SYNTHETIC PEPTIDES: APPROACHES TO BIOLOGICAL PROBLEMS

Organizer: James P. Tam

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Synthetic Peptides: Approaches to Biological Problems

Synthesis and Characterization

CK 001 LASER DESORPTION/IONIZATION MASS SPECTROMETRY OF LARGE PROTEINS: MOLECULAR WEIGHT DETERMINATION AND STRUCTURAL ANALYSIS,

F. Hillenkamp and Michael Karas, Institut für Medizinische Physik, Universität Münster, Hüfferstr. 68, D-4400 Münster, Fed. Rep. Germany

Laser desorption is one of the soft techniques for ion generation of nonvolatile, thermally labile bioorganic compounds. It has recently been shown by the authors that the mass range of ions which can be desorbed and ionized can be extended to several hundred thousand Daltons, thus covering that of the majority of biomolecules if analyte molecules are isolated in a dilute solution of in a suitable matrix such as nicotinic acid <1, 2, 3>. Preferentially the matrix should have a resonant absorption at the ultraviolet laser wavelength far exceeding that of the analyte molecules. Resonant absorption of ligands or prosthetic groups of analyte molecules may lead to partial or full cleavage of such groups <4>. Sensitivity has been shown to be in the femtomole range of material needed for preparation and is believed to be in the atomole range for the amount of material consumed <5>. Besides singly charged parent molecular ions spectra typically show signals of oligomers and multiply charged ions, the relative abundance of which can be influenced to some extent by a variation of the concentration and the laser irradiance. Some basic principles, typical technical approaches for sample preparation and mass analysis and a variety of typical applications will be presented. Expectations for future developments and applications will be discussed, based on the current state of the art.

1. **Karas, M. and Hillenkamp**, F.: Laser Desorption Ionization of Proteins with Molecular Mass Exceeding 10.000 Daltons, Anal. Chem. 60 (1988) 2299-2301.
2. **Karas, M., Bahr, U. and Hillenkamp**, F.: UV-Laser Matrix Desorption Mass Spectrometry of Proteins in the 100,000 Dalton Range, Int. J. Mass Spectrom. Ion Proc. 92 (1989) 231-242.
3. **Karas, M, U. Bahr, A. Ingendoh and F. Hillenkamp**, Laser Desorption/Ionization Mass Spectrometry of Proteins of Mass 100.000 to 250.000 Dalton, Angewandte Chemie, Int. Ed. Engl. 28 (1989) 760.
4. **Karas, M., A. Ingendoh, U. Bahr and F. Hillenkamp**, UV-Laser Desorption/Ionization Mass Spectrometry of Femtomol Amounts of Large Proteins. Biomed. Environm. Mass Spectrometry, 18 (1989) 841 - 843.
5. **Hillenkamp, F., M. Karas, A. Ingendoh and B. Stahl**, Matrix Assisted UV-Laser Desorp tion/Ionization: A New Approach to Mass Spectrometry of Large Biomolecules. Proceedings of the Second Int. Symposium on Mass Spectrometry in the Health and Life Sciences, A. Burlingame and J. A. McCloskey, eds. Elsevier Pub. Comp., in press.

Structure Determination

CK 002 THE USE OF ISOTOPES IN NMR STUDIES OF ENZYMES/INHIBITOR COMPLEXES, Stephen W. Fesik, Erik R.P. Zuiderweg, R.T. Gampe, Jr., Hugh L. Eaton, and Edward T. Olejniczak, Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL 60064.

Enzyme/inhibitor complexes are difficult to study by NMR due to the overlap of NMR signals. However, by labeling the enzyme and/or inhibitor with stable isotopes (^{15}N, ^{13}C, ^{2}H), the proton NMR spectra of macromolecular complexes can be simplified and made interpretable. Approaches will be presented for studying enzyme/inhibitor complexes that involve the use of isotopes. These include: 1) the selective detection of protons attached to ^{13}C and ^{15}N labeled ligands, 2) 2D NOE difference spectroscopy on enzyme/inhibitor complexes prepared with either protonated or deuterated inhibitors, 3) the selective detection of the proton NMR spectra of ligands bound to perdeuterated enzymes, and 4) heteronuclear three-dimensional NMR spectroscopy. The relative merits of each of these approaches for providing detailed structural information on enzyme/inhibitor complexes will be discussed.

CK 003 CALCIUM REGULATION IN MUSCLE CONTRACTION; NMR STUDIES OF PROTEIN-PROTEIN AND PROTEIN-CALCIUM INTERACTIONS, Brian D. Sykes, A. Patricia Campbell, Gary S. Shaw and Robert S. Hodges, Department of Biochemistry and MRC Group in Protein, Structure and Function, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

Calcium plays an ir..portant regulatory role in many biological systems such as muscle where it effects a structural change in regulatory proteins such as troponin and tropomyosin. We have focussed on two aspects of this regulation; the binding of metals to the calcium binding protein troponin-C (TnC) and the calcium mediated interaction of troponin-I (TnI), troponin-C and actin. The main focus is to determine what structural changes are produced in troponin-C upon calcium binding, and how are they propagated to the other proteins in the thin filament regulatory system. In both of these areas our approach has been to use synthetic peptide analogs to simplify the NMR spectrum and allow us to focus on one aspect of the system. We have used high resolution 500 MHz ^{1}H NMR techniques to determine the structure of helix-loop-helix peptides in solution. We are then able to modify any residue in these peptides at will to help elucidate which factors govern the affinity, selectivity, and kinetics of metal binding. It is necessary for these studies to prove that these peptides have the same structure as the loop in the intact protein. Structural constraints were obtained from relaxation studies with the paramagnetic calcium analogue gadolinium, from 2D NOESY studies, and from NH-αCH NMR coupling constants. The comparison of distance restraints with the equivalent distances calculated from the X-ray structure suggests the structures are very similar. We have also studied a synthetic analogue of the inhibitory region of Tn-I, which represents the minimum sequence necessary for inhibition of actomyosin ATPase activity. Conformational changes in the peptide induced by the formation of the synthetic peptide Tn-C complex were followed by the transferred 2D NOESY. This technique also allows one to determine which residues on the protein make contact with the bound peptide. We have also used gadolinium relaxation measurements to locate the position of the TnI peptide when bound to TnC and used computer modelling techniques to dock the TnI peptide on TnC.

CK 004 NMR INVESTIGATIONS OF PEPTIDE/ANTIBODY INTERACTIONS, Peter E. Wright, H. Jane Dyson, Richard A. Lerner, Lutz Riechmann and Pearl Tsang, Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037

With recent advances in methodology, it now appears that NMR can be used at an unprecedented level of sophistication to obtain new insights into the solution structure and dynamics of the antibody combining site, both free and in its complex with antigen. A problem of particular interest is the molecular basis for recognition by antipeptide antibodies. NMR studies of a variety of immunogenic peptides have revealed a marked tendency to adopt highly preferred conformations in aqueous solution. These observations have led us to suggest that there is a relationship between the ability of a peptide to adopt secondary structure in water solution and induction of antipeptide antibodies that cross react with elements of secondary structure in the folded protein. NMR can also be used to investigate the conformation and dynamics of peptides bound to the Fab fragment (molecular weight ~50 kDa) of antipeptide antibodies. Such experiments rely upon isotopic labeling of the peptide, primarily with ^{15}N, and use isotope-edited NMR techniques to selectively observe resonances of the bound peptide. Application of the tools of molecular biology now make it possible to generate Fv fragments of almost any desired antibody in a form suitable for detailed NMR studies. There now exist new opportunities to investigate the solution structure and dynamics of the antigen binding domain, both free and in its complexes with protein, peptide or other antigens.

CK 005 **THREE-DIMENSIONAL STRUCTURE DETERMINATION OF PEPTIDES AND PROTEINS BY NMR IN SOLUTION.** Kurt Wüthrich, Institut für Molekularbiologie und Biophysik, Eidgenössische Technische Hochschule-Hönggerberg, CH-8093 Zürich, Switzerland.
Compared to the data available on amino acid sequences, relatively few three-dimensional protein structures have been determined. Furthermore, until recently three-dimensional protein structure determination was limited to the crystalline state. This scarcity of experimental data on protein conformations is one of the principal bottle-necks in protein design and protein engineering, and the same situation is encountered in work with polypeptides of smaller size. The introduction of nuclear magnetic resonanc spectroscopy as a second technique for protein structure determination besides X-ray diffraction in single crystals (K. Wüthrich, NMR of Proteins and Nucleic Acids, Wiley, New York, 1986) promises to improve the situation both by providing complementary information to that contained in crystal structures and by the addition of new structures. In the first part of this lecture selected new solution structures will be described for several proteins which have so far not been crystallized. The second part will be devoted to an assessment of the quality of structure determinations by NMR, where important recent improvements resulted from stereospecific assignments of diastereotopic groups of protons.

