology, The Salk *Institute, 10010* N. Torrey Pines Rd., La Jolla, CA 92307; [3]Biosym Technologies, Inc., 9685 Scranton Rd., San Diego, CA 92121.

Synthesis and conformational study of constrained gonadotropin releasing hormone (GnRH) analogs has led to a model for the active conformation of GnRH [Rizo et al. (1992) *J. Am. Chem. Soc.* **114**, 2860] and to the development of highly potent, bicyclic GnRH antagonists. In order to refine our putative binding model, we have analyzed the structure of a bicyclic antagonist containing (4-10) and (5-8) bridges, bicyclo (4-10,5-8)[Ac-D-2Nal[1],D-Cpa[2],D-3Pal[3],Asp[4],Glu[5] ($c^{\beta5}$,$N^{\Delta8}$-Gly),D-Arg[6],Dbu[8],Dpr[10]]GnRH, using nuclear magnetic resonance (NMR). Simulated annealing and distance geometry calculations, incorporating 65 interproton distance constraints, yielded a set of structures consistent with all the NMR data, which have some similarities and some differences with respect to the conformations found for constrained GnRH antagonists studied previously. Although the tail formed by residues 1-3 is mobile, as it is in related GnRH analogs, it appears to be pointing outwards with respect to the ring, while it was previously found to be oriented above the ring. The bicyclic part of the peptide is highly rigid and contains a Type I' β-turn around residues 6-7, while a Type II' β turn around these positions appeared to be essential for antagonist activity. In light of these observations, we are performing a detailed comparison of the shape and flexiblity of all constrained GnRH antagonists whose conformations have been studied, to develop a refined model for the GnRH pharmacophore that is consistent with all available data.

L 325 **ADAMANTOYL-Lys-Pro-Tyr-Ile-Leu (ADA-KPYIL), A SYSTEMICALLY-ACTIVE NEUROTENSIN 9-13 ANALOG WITH AN ANALGESIC AND ANTIPSYCHOTIC PROFILE IN MICE AND RATS,** William K. Schmidt, Gary A. Cain, Thomas E. Christos, Pat N. Confalone, Alexander L. Johnson, Bruce J. Aungst, and S. William Tam, The Du Pont Merck Pharmaceutical Company, Inc., CNS Diseases Research, Wilmington, DE 19880-0400

Neurotensin (NT) is a naturally occurring 13 amino acid peptide isolated from brain and intestinal tissue. As part of our program to prepare parenterally-active peptidomimetic analogs of NT 9-13, we found that the Nα addition of bulky, lipophilic acyl groups to residue 9 in NT 9-13 produces compounds with potent i.v. parenteral activity. Compared to native analogs without the adamantanecarbonyl group, ADA-KPYIL has moderately high affinity for the NT receptor in rat brain homogenates (Ki = 117 nM vs. 73 and 688 nM for NT 9-13 and Lys-9 NT 9-13, respectively) and has potent full agonist activity in the guinea pig ileum longitudinal muscle preparation (EC50 = 2.9 nM vs. 216 and 648 nM). Injected systemically, ADA-KPYIL produces potent, long-lasting analgesic activity in mouse and rat phenylquinone writhing tests (ED50's = 2.2 and 0.44 mg/kg i.v.). ADA-KPYIL analgesic effects are not reversed by naloxone, indicating a non-opioid mechanism of action. ADA-KPYIL blocks amphetamine-stimulated locomotor activity and has potent anti-mescaline activity in the mescaline scratch test in mice, indicating potential *antipsychotic* activity. ADA-KPYIL is not active following oral administration. Absorption/metabolism studies show that ADA-KPYIL is stable to gastric acid and pepsin but is readily degraded by pancreatic enzymes. We hypothesize that adamantanecarbonyl enhances penetration of ADA-KPYIL through the blood brain barrier and further protects it against endopeptidase degradation following systemic injection in rodents.



L 326 **IODINATED MELANOTROPIN PEPTIDES FOR RADIORECEPTOR STUDIES : BIOLOGICAL EVALUATIONS,** Shubh D. Sharma, Jinwen Jiang@, Mac E. Hadley@, and Victor J. Hruby, Departments of Chemistry and Anatomy@, University of Arizona, Tucson, Arizona 85721, U.S.A.