Peptide and Protein Modelling

CK 006 CATALYTIC ANTIBODIES: PERSPECTIVES AND PROSPECTS, Donald Hilvert, Departments of Chemistry and Molecular Biology, Research Institute of Scripps Clinic, 10666 North Torrey Pines Road, La Jolla, CA 92037

Recent attention has focused on the mammalian immune system as a source of tailored catalysts with designed specificities and activities. Preparation of enzyme-like antibodies involves: (*i*) synthesizing compounds that mimic the transition state structure of a particular reaction, (*ii*) eliciting an immune response against such substances, and (*iii*) screening the resulting immunoglobulins for the desired activity. We have targeted concerted chemical reactions for catalysis by antibodies, because they are expected to be sensitive to induced strain and proximity considerations, and because they do not require the participation of specifically oriented catalytic groups within the binding pocket. Such processes are, moreover, of enormous practical and theoretical interest. Our progress in generating immunoglobulins that catalyze concerted reactions, particularly sigmatropic rearrangements and Diels-Alder cycloadditions, will be discussed. Special emphasis will be given to the use of chemical and molecular biological techniques to study and improve first-generation antibody catalysts.

CK 007 LESSONS FROM PEPTIDE SYNTHESIS: SYNTHESIS AND CONFORMATIONAL STUDIES OF THE AMYLOID-FORMING PROTEIN OF ALZHEIMER'S DISEASE, Peter T. Lansbury, Jr., Julia C. Hendrix, and Kurt J. Halverson, Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

The brains of Alzheimer's disease (AD) patients are characterized by the presence of insoluble proteinacious deposits known as amyloid plaque. The plaque is composed of a single, 42-amino acid protein , known as the β-protein. Small quantities of the plaque have been isolated from brain, however, the protein is extremely difficult to solubilize. X-ray diffraction studies of the plaque reveal the presence of cross-β fibrillar structure. We hope to elucidate the molecular details of fibril formation and to determine which portion(s) of the β-protein sequence initiate structure formation. In order to obtain material for conformational analysis, a total synthesis of the β-protein has been initiated. The chemical synthesis is being attacked using a solid-phase fragment-coupling strategy. The oxime resin developed by Tom Kaiser is being employed, both for the synthesis of the protected fragments and for assembly of these fragments. Details of these procedures will be discussed. In addition, conformational studies of synthetic fragments of the β-protein have provided clues as to the molecular nature of the unusual solubility properties of the β-protein. These studies will be discussed.

CK 008 SOLUTION NMR STUDIES OF A MODEL 4 HELIX BUNDLE PROTEIN, David Live[1], Robert C. Long, Jr.[1], William F. DeGrado[2], Tracy Handel[2], Jeffrey Hoch[3], John Osterhout[3]
[1]Department of Chemistry, Emory University, Atlanta, GA 30322, [2]E. I. DuPont, de Nemours & Co., Central Research and Development, Experimental Station, Bldg. 328, Wilmington, DE 19898, [3]Rowland Institute for Science, 100 Cambridge Parkway, Cambridge, MA 02142.

Using a rational strategy, a 16 amino acid peptide was designed to favor the formation of amphiphilic helicies such that the hydrophobic side chains could interdigitate with each other enabling the spontaneously formation a 4 helix bundle aggregate (1). The synthetic peptide was subjected to size exclusion chromatography and CD investigation which indicated the formation of a 4 helix bundle, and molecules synthesized with the four helix-preferring peptide segments linked covalently by loops gave comparable results. These data provide a global measure of the molecular conformation but not the conformational detail useful for further design refinement. In this study, we have applied NMR techniques to establish conformational details at atomic resolution of a bundle formed by 4 individual peptide molecules. Examination of NOE's, coupling constants, and amide exchange rates, has shown that a stable helix structure exists essentially along the full length of the peptide chain. Further, the 4 helicies are found to display the same conformation individually and to be related by symmetry, at least on the NMR time scale. Progress toward a complete description of the inter- and intrachain interactions will be reported.

1. Ho, S. P., DeGrado, W. F.; **J. Amer. Chem. Soc.**, 109 6751 (1987).

CK 009 DETERMINING THE FUNCTIONAL CONFORMATIONS OF BIOLOGICALLY ACTIVE PEPTIDES‡, John W. Taylor, George Ösapay and Marlène Bouvier, Laboratory of Bioorganic Chemistry and Biochemistry, The Rockefeller University, New York, NY 10021

Many biologically active peptides exist as multiconformational, disordered structures in aqueous solution. However, these peptides are expected to have highly ordered structures in their functional environment that can participate in specific recognition processes. Since it is not usually possible to investigate these structures directly, we are developing several indirect approaches to this problem. The first is the study of peptide behavior at interfaces, by which we hope to identify surface-induced conformations in peptide hormones that might resemble their functional conformations at cell-surface receptors. Idealized model amphiphilic peptides have been used to define characteristic compression isotherms for monolayers of α-helical, β-sheet and disordered structures at the air-water interface, and CD spectra for these peptide films transferred onto siliconized quartz slides have also been obtained. Several peptide hormones with the potential to form amphiphilic α-helical structures, including β-endorphin, calcitonin, glucagon, neuropeptide Y and parathyroid hormone 1-38, behave as helical peptides at these interfaces. In contrast, dynorphin A(1-17) binds to the air-water interface and siliconized slides in a predominantly disordered conformation, and LH-RH does not bind well to either interface. Another approach, developed by Kaiser, Kézdy and coworkers[1], involves the design and study of peptide analogues incorporating minimally homologous models of hypothesized structural domains. This method has been used to define the general structural requirements of β-endorphin and dynorphin A(1-17) that are sufficient and, in some cases, necessary to reproduce the contrasting selectivities of these hormones for μ-, δ- and κ-opioid receptors. The third approach we are developing involves the synthesis of peptide analogues that have multiple conformational constraints. An oxime resin support is used for the solid-phase synthesis of fully protected, cyclic peptide fragments by a novel and efficient method. These are then condensed together to prepare the desired multicyclic analogues. The synthesis and initial studies of amphiphilic α-helical peptides with multiple amide bridges between side chains that are adjacent to one another on the helix surface will be described.

1. Kaiser, E. T. & Kézdy, F. J. Science 223, 249-255 (1984).

‡Dedicated to the memory of Emil Thomas Kaiser.

Pattern Recognition and Structure Prediction

CK 010 PATTERN RECOGNITION AND STRUCTURE PREDICTION, Fred E. Cohen, Bruce I. Cohen, Nathalie Colloch and Scott R. Presnell, Departments of Pharmaceutical Chemistry & Medicine University of California, San Francisco, CA 94143.

Protein sequences code for local structures and the global assembly of these structures to form a unique tertiary structure. Through a systematic study of proteins which are members of well defined classes [all α -helical (α/α), all β -sheet (β/β) and alternating α helix and β strand (α/β)] potential sequence-structure correlates are identified. These features include loops, the hydrophobic core of helices, and the N and C terminal caps of α -helices and β -strands. A computer program (match-point) was created to facilitate the expression and evaluation of general sequence patterns. A collection of all helical proteins was divided into a training set for algorithm development and a test set which was sequestered during the development phase. Helical structure can be recognized with 87% accuracy in the training set and 82% accuracy in the test set. Loops are located with 89% and 84% accuracy respectively. N and C terminal capping patterns are more problematic. Our ability to predict protein tertiary structure solely from amino acid sequence will be discussed.

CK 011 PREDICTION OF PROTEIN INTERACTION SITES, Thomas P. Hopp,
Protein Research Laboratories, 4411 53rd Avenue SW, Seattle, WA 98116

The hydrophilicity plotting method of Hopp and Woods was the first of the so called hydropathy plotting procedures to use a full scale of amino acid values in determining the local hydrophilic/hydrophobic nature of polypeptide chains. It remains the most generally useful of these procedures, and can be used to determine likely sites of interaction of proteins with each other, with DNA and RNA, and with membranes. When used properly, it can also give information regarding the extent of α helical and β stranded secondary structures (reviewed in reference 1).

Recently, the method has been shown to be useful also in *de novo* protein/peptide design. An extremely hydrophilic peptide segment was created by chemical synthesis and used to raise a monoclonal antibody. Because of its hydrophilic nature, the peptide could be appended to a variety of recombinant proteins without causing unwanted folding or denaturing effects. Thereafter, the antibody was used for immunodetection and purification of the native peptide-protein conjugates. Finally, due to its surface exposed position, the peptide segment could be removed easily by limited proteolysis. This has led to the establishment of a universal detection and purification process for such "flagged" proteins.

The FLAG™ system is one of the first useful products to be made via *de novo* protein design, and represents a marriage between chemical peptide synthesis and recombinant protein production.

1. Hopp, Thomas P. (1986) Protein surface analysis: Methods for identifying antigenic determinants and other interaction sites. J. Immunol. Methods 88, 1-18.

CK 012 THE IDENTIFICATION OF PROTEIN FUNCTION FROM PRIMARY SEQUENCE INFORMATION, Temple F. Smith, Molecular Biology Computer Research Resource at Dana Farber Cancer Institute and Harvard School of Public Health, 44 Binney Street, Boston, MA. 02115.

The idea that a common enzymatic function within different proteins is generally encoded by a similar structure and uses the same biochemistry arose from our understanding of the evolutionary relationships among protein families which carry out similar functions. It is not surprising.therefore, that one is able to associate a sets of conserved primary sequence elements with given protein functions. A number of techniques have been developed to identify such sets of conserved primary sequence elements. Nearly all involve the alignment of some initial set of "related" sequences from which an initial pattern regular-expression, profile weight-matrix, consensus sequence or complex hierarchical descriptor is generated. These can then be measured for and refined by their diagnostic ability --their sensitivity and specificity-- on appropriate database control sets. The pattern construction methods have many properties in common and can be shown to reduce to one another in certain limiting cases. They have proved useful in identifying the probable function (and structure) of many newly sequenced genes. Their utility may prove to be even more general, and allow the accurate prediction of some protein substructures and subfunctions not arising from a common inheritance but from common biochemical and structural constraints. These patterns, thus appear to represent a complex mix of history and physical chemistry which we are just beginning to understand.

Immune Modulatory Peptides

CK 013 ANTIBACTERIAL PEPTIDES FROM PIG INTESTINE. ISOLATION OF A MAMMALIAN CECROPIN[1], Hans G Boman*, Jong-Youn Lee*, Anita Boman*, Sun Chuanxin*, Mats Andersson†, Hans Jörnvall§ and Viktor Mutt†, *Department of Microbiology, University of Stockholm S-106 91 Stockholm, Sweden, †Department of Biochemistry II and §Department of Chemistry I, Karolinska Institutet, Box 60400, S-104 01 Stockholm, Sweden. Pig small intestine was used as starting material for a batch-wise isolation of a peptide fraction enriched in antibacterial activities against *Escherichia coli* (anti-E.coli factor) and against *Bacillus megaterium* (anti-Bm factor). Separation and further purification was by different types of chromatography. Sequence analysis showed the anti-Bm factor to be apparently similar to vasoactive intestinal peptide (VIP). The anti-E. coli factor was found to have a 31 residue sequence that was cecropin-like. It was named cecropin P1 and its structure was confirmed by solid phase synthesis. Synthetic cecropin P1 with and without C-terminal amide was assayed on 8 different bacteria. The results indicate that cecropin P1 could be useful as a theraputic agent. Mobility comparison between synthetic and natural cecropin P1 indicate that the natural peptide has a free C-terminal carboxyl group. The molecular cloning of cecropin P1 is in progress.

1) In press in Proc. Natl. Acad. Sci. USA, Dec. 1989.

CK 014 REGULATION OF LYMPHOCYTE ACTIVATION BY THE CONSTANT REGION OF IMMUNOGLOBULIN, Edward L. Morgan Department of Immunology, Research Institute of Scripps Clinic & Division of Cellular Immunology, Immunetech Pharmaceuticals, San Diego, CA 92121

The biological activities associated with antibody can be categorized as primary or secondary functions. Complexing of antibody with antigen through the Fab portion of the antibody molecule constitutes the primary function. Formation of Ag-Ab complexes also results in the appearance of secondary functions not associated with the free antibody molecule. These secondary effector functions include; control of antibody catabolism, complement fixation, Fc receptor binding, anaphylaxis, opsonization, placental and gut transfer, mediator release, and immunoregulation. Many of these activities are associated with the Fc region of the antibody molecule.

Proteolytic cleavage of Ig has been long used as a method for the localization and characterization of biologically active regions within the Ig molecule. Enzymes such as trypsin, papain, pepsin, elastase, and plasmin have been used to cleave the Fc region of Ig into various size fragments which exhibit different biological activities. In addition, biologically active fragments and peptides can be derived from cellular and parasitic enzymatic digestion of Ig .

The bifunctional regulatory effects of Fc fragments on the cellular and molecular events of lymphocyte and monocyte/macrophage activation suggest a participation of the Fc region of antibody in Ab-Ag-mediated immunoregulation. The fact that Fc region fragments and peptides and Ag-Ab appear to activate mononuclear cells by similar mechanisms also suggests a common immunoregulatory pathway. Thus the release of biologically active peptides derived from the Fc region of antibody may form part of a nonspecific immunoregulatory network active in normal and disease states.

CK 015 INTERACTIONS BETWEEN MHC MOLECULES AND IMMUNOGENIC PEPTIDES.

Jonathan B. Rothbard, C. Mark Hill, Julian Hickling, and Robert Busch. ImmuLogic Pharmaceutical Corporation, 855 California Avenue, Palo Alto 94304.

The identification and sequencing of the antigen receptor of T cells (1,2) coupled with the demonstration that MHC proteins specifically bind immunogenic peptides(3), and the solution of the crystal structure of a MHC class I molecule (4,) collectively have led to a working model of how T cells recognize protein antigens. This unique recognition mechanism has evolved to allow receptors on two separate cells to contact a common peptide ligand. As valuable as these experimental results have been for increasing our understanding of T cell recognition, they also have raised several different, but equally perplexing questions. The principal issue our laboratory has concentrated on is how can a single, conserved MHC antigen combining site specifically recognize a very large number of diverse peptides?

We have studied the molecular interactions between immunogenic peptides and HLA DR antigens by analyzing the ability of peptide analogues to bind class II molecules on the surface of cells. Complexes formed between MHC class II molecules and biotinylated peptides were detected using fluorescently labeled avidin and flow cytometry. The rapid, simple, and quantitative binding assay has been used to show that immunogenic peptides can bind many alleles of both MHC class I and II proteins. This interesting degeneracy in binding will be discussed in the context of the MHC antigen combining site.

In addition, information on the conformation of the bound peptide and identification of the critical peptide residues in the formation of the complex can be determined by substituting a biotinylated lysine at each position in the sequence. When this set of peptides was incubated with cells, marked differences in the resultant fluorescent signal were present. The variations in fluorescence appeared to have arisen from the differential capacity of the DR molecules to bind antigen, reflecting the effect of biotinylation at each position had on the affinity of the interaction. The resultant fluorescent profile was consistent with the central core of the peptide adopting a helical conformation with the terminal residues less constrained. Comparison with the profiles generated by other T cell determinants revealed that such a conformation was common. However, interesting differences were found when the same determinant bound to closely related DR alleles. The variations in binding appeared to be important in T cell recognition. Details of these differences will be discussed.

1. Haskins, K., J. Kappler, P. Marrack. 1983. J. Exp. Med. 157:1149.
2. Hedrick, S, E. Nielsen, J. Kavaler, D. Cohen, and M. Davis. 1984. Nature 308:153.
3. Babbitt, B., P.M. Allen, G. Matsueda, E. Haber, and E. Unanue. 1985. Nature 317:359.
4. Bjorkman, P., M. Saper, B. Samraoui, W. Bennett, J. Strominger, and D. Wiley. 1987. Nature 329: 506.

CK 016 CD8 AS AN IMMUNOMODULATORY PEPTIDE, Mark L. Tykocinski and David R. Kaplan, Institute of Pathology, Case Western Reserve University, Cleveland, OH 44106

CD8 is a cell surface-associated glycoprotein that is expressed by subsets of thymic and peripheral T lymphocytes. This molecule exists in both homodimeric α/α and heterodimeric α/β forms. Our early studies, predicated upon the linking of antisense transfection and human T cell cloning technologies, confirmed the notion that CD8 functions as a receptor/adhesin in an accessory molecular role for the activation of cytotoxic T-cells. More recent studies, using a variety of antisense and sense CD8 transfectants, have established a second function for CD8 whereby it acts as an inhibitory ligand to mediate T-cell immunoregulation. These studies have entailed the use of mixed cell cocultures to monitor the effects of CD8-positivity of the cells added to the system. One line of investigation has shown that irradiated CD8- cells, but not CD8+ phenocopies, can serve as antigen-specific stimulators of proliferation and the generation of cytotoxicity. Another line of investigation has demonstrated that irradiated CD8+ cells, but not CD8- phenocopies, can inhibit antigen-specific responders, in a "veto-like" fashion. Glycoinositolphospholipid-modified CD8, lacking the native CD8 hydrophobic transmembrane domain and cytoplasmic extension, retains the inhibitory capacity of the native CD8 molecule. These novel findings have provided significant molecular insights into the immunoregulation mediated by CD8-positive T-cells. Current efforts are directed at exploiting CD8's pharmaceutical potential as an immunomodulatory peptide. Moreover, the multifunctional perspective for CD8 has prompted a reassessment of the functional repertoire of other lymphoid cell surface-associated molecules.