Alpha melanotropin, a tridecapeptide, has a wide range of physiological activities both in the central nervous and peripheral systems. The control of melanin pigmentation of the skin by melanotropic peptides is now very well established. Recent studies have provided evidence that melanoma tumor cells possess receptors for melanotropins. Thus, the biochemical characterization of these receptors has become central to melanoma research. The use of radioiodinated melanotropins in this endeavor has, however, yielded misleading results. It has been reported that the evidence for the presence or absence of these receptors was dependent on the type of the radioiodinated ligand employed in these studies. Further, it was earlier shown that the iodination of melanotropin peptides by classical chloramin-T method can cause severe damage to the certain amino acids that are critical for the biological activity of the peptide. In the present study a series of specifically conjugated, iodinated melanotropin analogues have been synthesized and evaluated for their biological activity profiles. The radioiodinated versions of these conjugated peptides were readily prepared under non-oxidative conditions. The full chemical and biological characterization of these iodinated analogues employed in radioreceptor studies assure an unambiguous characterization of the melanotropin receptors on various melanoma cell lines. This approach may also become of direct relevance in the identification and characterization of various receptor subtypes. Supported by grants from the U.S. Public Health Service.

L 327 DEVELOPMENT OF HIGHLY POTENT THROMBIN INHIBITORS BASED ON AMIDINOPHENYLALANINE DERIVATIVES, Werner Stueber, Gerhard Dickneite, Rainer Koschinsky, Martin Reers, Dieter Hoffmann and Eric-Paul Pâques, SBU Therapeutics, Behringwerke AG, D-W 3550 Marburg, GERMANY

Amidinophenylalanine has been used as a building block for the development of thrombin inhibitors for more than ten years. So far reported NAPAP (8-Naphthylsulfonyl-glycyl-D,L-4-amidinophenylalanylpiperidide) is the most potent thrombin inhibitor with a Ki of 6 nM based on the amino acid phenylalanine. Additionally, derivatives on the basis of 3-amidinophenylalanine led to thrombin inhibitors with a reduced potency against thrombin. The drawback of all these substances are their limited efficacy in vivo, their side effects in vivo and the low bioavailability. We report herein on the development of new and highly potent thrombin inhibitors with the aim to overcome these shortcomings. For this purpose, we optimized the inhibitor structure of amidinophenylalanine derivatives employing CAMD and synthesized and characterized a series of inhibitor molecules. These inhibitors show an improved potency and specificity against thrombin (ki<1nM). In vivo these inhibitors show reduced side effects and due to their high stability oral resorption has been observed.

L 328 "PEPTIDOMIMETIC PRODRUGS. SOLUBILITY, STABILITY, AND TARGETED RECONVERSION OF AMINO ACID ESTERS OF RENIN INHIBITORS"
M. D. Taylor, J. L. Wright, J. T. Repine, X. M. Cheng, B. H. Stewart, K. D. Massey, A. R. Kugler, Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48106-1047.

A variety of approaches have been used to improve drug delivery of peptide and protein based drugs, particularly for oral administration with limited success. Renin inhibitors are one example of peptidomimetic drugs that are still limited by low bioavailability even though an enormous effort in molecular modification has resulted in a substantial reduction in size and peptide character.

Low oral bioavailability of peptide-like drugs may result from rapid enzymatic degradation, poor absorption, high first pass clearance, or a combination of these. While enzymatic stability of renin inhibitors has been addressed successfully, they are subject to extensive hepatic clearance and may be limited by poor absorption, particularly in cases where aqueous solubility is low.

We evaluated a series of amino acid prodrugs of renin inhibitors designed to be cleaved by intestinal brush border peptidases. This strategy combines greatly improved solubility of the prodrug with targeted release of the drug at the site of absorption producing a concentration gradient that increases passive transport of the agent. This approach hasbeen applied successfully to highly permeable, but poorly soluble steroids (Amidon, et al. J Pharm Sci, 1980, 69, 1363).

Renin inhibitors were prepared that incorporated a hydroxy group at various sites for attaching amino acid auxilliaries. Esters of basic, acidic, and neutral amino acids were prepared and evaluated for aqueous solubility, stability in buffer solution, and rate of hydrolysis in intestinal perfusate and brush border membrane suspensions. Steady-state permeabilities of the parent compounds and several prodrugs were determined in a rat single-pass intestinal perfusion model.

The amino acid ester prodrugs had substantially increased aqueous solubility. Appropriate selection of amino acid auxilliaries produced prodrugs with adequate stability in buffer solution, while still having sufficient susceptibility to hydrolysis by intestinal brush border aminopeptidases to release active drug. The best selectivity was exhibited by the aspartate esters. Several prodrugs also had the unexpected effect of improving stability toward proteolysis at the P_3-P_2 peptide bond by pancreatic enzymes.