Peptides Related to AIDS

CK 017 THE DESIGN AND SYNTHESIS OF HIV PROTEASE INHIBITORS, J. R. Huff, P. S. Anderson, S. F. Britcher, P. Darke, S. J. deSolms, R. A. F. Dixon, E. Emini, J. M. Hoffman, T. A. Lyle, W. C. Randall, C. S. Rooney, I. S. Sigal, C. S. Sweet, W. J. Thompson, J. P. Vacca, S. D. Young, Merck Sharp and Dohme Research Laboratories, West Point, PA 19486.
The gene products of human immunodeficiency virus (HIV) are expressed as polyproteins which undergo post-translational cleavage to produce mature viral proteins. A virus-encoded protease is capable of cleaving the gag and pol gene products to produce, respectively, structural proteins p24, p17, p15 and reverse transcriptase and integrase. The HIV protease has been characterized biochemically and structurally as a member of the aspartyl protease family. Inactivation of this protease results in the production of non-infectious virons and consequent inhibition of the spread of viral infection in susceptible cells, making the enzyme an attractive therapeutic target. Dipeptide isosteres of the enzymatic reaction's presumed transition state have served as the basis for the design and synthesis of a series of potent HIV protease inhibitors.

CK 018 STRUCTURE OF A COMPLEX OF SYNTHETIC HIV-1 PROTEASE WITH A SUBSTRATE-BASED INHIBITOR AT 2.3 Å RESOLUTION, Alexander Wlodawer, Maria Miller, Bangalore K. Sathyanarayana, Crystallography Laboratory, NCI-Frederick Cancer Research Facility, BRI-Basic Research Program, P.O. Box B, Frederick, MD 21701; Mihaly V. Toth, Garland R. Marshall, Department of Pharmacology, Washington University School of Medicine, St. Louis, MO 63110; Leigh Clawson, Linda Selk, Jens Schneider, Stephen B.H. Kent, Division of Biology, 147-75, California Institute of Technology, Pasadena, CA 91125
A peptide inhibitor with the sequence N-acetyl-Thr-Ile-Nle-Ψ[CH_2-NH]-Nle-Gln-Arg.amide, with a reduced peptide bond between the two adjacent norleucine residues, has been cocrystallized with chemically synthesized HIV-1 protease. The structure has been refined to an *R*-factor of 0.176 at 2.3 Å resolution and has provided a clear picture of the interactions between the inhibitor and the enzyme. Despite the symmetric nature of the unliganded enzyme, the asymmetric inhibitor lies in a single orientation, and makes extensive interactions at the interface between the two subunits of the homodimeric protein. Compared to the unliganded enzyme, the protein molecule has undergone substantial changes, particularly in an extended region corresponding to the "flaps" (residues 35-57 in each chain), where backbone movements as large as 7 Å are observed.

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Growth Factors and Lymphokines

CK 019 STRUCTURE-FUNCTION RELATIONSHIPS IN BASIC FIBROBLAST GROWTH FACTOR, William F. Heath, Amanda S. Cantrell, and S.Richard Jaskunas, Lilly Research Laboratories, Indianapolis, IN 46285

Basic fibroblast growth factor is a member of a family of heparin-binding polypeptide growth factors which have been shown to be mitogens for a wide variety of cells of mesodermal and neuroectodermal origin. In addition, basic FGF has been shown to be a potent stimulator of angiogenesis in vivo and to stimulate collagenase and plasminogen activator secretion, chemotaxis and mitogenesis in capillary endothelial cells in vitro. Interaction with sulfated glycosaminoglycans such as heparin sulfate has been shown to be important to the biological activity of fibroblast growth factor. Eleven structural analogs of human basic fibroblast growth factor (bFGF) have been prepared by site-directed mutagenesis of a synthetic bFGF gene to examine the effect of amino acid substitutions in the heparin-binding domains on biological activity. After expression in *Escherichia coli*, the mutant proteins were purified to homogeneity using Heparin-Sepharose chromatography and analyzed for their ability to stimulate DNA synthesis in human foreskin fibroblasts. The results of these experiments will be presented and discussed in the context of a rational approach to protein analog design.

CK 020 AMPHIREGULIN: A NEW MEMBER OF THE EGF/TGF-α FAMILY OF GROWTH FACTORS, GD Plowman, M Shoyab, and GJ Todaro, Oncogen, Seattle, WA 98121

Amphiregulin (AR) is a novel growth regulatory peptide that exhibits structural homology with epidermal growth factor (EGF), transforming growth factor alpha (TGF-α), and related molecules. The name amphiregulin reflects the bifunctional nature of the regulatory effects of AR: it stimulates the growth of normal fibroblasts and keratinocytes but inhibits many human tumor cells. AR was initially isolated from the human breast carcinoma cell line, MCF-7, after treatment with the phorbol ester TPA.

Molecular cloning and sequence analysis have been completed for AR, and recombinant protein has been expressed in mammalian cells. Alignment of the AR sequence with sequences of other members of the EGF/TGF-α family shows that AR contains the conserved spacing of the 6 essential cysteine residues, as well as some other characteristic amino acids. The EGF-like domain of AR shows 38% homology to EGF and 32% homology to TGF-α. In addition to this region, however, AR possesses a unique 43-amino acid N-terminal extension that is extremely hydrophilic, containing high numbers of lysine, arginine, and asparagine residues. Also contained in this region are nuclear targeting motifs that enable AR to interact directly with DNA. Like other members of the EGF/TGF-α family, AR is synthesized as a larger precursor protein containing a potential membrane-spanning domain. The position of AR's transmembrane region, relative to the mature growth factor, is similar to that seen with EGF, TGF-α, and vaccinia growth factor (VGF). Immunostaining studies of proliferating breast carcinoma cells showed that the membrane-bound AR precursor was localized at the proliferating edge of the cell mass.

In the growth of murine keratinocytes, AR can supplant the requirement for TGF-α and/or EGF. The effect of AR on the growth of tumor cells, however, is quite different from that of TGF-α. Published studies have suggested that the coexpression of TGF-α and the EGF receptor provides a selective growth advantage to tumor cells. In contrast, AR dramatically inhibits the growth of two human breast cancer cell lines that express high levels of both TGF-α and the EGF receptor. Amphiregulin is capable of binding to the EGF/TGF-α receptor, but that binding is of lower affinity and only partially competes with the other ligands.

Amphiregulin is constitutively expressed in some normal adult tissues, and may thus play a role in normal growth regulation. The elucidation of its normal activity, which could potentially occur via binding directly to DNA as well as to receptors on the membrane, could lead to the development of AR as an anti-neoplastic therapeutic agent.

CK 021 BIOLOGICALLY ACTIVE PEPTIDES FROM LAMININ Yoshihiko Yamada, Kenichiro Tashiro, Gregory Sephel, David Greatorex, Norio Shiraishi, George R. Martin, and Hynda K. Kleinman. Laboratory of Developmental Biology and Anomalies, National Institute of Dental Research, NIH, Bethesda, MD 20892.

Laminin (Mr=800,000) is the major glycoprotein of basement membranes, the thin extracellular matrix which underlies epithelial cells and surrounds nerve, muscle, and fat cells. The predominant form of laminin consists of three chains, A (Mr=400,000), B1 (Mr=210,000), and B2 (Mr=200,000) which have been cloned and sequenced. Laminin has diverse biological activities including stimulating cell adhesion, migration, growth, and differentiation as well as neurite outgrowth and collagenase IV production.

Using synthetic peptides, we have identified several active sites of laminin. These include a site of five amino acids, YIGSR on the B1 chain, an RGD containing site on the short arm of the A chain and an IKVAV site on the end of the long arm of the A chain. While all these sites are active for adhesion of a variety of cell types, the IKVAV containing site is unique in stimulating neurite outgrowth of PC12 cells, and septal and cerebellar neurons, and in increasing collagenase IV production of tumor cells. More recently, we have identified another site near the IKVAV sequence which is active for neurite outgrowth, heparin binding, and in stimulating neuronal cell growth. In addition, several sites on the carboxyl terminal globular domain of the A chain have also been identified as active for cell adhesion, neurite outgrowth, and heparin binding. It is likely that these active sites on laminin interact with different cell surface receptors. Distinct regions of laminin, utilizing different receptors on cells may be acting sequentially, and/or in concert during development and repair.

Hormones and Neuropeptides

CK 022 NOVEL, NATURAL PRODUCT-BASED LIGANDS FOR PEPTIDE RECEPTORS, R. M. Freidinger,[a] B. E. Evans,[a] M. G. Bock,[a] K. E. Rittle,[a] R. M. DiPardo,[a] W. L. Whitter,[b] G. F. Lundell,[a] D. F. Veber,[a] R. S. L. Chang,[b] V. J. Lotti,[b] D. J. Pettibone,[b] B. V. Clineschmidt[b] Departments of Medicinal Chemistry[a] and New Lead Pharmacology,[b] Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486.

Potent, orally active agents of long duration that interact competitively at peptide receptors are needed for elucidating the roles of peptide hormones and neurotransmitters. Such compounds are also important for developing the therapeutic potential of these peptides. In recent years, advances have been made in the design of protease stable peptide analogs of improved duration, but the goal of good oral bioavailability remains difficult to achieve. Solutions to this problem have now been developed for the cases of cholecystokinin (CCK) and gastrin with the design of nonpeptide antagonists based on the natural product asperlicin.[1] Two classes of these 3-substituted-5-phenyl-1,4-benzodiazepines having different receptor subtype specificities have been designed. MK-329 exhibits nanomolar potency, greater than one thousand fold selectivity for the CCK-A (peripheral) receptor, and good oral activity.[2,3] On the other hand, L-365,260 binds selectively to the CCK-B (brain) receptor and the peripheral gastrin receptor.[4,5] A key element in these studies was the recognition of the utility of substituted benzodiazepines as ligands for peptide receptors. The structural features important for CCK-A or CCK-B/gastrin selectivity, syntheses of key compounds, and certain pharmacological properties of these antagonists will be discussed. Progress is also being made in the area of oxytocin antagonists with the discovery of the novel fungal-based cyclic hexapeptide L-156,373.[6] Synthetic modifications leading to the nanomolar potency analog L-365,209 will be described.