L 329 ANTI-INFLAMMATORY PEPTIDE AGONISTS,
Holly A. Thomas and Edward T. Wei, School of Public Health, University of California, Berkeley, CA 94720

Vascular leakage is a characteristic response of living tissues to injury. Several peptide hormones inhibit this inflammatory process: corticotropin-releasing factor (CRF) being a prototype. For example, CRF (30 to 60 μg/kg) decreased edema and swelling in the anesthetized rat's paw after exposure to heat or to extreme cold, in trachea mucosa after exposure to formaldehyde, in lung alveoli after an intravenous injection of epinephrine, in skeletal muscle after a knife cut, and in brain cortex after freezing. To search for sequences in CRF, a 41-residue peptide, that might be associated with anti-inflammatory activity, we tested peptides similar to the carboxyl-termini of ovine and human/rat CRF. Because h/rCRF(35-39), -Arg.Lys.Leu.Met.Glu-, resembles -Arg-Lys.Leu.Leu.Glu-, a sequence found in many intermediate filament proteins, analogous peptides were also evaluated. Ovine CRF(21-41), CRF(26-41) and CRF(30-41) were inactive when tested at 5 mg/kg i.v. on thermal edema. Crude peptides with D-amino acid substitutions (denoted by the lower case of the single letter code), *a*HS*n*RK(L/M)E*l*/-NH_2 and /ATyRKLLE*l*/-NH_2, were found to have anti-inflammatory activity. Characterization of structures within the crude mixture revealed that substitution of the glutamic acid residue (E) with an anisoylated glutamic acid derivative (*) increased overall anti-inflammatory potency. The anisole derivative was apparently a by-product of the temperature-dependent Friedel-Crafts acylation reaction which occurs during hydrogen fluoride cleavage of glutamyl-containing peptides. Several undecapeptides containing D-amino acids and the anisoylated glutamic acid derivative were synthesized and characterized by HPLC, amino acid analysis and sequencing, mass spectrometry, and nuclear magnetic resonance. The more potent peptides, /ATyRKLL*l/-NH_2 and *a*HS*n*RKL(L/M)*l/-NH_2, reduced pulmonary and thermogenic edema with ED_{50} values of 40 to 240 μg/kg i.v., respectively. These short anti-inflammatory peptides containing the sequence -RKL(M/L)*l/-NH_2 were called mystixins because their powerful mode of action is unknown, mysterious, and intriguing.

L 330 DESIGN OF MIMETIC LIGANDS WITH SPECIFICITY FOR PROTEASES AND PROTEASE INHIBITORS.
Janette A. Thomas, N. P. Burton, C. R. Lowe. Institute of Biotechnology, University of Cambridge, Tennis Ct Rd, Cambridge, UK.

The interactions which determine the specificity of proteases and protease inhibitors are being probed by the design of ligands that mimic their natural substrates. Computer modelling is used to assist in the design of the mimetic ligands for proteins whose crystal structures are available in the Brookhaven database.

Ligands have been designed to bind the trypsin-like enzymes, where specificity is based on their ability to discriminate between the side chains of amino-acids through a number of binding pockets near the catalytic apparatus. Pancreatic Kallikrein is specific for an arginine residue binding in the primary binding pocket (the active site) and has strong specificty for hydrophobic amino acids binding in the secondary binding pocket, thus pancreatic Kallikrein has a high specificity for the dipeptide Arg-Phe. The small ligand benzamidine is known to bind Kallikrein by mimicing the arginine side chain. However, ligands have now been designed which mimic the hydrophobic binding site as well as the Arg binding site to display a higher affinity for Kallikrein than benzamidine alone.

Work is also in progress to model interactions of alpha1-proteinase inhibitor based on the X-ray crystal structure of the cleaved form of this protein. When cleaved, the binding loop of the inhibitor unfolds and integrates into the protein to form a central strand of a large beta sheet. Peptides based on the sequence of the binding loop are known to mimic the cleaved strand and bind to the intact form of alpha1-proteinase inhibitor under suitable conditions. When the peptide is bound, alpha1-proteinase inhibitor cannot bind its substrate in spite of the presence of the intact binding loop. Ligands are being designed to mimic this peptide.

L 331 THE EFFECT OF CONFORMATIONAL ISOMERISM ON MEMBRANE PERMEABILITY OF MODEL PEPTIDES
David G. Vander Velde, Phillip R. Burton,[+] and Ronald T. Borchardt, Department of Pharmaceutical Chemistry, University of Kansas, Lawrence KS 66045, and [+]Drug Delivery Research, The Upjohn Company, Kalamazoo MI 49001.

The great majority of drug substances cross the biological barriers posed by lipid membranes via passive diffusion en route to their site of action. However, transmembrane permeability of peptides and peptide-like molecules is generally poor, resulting in significant drug delivery problems even in the absence of enzymatic degradation. This has been attributed to the solvation of the hydrogen bonding groups in the amide backbone; these groups must be desolvated at substantial energy cost in order for the peptide to leave the aqueous media and enter the membrane. Recent work on model peptides has shown that simply counting the number of hydrogen bonding sites better predicts membrane permeability than the octanol:water partition coefficent, which is commonly used to predict the permeability of small organic molecules.[1] In this same series of model peptides (trimers of D-Phe with varying numbers of N-methyl substituents), we have found that the *cis-trans* equilibrium around the N-methylated amide bonds, as identified by NMR, appears to be an additional modulator of transport behavior. Within series of structural regioisomers, the peptides showing higher amounts of the *cis* isomer showed 2-3 fold enhanced membrane permeability *in vitro* using an intestinal epithelial cell line (CACO-2). The two-dimensional NMR data by which the isomers were identified will be presented. The *cis* isomers are stabilized by favorable dipolar interactions between carbonyl and aromatic groups, thereby reducing their affinity for solvent, and are also more compact structures. Both properties would be expected to correlate with enhanced permeability. These results suggest than in addition to such factors as size, charge, lipophilicity, and hydrogen bonding potential, that conformation is important in determining membrane permeability.

[1] R. A. Conradi *et al.* ***Pharm. Res.*** **1992**, *9*, 435-439.

L 332 **CONFORMATIONAL FEATURES OF HIGHLY POTENT, RIGID BICYCLIC OXYTOCIN ANTAGONISTS.**
Susanne Wilke#, Katalin E. Kover#, Petr Malon, Nathan Collins#, and Victor J. Hruby#.* University of Arizona, Dept. of Chem., Tucson, AZ 85721, U.S.A. *Czechoslovak Academy of Sciences, Dept. of Org. Chem. and Biochem., 166 10 Prague 6, *Czechoslovakia.*

Considerable clinical interest in the design of new drugs based on endogenous peptide hormones and neuropeptides has increasingly stimulated research determining the conformational features important for peptide agonism and antagonism. Also, the production of highly specific peptide drugs is substantially aided by knowledge of solution conformations of sterically restricted highly potent antagonists.

In our laboratory, a new class of potent rigid bicyclic antagonists of oxytocin (OT) has been developed (Hill et al., *JACS*, 1990). They are among the most potent antagonists of OT at the uterotonic receptor. OT is the neurohypophyseal hormone generally accepted as important for milk-ejection and uterine contracting activities. OT antagonists are important in the prevention of premature labor.

We investigated the global constraints imposed by bicyclization on these analogs using advanced methods of 2D-NMR including z-filtered ^{1}H TOCSY, NOESY and HMQC heteronuclear z-filtered TOCSY experiments. We found that these steric constraints forced an exclusive cis-conformation around the Cys^6-Pro^7 peptide bond, not found thus far in OT analogs. Furthermore, using CD, modelling, and structure-activity relationships of relevant OT analogs, we will discuss the influence of these constraints on disulfide helicity, Tyr^2 rotamer populations and turn conformations.

(This work was supported by the National Department of Health)

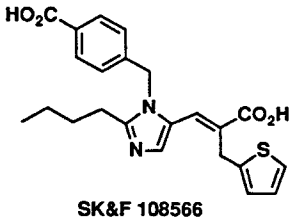
Late Abstracts

DESIGN OF HIGH AFFINITY DNA BINDING MYC ANALOGS: CRITICAL SIDE CHAIN AND CONFORMATIONAL REQUIREMENTS, David E. Fisher and Phillip A. Sharp, Center for Cancer Research, MIT, Cambridge, MA 02139 and Dana Farber Cancer Institute, Harvard Medical School, Boston, MA 02115