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CK 023 **NEUROPEPTIDE Y: TEST OF A STRUCTURAL MODEL**

John L. Krstenansky, Larry R. McLean and Stephen H. Buck
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The structure of neuropeptide Y (NPY) and its interaction with lipid were examined by means of a series of NPY analogs, devoid of certain central residues and constrained by a disulfide bridge. NPY has been proposed to be an intramolecularly stabilized structure shaped much like a hairpin with an N-terminal polyproline helix opposing an amphipathic α-helix. For the postsynaptic receptor subtype, of which the mouse brain receptor is an example, both the N- and C-termini of the peptide are important for binding potency. Removal of residues in the turn portion of the "hairpin" model of NPY with a spacer residue (Aoc = 8-aminooctanoic acid) and a disulfide linkage of the N- and C-terminal helices leads to an analog ([D-Cys^{7},Aoc$^{8-17}$,Cys^{20}]-NPY = C7-NPY) having full binding potency at the mouse brain receptor. The interaction of C7-NPY with lipid is substantially different from that of NPY which suggests that the receptor binding potency of NPY does not involve lipid interaction. This is of interest because lipid interaction is considered to be important for many peptides which incorporate amphipathic α-helices (e.g. glucagon and endorphin) and NPY is highly disruptive of lipid structure. NPY may have actions in addition to its receptor binding where lipid binding may play a role.

CK 024 The Conformational Restriction of Synthetic Peptides with Covalent Hydrogen Bond Mimics: The Reverse Turn and Alpha Helix. Arnold C. Satterthwait, Lin-Chang Chiang, Edelmira Cabezas, Thomas Arrhenius and Richard A. Lerner, Department of Molecular Biology, The Research Institute of Scripps Clinic, La Jolla, CA 92037

Biologically active peptides identify critical regions of proteins and provide targets for drug and vaccine design. It is likely that new activities can be identified and others enhanced through the process of conformational restriction. Two fundamental problems require solutions before this thesis can be fully tested. First, it will be necessary to translate primary amino acid sequence information into secondary structures and second, synthetic methods must be developed for restricting peptide conformations to these structures for biological testing.

Whereas algorithms for predicting secondary structure have accuracies of >50% and can be expected to improve, a general strategy for shaping peptides into diverse structures is needed. One approach may emerge from a search for covalent hydrogen bond mimics. On average, every other amino acid in a protein engages in an amide-amide hydrogen bond (NH··O=C(R)NH) and different hydrogen bonding patterns define different secondary structures. The substitution of putative hydrogen bonds with a covalent mimic could serve as a general method for conformationally restricting peptides.

Syntheses have been developed for substituting an amidinium link (N-C(R)=NH-CH_2-CH_2) and a hydrazone link (N-N=CH-CH_2-CH_2) for structure defining amide-amide hydrogen bonds in selected peptides. The centerpiece of each mimic is a carbon-nitrogen double bond which replaces the putative hydrogen bond. It's been found that the amidinium link restricts peptides to the Type 1 reverse turn, while the hydrazone link can substitute for the (i, i + 4) hydrogen bond in alpha helices. These results establish that covalent hydrogen bond mimics can substitute for the hydrogen bond and conformationally restrict peptides to common secondary structures.

CK 025 BRADYKININ ANTAGONISTS: DESIGN AND APPLICATIONS. John M. Stewart and Raymond J. Vavrek, Department of Biochemistry, University of Colorado School of Medicine, Denver, CO 80262

The kinin peptides bradykinin (BK: Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) and its homolog kallidin (Lys-BK) are involved in regulation of every major physiological system in the body. Overproduction of BK or kallidin characterizes many kinds of pathology including pain, rhinitis, lung disease (asthma, adult respiratory distress syndrome) and most inflammation, including joint disease (arthritis), inflammatory bowel disease, septic shock and inflammation subsequent to trauma. Development of potent, specific antagonists of BK has made possible new understanding of fundamental physiology and offers the potential for development of new kinds of anti-inflammmatory drugs. Kinin effects are mediated by several classes of receptors, most important being the B-2 receptors, which can be coupled to all known second messenger systems in various tissues. B-1 receptors recognize BK(1-8) and are present particularly in damaged smooth muscle. BK B-2 antagonists are obtained by replacing the 7-Pro residue with a D-aromatic amino acid, such as Phe. Potency and resistance to enzymatic degradation are increased by making further changes in the peptide. Many details of normal physiology and of pathology are being elucidated by use of BK antagonists, and one antagonist is in human trial for rhinitis, pain and asthma. (Aided by NIH grant HL-26284)

Peptide Structure

CK 100 THE EFFECT OF COVALENTLY-LINKED OLIGOSACCHARIDE ON PEPTIDE CONFORMATION, Shimon T. Anisfeld and Peter T. Lansbury, Jr., Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139

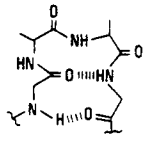
Various roles have been ascribed to the oligosaccharide portion of glycoproteins, but few of these roles are fully understood. We are interested in exploring the possibility that the oligosaccharide may influence the conformation of the protein chain, thus playing a role in protein folding. The oligosaccharide may stabilize certain units of secondary structure--*e.g.*, it may stabilize an amphiphilic α-helix by hydrophobic interactions--thus permitting the formation of stretches of secondary structure which can serve as intermediates in folding. We are synthesizing peptide models which have the potential to form amphiphilic α-helices and attaching oligosaccharides to them by an N-glycosyl linkage. Structural studies (by CD and NMR) of the peptides with and without attached oligosaccharide will enable the determination of the effect of the oligosaccharide--including the pinpointing of specific interactions between the sugar and the peptide.

CK 101 SYNTHESIS AND SOLUTION CONFORMATION OF PEPTIDES CONTAINING TWO DEHYDROPHENYLALANINES: MODEL HELICAL PEPTIDES, Virender S. Chauhan, Ashima Bhardwaj & Alka Gupta, International Centre for Genetic Engineering & Biotechnology, NII Campus, Shaheed Jeet Singh Marg, New Delhi - 110 067, INDIA.

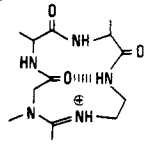
We have earlier shown using NMR and X-ray differaction that dehydrophenylalanine and dehydroleucine residues induce a β-turn structure and are easily accommodated at (i+2)th position in model peptides. We have synthesised peptides containing more than one dehydrophenylalanine and now present their solution conformations using ^{1}HNMR. Studies of NH group solvent accessibility in pentapeptide (Boc-G-ΔF-L-ΔF-A-NH-CH_3) and heptapeptide (Boc-G-ΔF-A-L-ΔF-A-$NHCH_3$) in $CHCl_3$ showed that 4NH groups in the pentapeptide and 5 NH groups in the heptapeptide respectively, from the C-terminal end, are involved in intramolecular hydrogen bonds. Observation of sequential intraresidue $N_iH \leftrightarrow N_{i+1}H$ nuclear Overhauser effects suggest a major population of folded helical conformations. However, in highly polar solvent, $(CD_3)_2SO$, some $C_i^{\alpha}H \leftrightarrow N_{i+1}H$ were also observed indicating the presence of a population of partially extended conformers. These results indicate that two dehydrophenylalanines in both the arrangements, favor a helical conformation in solution. Such arrangements may be used in designing peptides with favored secondary structures.

CK 102 THE CONFORMATIONAL RESTRICTION OF PEPTIDES TO THE TYPE I REVERSE TURN WITH THE AMIDINIUM COVALENT HYDROGEN BOND MIMIC, Lin-Chang Chiang, Richard A. Lerner and Arnold C. Satterthwait, Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037

Covalent hydrogen bond mimics have a potential for serving as a general means for the conformation restriction of peptides. Perhaps the most common secondary structure found on protein surfaces is the Type 1 reverse turn. An amidinium link (N-C(R)=NH(+)-CH_2-CH_2) has been substituted for the penultimate amide-amide hydrogen bond (N-H•••O=CR-NH) in small loops and folds the corner amino acids into an ideal Type 1 reverse turn enforcing an (i, i + 3) hydrogen bond (A). Each of the amino acid residues in the loop have been substituted with L-alanine and glycine. Conformational analyses of these loops in dimethylsulfoxide and water with nuclear magnetic resonance experiments indicate little change in structure implying that they are rigid.