The Myc oncoproteins belong to the b-HLH-ZIP family and recognize the palindromic core sequence CACGTG. This family of proteins forms homo- or heterodimers via interactions in helix-loop-helix and leucine zipper domains and contacts DNA via an adjacent "basic domain" of *approximately* 20 amino acids. Using gel filtration and sedimentation analyses, these proteins have been shown to form tetramers in solution but bind DNA as dimers. This DNA contact has been analyzed in detail to shed light on biochemical features of this recognition process. Methylation interference studies demonstrated that binding occurs within the major groove of DNA. To determine critical amino acids for DNA recognition, the basic domain sequences of CACGTG-binding proteins were analyzed for homologies. Conserved positions were detected every 3-4 residues. This spacing is reminiscent of consecutive turns of alpha-helical peptide and suggested that the basic domain utilizes one face of an alpha-helix to contact DNA. Using the protein TFEB as a homodimeric model, circular dichroism was performed in the absence and presence of target DNA. Alpha-helical content was shown to increase significantly upon addition of DNA, suggesting that the basic domain is disordered in the absence of DNA, but alpha-helical in its presence. Exhaustive "alanine scanning" mutagenesis was undertaken in the basic domain. Four conserved amino acids were shown to be critical as demonstrated by obliteration of DNA binding by their mutation. Three other residues strongly stabilized the interaction. Based on their positions, the presence of a DNA "anchoring" subdomain has been postulated. DNA bending was also analyzed, electrophoretically, for 5 proteins in this family. All were found to produce minor groove oriented bends of 74°-82°. Utilizing the structural information from these studies, artificial Myc basic domain analogs were designed. Basic region positions predicted not to contact DNA were systematically mutated to alanine. Six wild-type residues ultimately remained within a polyalanine backbone. Binding affinities as much as 35 fold higher than c-Myc's basic region were obtained. Stronger DNA binding may arise from enhanced alpha-helicity of polyalanine. This design strategy may be applicable to other helix-dependant biomolecular interactions. High affinity Myc family analogs may be useful in the design and study of protein:DNA interactions and biological effects of Myc induced oncogenesis.

POTENT NONPEPTIDE ANGIOTENSIN II RECEPTOR ANTAGONISTS: 1-(CARBOXYBENZYL)IMIDAZOLE-5-ACRYLIC ACIDS, R. M. Keenan, J. Weinstock, J. A. Finkelstein, R. G. Franz, D. E. Gaitanopoulos, G. R. Girard, D. T. Hill, T. M. Morgan, J. M. Samanen, C. E. Peishoff, N. Aiyar, D. P. Brooks, E. Griffin, E. H. Ohlstein, E. J. Stack, E. F. Weidley and R. M. Edwards, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406

Nonpeptide angiotensin II (AII) receptor antagonists containing a unique thienylmethyl-substituted (E) acrylic acid attached to an imidazole nucleus have been developed using overlay hypotheses of nonpeptides on a novel pharmacophore model of AII. Modeling suggests that the newly introduced acrylic acid sidechain may mimic the C-terminal phenylalanine region of AII. Modification of the N-benzyl ring substitution in an effort to mimic the Tyr^4 residue of AII resulted in the discovery of N-carboxybenzyl compounds with nanomolar affinity and good oral activity. SAR studies of these potent diacid antagonists revealed that a number of structural elements, such as the thienyl ring, the (E) acrylic acid, and the imidazole ring, in addition to the two acid groups were important for high potency. Overlay comparisons with a representative biphenyltetrazole-substituted imidazole nonpeptide antagonist suggest that these two apparently similar series of antagonists may in fact bind in a different fashion. The parent diacid analog, SK&F 108566, a pure competitive antagonist selective for the AT-1 AII receptor, is in clinical development for the treatment of hypertension.



SK&F 108566

Evaluation of the NOE Derived Structures of Flexible Peptides: Application to *Desmopressin*

Jianjun Wang, Frank D. Sönnichsen, Robert Boyko and Brian D. Sykes

Protein Engineering Network of Centres of Excellence
MRC Group of Protein Structure and Function
Department of Biochemistry, University of Alberta
Edmonton, Canada T6G 2S2

Desmopressin is a nine-residue peptide hormone analog with strong antidiuretic and antibleeding activities. It consists of a six-residue disulfide bonded loop and a three residue tail. The solution structure of *desmopressin* has been generated by two different methods, simulated annealing and distance geometry, based on NMR nOe data. The structures generated by these two methods generally are similiar for the backbone of the disulfide-bonded loop, but show differences for the side-chains as well as for residues in the tail. In order to evaluate how well these two families of structures actually reproduce the experimental data and represent the flexibility of *desmopressin* , a full relaxation matrix approach (PEPFLEX) has been developed for peptides which may show multiple conformations in solution. Using PEPFLEX, we have been able to evaluate the two families of structures generated by simulated annealing and distance geometry and find that better RMS deviations between calculated and experimental nOes are obtained for the simulated annealing structures. We are now trying to determine which family of structures gives the better indication of the flexibility of *desmopressin*. A procedure of generating the families of structures for flexible peptides by using simulated annealing and PEPFLEX is discussed.

PREDICTING PEPTIDE BINDING AFFINITY: STERIC FIT IN RNase-S PEPTIDE INTERACTIONS, Chris Lee, Dept. of Cell Biology, Stanford University Medical Center, Stanford, CA 94305.

Understanding binding specificity is an important goal for drug design. Part of the complexity of this problem arises from the fact that many types of interactions contribute to specificity-- electrostatic, hydrogen bonding, van der Waals, and hydrophobic. It would be very useful to develop a clear picture of their relative importance in determining specificity. Van der Waals interactions, mediating the *steric fit* of a ligand to its binding site, would appear to be a very simple but very important specificity determinant, very strictly excluding ligands that in any way "do not fit".

To test this idea, we have developed methods to predict the relative "steric fit" of a peptide to a site, repacking the substituted sidechain(s) and surrounding peptide and binding-site sidechains, to locate the best possible packing for the given sequence. The repacking method allows all possible sidechain torsional rotations at 10 degree steps, and uses a self-consistent mean-field ensemble approach to locate the packing global minimum in a few minutes computation, even starting from a completely random "guess" conformation.

Recent experiments measuring the effects of peptide mutations on RNase-S peptide binding affinity (Connelly et al., 1990,Biochemistry 29,6108-6114) provide an excellent model system for testing these ideas. These experiments showed a surprising divergence of mutant affinities from those expected on the basis of hydrophobicity. Packing appears to play an extremely important role in RNase-S peptide binding specificity, and provides an excellent predictor of peptide affinity. The predicted packing energies of the 8 mutant peptides correlate closely with the experimentally measured affinities (0.85 correlation coefficient). Using this method, we have generated specificity predictions for substitutions of the neighboring residue Phe 8, which appears to contribute more to the overall binding energy than Met 13, and to be much less tolerant of beta-branched substitutions. Further work on mutations in the binding site indicates that its specificity can be "tuned", to produce a specific preference for any of a variety of hydrophobic amino acids at peptide residue 13.

STRUCTURAL INVESTIGATION OF THE GAL4 DIMERISATION DOMAIN IN SOLUTION BY NMR SPECTROSCOPY, Peter Schmieder, Ronen Marmorstein, James D. Baleja, Stephen C. Harrison, Gerhard Wagner, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, 02115; Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA, 02138.

The yeast protein Gal4 activates transcription of genes required for catabolism of galactose[1]. The protein consists of 881 amino acids and binds as a dimer to a DNA sequence of 17 base pairs. Functions assigned to parts of the protein are DNA-binding (residues 1-50), dimerisation (50-94) and activation (94-106,148-196,768-881)[2]. The structure of the DNA-binding domain (residues 1-65) has been studied using NMR-spectroscopy[3] as well as X-ray methods[4], revealing a new DNA-binding motif (9-40). In the X-ray structure the residues 50-64 reveal a weak dimerisation function that extends away from the bound DNA. The full dimerisation domain consisting of residues 50-106 has been studied in solution by NMR-spectroscopy. Our preliminary results indicate that the protein is a dimer in solution and its spatial structure is stable to temperatures well above 45°. The protein is in part α-helical and there are extensive contacts between aromatic and methyl-groups side-chains. Structural questions of special importance whether the region from 50-64 resembles the X-ray structure and if any part of the region from 65-106 folds back to contact the DNA again. Complete sequence specific assignments and the determination of the structure in solution are under way. Aspects of dimerisation will be investigated with the aid of heteronuclear experiments of mixtures of labeled and unlabeled molucules.

1. M. Johnson, *Microbiol. Rev.* **51**, 458-476 (1987).
2. M. Carey, H. Kakidani, J. Leatherwood, F. Mostashari, M. Ptashne, *J. molec. Biol.* **209**, 423-432 (1989).
3. J.D. Baleja,R.Mamorstein, S.C.Harrison, G.Wagner, *Nature* **356**, 450-452 (1992); P.J. Kraulis, A.R.C. Raine, P.L. Gadhavi, E.D. Laue, *Nature* **356**, 450-452 (1992).
4. R. Mamorstein, M. Carey, M. Ptashne, S.C. Harrison, *Nature* **356**, 408-414 (1992).