Loop



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CK 103 STRUCTURAL STUDIES OF PEPTIDES DERIVED FROM ANTITHROMBIN III; A MODEL FOR THE HEPARIN-ANTITHROMBIN III INTERACTION, Annemarie I. Coffman and Peter T. Lansbury Jr., Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139. The inhibitory function of Antithrombin III (ATIII), a serine protease inhibitor found in the blood clotting cascade, is attenuated by the binding of heparin, a polysulfated proteoglycan. Spectroscopic studies have shown that heparin binding induces a conformational change in ATIII which includes the stabilization of secondary structure. In order to test the idea that heparin stabilizes structure by neutralizing charged residues on ATIII, peptides representing putative heparin binding sequences in ATIII have been synthesized. Circular dichroism spectroscopy is being employed to determine the effects of heparin, high pH, and other charge-neutralizing environments on the structure of these synthetic peptides.

CK 104 CONFORMATIONS OF MACROMOLECULES IN RESTRICTED SPACES David G. Covell, & Robert L. Jernigan, ASCL/PRI/FCRF, Frederick, MD 21701, NCI/DCDB/LMMB, Bethesda, MD 20892. We present a new method to examine the complete range of folded topologies accessible in the compact state of globular proteins. The procedure is to generate all conformations, with volume exclusion, upon a lattice in a space restricted to the individual protein's known compact conformational space. Using one lattice point per residue we find 10^2 to 10^4 possible compact conformations for the five small globular proteins studied (avian pancreatic polypeptide, 36aa, crambin, 46aa, rubredoxin, 52aa, ferredoxin, 54aa and neurotoxin, 62aa). Subsequently, these conformations are evaluated in terms of residue-specific, pairwise contact energies that favor non-bonded, hydrophobic interactions. Native structures for the five proteins are always found within the best 2% of all conformers generated. This novel method is simple and general and can be used to determine most favorable overall packing arrangements for the folding of specific amino acid sequences within a restricted space.

CK 105 PROTON NMR STUDIES OF PEPTIDE FRAGMENTS OF TWO PROTEINS, H. Jane Dyson, Jonathan P. Waltho, James R. Sayre, Richard A. Lerner and Peter E. Wright, Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037
A considerable body of experimental work now indicates that proton NMR methods are capable of detecting significant populations of peptide molecules in aqueous solution which have conformational preferences for other than extended-chain structures. This has led to renewed interest in the study of short synthetic peptides as model systems for very early stages of protein folding. We have recently completed an NMR study of sets of peptide fragments with sequences derived from two proteins, plastocyanin and myohemerythrin. Plastocyanin consists of a Greek-key β-barrel; myohemerythrin is a four-helix bundle protein. Both proteins contain metal ions. The peptide fragments of the two proteins exhibit very different characteristics in aqueous solution. In general, the plastocyanin peptides show very little tendency to form anything but extended-chain structures, even in regions which contain loops and turns. By contrast, the myohemerythrin peptides show a tendency towards helical and nascent-helical structures in solution. These results suggest that the initiation of folding in these two protein types may be significantly different.

CK 106 REPEAT PEPTIDE MOTIFS WHICH CONTAIN REVERSE TURNS AND MODULATE DNA CONDENSATION IN CHROMATIN, Monique Erard, Faouzi Lakhdar-Ghazal, Antoine Zwick* and François Amalric, Centre de Recherche de Biochimie et de Génétique Cellulaires du C.N.R.S. et *Laboratoire de Physique des Solides, Université Paul Sabatier, 118 route de Narbonne, 31062 Toulouse, Cédex France. The amino-acid sequence of the N-terminal third of nucleolin, a major nucleolar protein involved in the control of ribosomal RNA sysnthesis, presents the characteristic bipolar pattern of an HMG-like domain, comprising lengthy acidic stretches interspersed with basic lysine residues. The disposition of lysine residues in nucleolin within a short repeated sequence is reminiscent of that found in the histone H1 C-terminal domain. This rather intriguing homology between the basic repeats of these two proteins has prompted us to make the synthesis of two peptides (ATPAKKAA)2 and (KTPKKAKKP)2, corresponding to nucleolin and histone H1 respectively, in order to undertake a comparative study of their structure and their mode of interaction with DNA. By using circular dichroism in conjunction with Fourier-transform infrared and Raman spectroscopies, we demonstrate that the binding of nucleolin repeat to DNA does not significantly alter its conformation ant that, on the reverse, H1 repeat induces a very marked DNA condensation giving rise to a Ψ^-type CD spectrum. The highly conserved H1 repeat performs that function by adopting a more rigid reverse-turn containing structure which binds to DNA minor groove.

CK 107 **De Novo Design and Structure of Zinc-Binding Four-Helix Bundle Proteins:** Tracy M. Handel and William F. DeGrado. Central Research and Development Department; E. I. DuPont de Nemours; Wilmington, DE, 19880-0328. In previous studies, NMR spectroscopy has been used to probe the conformation and dynamics of a 16-residue α-helical peptide, A_1B ($AcGELEELLKKLKELLKGNH_2$), that self-associates into a four-helix bundle. In particular, it was possible to define the secondary structure of the individual helices in the peptide using proton-exchange and 2-D experiments (W. Degrado, T. Handel, J. Hooch, D. Live, J. Osterhout, D. Weaver). However, because of the symmetry of the bundle and the degeneracy of the amino acid sequence, it is not possible to establish the orientation of the helices with respect to each other based on cross peaks observed in NOESY spectra. In this work we describe an analogue of the original peptide with Val and Phe at positions 3 and 13, respectively. Noe's between the Val and Phe protons of the "mutant" indicate that the helices have an antiparallel orientation in the tetramer, consistent with the original design. Additional inter-helical distances will be obtained by selective deuteration of the 4 Leu's in order to establish the 3-dimensional structure of the bundle. Similar studies on the full-length linked four-helix bundle are also in progress.

Assuming the correctness of the model for the four-helix bundle, we have also synthesized linked α-helical pairs containing Zn^{+2} coordination sites modelled after those found in carbonic anhydrase and carboxy peptidase. The sites contain 3 His residues, 2 His and 1 Glu, or 2 His and 1 Asp residue, with the ligating atoms occupying three positions of a tetrahedron. Preliminary CD studies indicate that the helicity and thermodynamic stability of the peptides are increased in the presence of zinc.

CK 108 SYNTHETIC AND CONFORMATIONAL STUDIES OF THE AMYLOID-β PROTEIN OF ALZHEIMERS DISEASE, Julia C. Hendrix, Peter T. Lansbury, Jr., and Kurt J. Halverson, Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139. Thr brains of persons afflicted with Alzheimer's disease are characterized by the presence of proteinacious aggregates called amyloid plaques. The major component of these aggregates is a 42 amino acid protein known as the amyloid-β protein, the sequence of which is shown below.

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

The total synthesis of this protein is in progress. A solid phase-fragment condensation strategy is being employed using the Kaiser oxime resin. Six fragments of the protein have been synthesized, purified, and deprotected. Conformational studies of several of these fragments using FTIR and x-ray diffraction indicate the presence of β-sheet structure of the C-terminus of the protein. The C-terminus portion of the protein also assumes a β-sheet structure while bound to the oxime resin. Other fragments do not assume this structure when bound to the resin. Solvent systems which disrupt the β-sheet structure have helped to overcome some of the difficulties in the synthesis.

CK 109 USE OF PEPTIDES AND PEPTIDE DERIVATIVES OF CD4 TO ASSESS THE RELATIVE CONTRIBUTIONS OF CDR-1, CDR-2 AND CDR-3 HOMOLOGY DOMAINS OF THE CD4 MOLECULE TO BINDING OF HIV ENVELOPE GLYCOPROTEIN AND HIV INFECTIVITY IN VITRO, Kou M. Hwang, Dianne M. Rausch, Mary Padgett, Vaniambadi S. Kalyanaraman, Peter L. Nara, Lee E. Eiden and Jeffrey D. Lifson, Genelabs, Inc., Redwood City, CA 94063 and LCB, NIMH, Bethesda, MD 20892.
The human cell surface antigen CD4 is an adhesion molecule that functions in T-cell recognition of antigen-presenting cells and as the T-cell and macrophage receptor for human immunodeficiency virus (HIV). This region has homology to the immunoglobulin light chain, including its three complementarity-determining (antigen-binding) regions CDR-1, 2 and 3. In vitro mutagenesis experiments have demonstrated that the N-terminal approximately 100 amino acids of CD4 function as the HIV binding domain of the molecule and that the region CD4(43-55) of CD4 is important for establishing the correct conformation of CD4 for binding HIV or is itself part of the HIV binding domain of CD4. We have demonstrated using synthetic peptide fragments covering the entire CD4(1-120) sequence, as well as additional C-Terminal CD4 peptides, that 1) only conformationally restricted peptide analogs of the region CD4(81-92), which contains a portion of the CDR-3 homology domain, block HIV-1, HIV-2 and SIV infectivity and fusigenic activity, as well as CD4/gp120 direct binding, in vitro and 2) peptide analogs of the CDR-2 domain including CD4(48-50) fail to block CD4/gp120 binding, in vitro but do not inhibit the ability of CDR-2-directed antibodies (OKT4A, anti-Leu-3A) to block CD4/gp120 interaction. Together, in vitro mutagenesis, antibody blockade, and synthetic peptide studies suggest a model for HIV infection in which the envelope glycoprotein gp120 of HIV binds to the CD4 molecule at or near both the CDR-2 and CDR-3 domains. One of these sites may be the major CD4 binding site, with the other functioning as an accessory or epitope positioning domain. Binding of gp120 to CD4 presumably allows the HIV virion to closely approach the cell membrane of the T-lymphocyte, where it can fuse with the cell via the gp41 fusigenic domain, enabling viral entry and infection.

CK 110 SEQUENCES COMPARING OF HOMOLOGOUS PROTEINS WITH THEIR FUNCTIONAL INDEXES(SCHPFI) --- A NOVEL ALGORITHM FOR DESIGN OF SITE-DIRECTED MUTATION WITHOUT ANY INFORMATION FROM 3D-STRUCTURE OF PROTEIN, Jian-ning Liu, Xu-wei Wu, Lin Du, Department of Biochemistry, Nanjing University, Nanjing 210008, P.R. China
The Design for Site-Directed Mutation (SDM) was always depended on the basis of 3d-structure of the target protein. But many structures of preteins have not been determinded, and they have not any homologous structures to be modelled. Now we try to develop a novel algorithm to solve this problem, which is called "Sequences Comparing of Homologous proteins with their Functional Indexes (SCHPFI)". It could directly give a design of SDM based on the relationship between the changes of sequences and that of a certain functional activity of homologous proteins. As to the procedure of this method, first, we collected amino acid sequences of the target protein and its homologues and their functional activity indexes such as Ki, Km, LD50, etc. as many as possible, and we could classify twenty amino acids to several groups and give a number as a mark of the substitution of amino acid residue from one group to another, Second, realigned them and got N(N-1)/2 numberal sequences with analysis substitution in every position of each two sequences from N homologous proteins. According to a certain change direction, if the substitution in some position of sequences was regular, that one was a FUNCTION-RELATED RESIDUE (FRR). Otherwise, if random, it was of no importance. FRRs were different from conservative residues at that the mutations in them only caused function change not loss. Now, we could determine which direction of substitution would increase functional activity and give a design for SDM.

CK 111 EFFECT OF AMINO ACID REPLACEMENTS ON A PEPTIDE HELIX/COIL TRANSITION, Gene Merutka and Earle Stellwagen, Department of Biochemistry, University of Iowa, Iowa City, IA 52242.
The peptide acetylYEAAAKEAAAKEAAAKAamide exhibits a thermal dependent helix/coil transition in aqueous solutions at neutral pH as observed by circular dichroism. Analogs of this peptide were synthesized by the T-bag method, purified by reversed-phase HPLC, and found to be at least 90% homogeneous by reversed-phase HPLC. Amino acid analysis as well as FAB mass spectroscopy confirm that the correct peptide was obtained. The thermal transitions at 222 nm were globally fit with a symmetrical sigmoidal curve having a span of 35,900 deg cm^2 $dmol^{-1}$. Replacements at positions 4, 9, or 14 have an equal effect on the melting transition, while multiple replacements at these positions have an additive effect. Replacement of ala 9 with the other 19 amino acids changes the thermal dependence of the helix/coil transition largely as predicted by residue helix propensity values obtained from analysis of crystallographic models of proteins. (Supported by NIH grant HE-14388).

CK 112 COMBINED USE OF STEREOSPECIFIC DEUTERATION, NMR, DISTANCE GEOMETRY, AND ENERGY MINIMIZATION FOR THE CONFORMATIONAL ANALYSIS OF THE HIGHLY DELTA OPIOID RECEPTOR SELECTIVE PEPTIDE, [*D*-PEN^2, *D*-PEN^5]ENKEPHALIN, Henry I. Mosberg, Katarzyna Sobczyk-Kojiro, Gordon M. Crippen, and Ronald W. Woodard, College of Pharmacy, University of Michigan, Ann Arbor, MI 48109

Comparison of ^{1}H and ^{13}C NMR parameters for the cyclic, conformationally restricted, delta opioid receptor selective enkephalin analog, Tyr-*D*-Pen-Gly-Phe-*D*-Pen ([*D*-Pen^2, *D*-Pen^5]enkephalin, DPDPE) in aqueous *versus* dimethylsulfoxide (DMSO) solution indicates that this peptide adopts similar conformations in these solvents. This suggestion that the conformation of DPDPE is relatively environment independent allows conclusions regarding the receptor bound conformation of this peptide to be drawn from studies performed on experimentally convenient DMSO solutions of the peptide, alone. Accordingly, 2D NOESY experiments were conducted on DPDPE in DMSO and the observed interproton interactions were utilized for the quantitative calculation of the appropriate interproton distances. A commonly encountered limitation, the general inability to stereospecifically assign diastereotopic and enantiotopic hydrogens within the amino acid residues, which results in increased error limits for calculated distances involving such hydrogens, was overcome by the synthesis of stereospecifically deuterated amino acids, (2*S*, 3*R*)-[3-^{2}H]tyrosine, (2*S*, 3*S*)-[4,4,4-$^2H_3$]penicillamine, (*R*)-[2-^{2}H]glycine, and (2*S*, 3*R*)-[3-^{2}H]phenylalanine, and their incorporation into DPDPE. As a result all resonances in the ^{1}H NMR spectrum of DPDPE were assignable and more stringent interproton distances were calculated from the observed NOE interactions. These interproton distances were employed as distance constraints for distance geometry calculations of conformations consistent with the experimental data. Energy minimization of conformers generated by distance geometry calculations was performed using the AMBER force field and the resulting low energy conformers were reexamined for agreement with distance constraints and other conformation dependent NMR parameters. From these studies a conformer was identified which displayed significantly lower energy than all others found, while maintaining good agreement with experimental data.

CK 113 **Coiled Coils: A Model System for Measuring Individual Contributions to Helix Stability.** Karyn T. O'Neil and William F. DeGrado. Central Research and Development Department; E. I. DuPont de Nemours; Wilmington, DE, 19880-0328. To identify and assess the individual contributions of various energetic parameters to the stability of an α helix we have designed a synthetic peptide with sequence (AcEWEALEKKLAALEXKLQALEKKLEALEHG-NH2) that is predicted to form a two stranded coiled coil. Twenty peptides have been synthesized with each possible amino acid in the guest position. By linking helix formation to dimerization we have been able to obtain equilibrium constants for the transition from monomeric random coil to dimeric helices and thereby ΔΔG values for individual side chain contributions to the overall stability of the coiled coil structure. Using our model coiled coil we have extended this analysis to examine other energetic contributions to the stabilization of a leucine zipper structure. We have synthesized six peptides with a single mutation in the fourth leucine residue, an "a" position leucine thought to be involved in stabilizing the coiled coil. The substitutions were selected based on their occurance in natural leucine zipper sequences from the *fos* and *jun* proteins. Hetero and homodimeric forms of these peptides have been prepared and their thermodynamic stabilities measured.

CK 114 STRUCTURE AND FUNCTION OF MUTANT AND WILDTYPE ZINC FINGER PEPTIDES FROM YEAST TRANSCRIPTION FACTOR ADR1: IMPLICATIONS FOR DNA BINDING. Grace Parraga, Jon R. Herriott, Suzanne J. Horvath* and Rachel E. Klevit. Dept. of Biochemistry SJ-70, University of Washington. Seattle WA 98195 and *Division of Biology, California Institute of Technology, Pasadena CA 91125.

We have been utilizing 2DNMR spectroscopy and distance geometry to determine the structure of chemically synthesised zinc finger peptides. The wildtype single zinc finger structure was shown to consist of a C-terminal amphiphilic α-helix, a hydrophobic core defined by the interaction of conserved leucine and phenylalanine residues, as well as a N-terminal turn or lasso structure and a flexible finger tip. These structural elements are linked thermodynamically and kinetically to tetrahedral zinc liganding as evidenced by temperature and pH titration studies of the zinc-bound domain. ADR1 mutants containing single missense mutations in the zinc finger domains have been isolated and these are either completely deficient in DNA binding or bind very weakly. We have chemically synthesized the wildtype double finger domain from ADR1 (ADR1c) as well as single finger point mutants (ADR1b114V and ADR1b118Y). Both of the mutant zinc finger peptides are capable of tetrahedral zinc binding and folding into a structure similar to that determined for the wildtype peptide. Temperature studies showed that the point mutations did not destabilize the domain structure and 2DNMR studies indicated that the amphiphilic helix proposed to be involved in DNA-binding is still intact in both ADR1b114V and ADR1b118Y. The high resolution structures of the mutants will be discussed as well as resulting implications for DNA binding.

CK 115 POSSIBLE STRUCTURAL AND FUNCTIONAL RELATIONSHIPS BETWEEN LYSOSOMAL PROSPHINGOLIPID ACTIVATOR PROTEIN, RAT SULFATED GLYCOPROTEIN 1 AND INFLUENZA VIRUS NEURAMINIDASE. Michel Potier, Service de Génétique Médicale, Hôpital Sainte Justine, Université de Montréal, Montréal, Canada H3T 1C5.
We report that human lysosomal prosphingolipid activator protein (proSAP) and rat sulfated glycoprotein 1 of seminal fluid (SGP-1) bear a remarkable degree of amino acid sequence similarity with the influenza virus neuraminidase (FLU NA) based on the conservation of several disulfide bond-forming cysteines and clusters of polar amino acid residues localized in or close to the sialic acid binding site of FLU NA. The similar repetitive nature of amino acid sequences in proSAP, SGP-1 (4-fold repeated domain) and FLU NA (3-fold repeated domain) indicates that these proteins probably share a similar secondary and tertiary structure composed of repeated ß-sheets. The mammalian and viral proteins probably originate from an ancestral gene coding for a protein of around 80 amino acid residues which duplicated twice during evolution. The most ancient repeated region in proSAP and SGP-1 (region 2) is also that which bear the most ressemblance to FLU NA and constitutes the active site of FLU NA. The evidence suggests that proSAP and SGP-1 probably have neuraminidase activity or are sialic acid-binding proteins and that the genetic material was exchanged between the mammalian and viral genomes at a relatively late stage of evolution.

CK 116 **ELECTROCHEMILUMINESCENCE: A NEW TECHNIQUE FOR THE STUDY OF ENZYME INHIBITOR INTERACTIONS.**
Michael J. Powell*, Haresh P. Shah, Richard C. Titmas, Anitha M. Reddy,
IGEN Inc., 1530 East Jefferson Street, Rockville, Maryland 20852.
Trisbipyridyl chelates of ruthenium and osmium can be stimulated to emit light upon electrochemical oxidation; i.e., they exhibit electrochemiluminescence. Covalent attachment of a ruthenium trisbipyridyl chelate via an N-hydroxysuccinimide ester linkage to the amino terminus of a tripeptide trifluoroketone inhibitor of chymotrypsin, structure: H_2N-GLY-VAL-PHE-$COCF_3$* has allowed the study of the binding interactions of other chymotrypsin inhibitors with the active site of the enzyme. The displacement of the metal chelate labelled inhibitor from the active site of the enzyme by other ligands or inhibitors can be quantitatively determined by the measurement of the electrochemiluminescence exhibited by the metal chelate labelled tripeptide inhibitor upon release from the active site of chymotrypsin immobilized on latex beads. Electrochemical oxidation and light emission detection was performed with an **ORIGEN™** analyzer (1). The technique can be applied to the rapid screening for tight binding inhibitors of proteolytic enzymes and can also be used to study the the kinetics of activation of proenzymes.
* Provided by Robert A. Abeles, Brandeis University, Massachusetts.
(1) J. Inorg. Biochem., 1989, 36, (3-4), pp 259 and 275.

CK 117 A COMPUTERGRAPHIC ANALYSIS OF CONFORMATIONALLY RESTRAINED CHEMOTACTIC FACTORS, Simon F. Semus*, Natesa Muthukumaraswamy*, Elmer L. Becker** and Richard J. Freer*, *Division of Biomedical Engineering and Department of Pharmacology, Medical College of Virginia, Box 694, Richmond, Virginia 23298, and **University of Connecticut Health Center, Farmington, Connecticut, CT 06032. A computergraphic investigation of a conformationally restrained cyclic chemotactic peptide, N-formyl-Met-cyclo-[Orn-Leu-Phe-Phe] has been undertaken in order to determine the bio-active conformation of this class of compound. The cyclic peptide was constructed using the Sybyl suite of programs and was energy minimised using the Tripos force field. N-Formyl-Met-Leu-Phe (FMLP), constructed as either a beta-sheet or a beta-turn was fitted to the cyclic peptide, indicating that the linear peptide in the beta-turn conformation will adopt an energetically preferential conformer to that when fitted as a beta-sheet. These conformational studies enforce the belief that the beta-turn is the preferred peptide conformation for receptor interaction. The cyclic pentapeptide has been synthesized and shown to retain full biological activity (ED_{50} lysosomal enzyme secretion = 2.9×10^{-10}M) when compared to FLMP itself (2.7×10^{-10}M).

CK 118 THE de novo DESIGN AND THE SYNTHESIS OF α/β BARREL PROTEIN, Toshiki Tanaka, Ken-ichi Fukuhara, Haruki Nakamura, Shin Saito, Toshiaki Tanaka, Mayumi Hayashi, Yoshio Yamamoto, Atuko Kohara, Masakazu Kikuchi, and Morio Ikehara, Protein Engineering Research Institute, 6-2-3, Furuedai, Suita, Osaka 565, Japan.
The construction of a designed α/β barrel protein typified by triose-phosphate isomerase (TIM) is described. This type of protein consists of a parallel β-barrel core of 8 strands with α-helices surrounding it. We designed the 8 α-helices with amphiphilic 11-residues and the 8 β-strands with hydrophobic 6-residues. Amino acid sequences for the loops connecting β-strands and α-helices were designed mainly based on those of TIM and Taka-amylase A. Two putative ATP binding sites and potentially susceptible sites to trypsin and kallikrein were designed to be placed on the loops. Secondary-structure prediction for the protein sequence agrees with the designed structure. A gene for the protein ,which consists of 223 amino acids, was chemically synthesized and expressed in Escherichia coli under the control of a trp promoter as a fusion protein with a portion of human growth hormone through methionine. Upon induction with 3-indoleacrylic acid, the fusion protein accumulated as an inclusion body in the cell. After centrifugation of the disrupted cell, the protein was cleaved from the fusion by treatment with cyanogen bromide. It was dissolved in 7M urea and subsequently refolded by dialysis and then purified with a combination of cation exchange and size-exclusion chromatographies. Results of gel filtration and circular dichroism shows that the protein has a folded structure with 24% α-helix and 32% β-sheet.

CK 119 RAMAN AND *AB-INITIO* STUDIES OF SOLUTION STRUCTURES OF ALA-X PEPTIDES
Robert W. Williams†, James L. Weaver†, and Alfred H. Lowrey‡. †Department of Biochemistry, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Rd, Bethesda, MD 20814-4799. ‡Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, D.C., 20375-5000. Raman spectra of aqueous solutions of alanine-X peptides show that the amide III band shifts systematically (over 20 cm^{-1}) to lower frequencies as the neutral sidechain of the X amino acid becomes larger. The amide I frequencies do not change. This shift may result from changes in: (a) the average conformational preference of the peptide, (b) vibrational coupling of the amide III modes with the X amino acid sidechain, or (c) vibrational force constants. Computational techniques have been evaluated for the purpose of interpreting this shift in terms of these possibilities. Optimized structures and frequencies have been calculated for ala-X peptides using GAUSSIAN 86 with the 4-31G basis, MOPAC, CHARMm, and normal-mode methods based on empirical force fields. (1) Optimized structures and amide III frequencies computed with MOPAC and CHARMm do not show systematic shifts, suggesting that molecular mechanics and semi-empirical approaches are not adequate for this problem. (2) Frequencies calculated from scaled GAUSSIAN 86 forces correlate well with the experimental observations, suggesting that *ab initio* methods are appropriate for studies of solution peptide structure. A shift in the computed dihedral angle ϕ suggests that the experimental observations result from a shift in the average conformations of the ala-X peptides. (3) Changes in the optimized peptide conformations from β-sheet to α-helix produce large changes in both calculated amide I and amide III frequencies, suggesting that a shift in the β-sheet$\rightleftharpoons$ α-helix equilibrium is not involved. (4) Computational changes in ϕ produce shifts in a normal mode amide III frequency that are consistent with the experimental observations.
This work was supported by the NCI Supercomputer Facility and USUHS grant C07147.

Peptides in Immunity

CK 200 DEVELOPMENT OF IMMUNOPOTENTIATING SYSTEMS FOR B-EPITOPE OF *PLASMODIUM FALCIPARUM* CS-PROTEIN, Tatyana M. Andronova, Boris B. Ivanov, Elena A. Meshcheryakova, Svetlana S. Guryanova and Vadim T. Ivanov, Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Moscow, USSR
Production of recombinant subunit antigen and synthetic peptide antigenic determinants paves the way for design of vaccines of the new generation. As a rule these antigens are weak immunogens, so additional stimulation of the immune system, namely, proper application of adjuvants and effective presentation of the antigen to the immune system, is required.
The paper deals with the synthesis of the constructions based on the B-epitope of *Plasmodium falciparum* CS-protein - $(NANP)_3$, glycopeptide adjuvant N-acetylglucosaminyl-N-acetylmuramyl-alanyl-D-isoglutamine (GMDP) and polymer carriers. Investigations of immunogenic properties of these complexes showed that GMDP with immunogenic constructions enhances the level of anti-peptide andibodies, whereas GMDP with nonimmunogenic conjugates have practically no effect. The conjugate of $(NANP)_3$ with polytuftsin and GMDP induces higher titres of antipeptide antibodies than standard glutaraldehyde conjugate $(NANP)_3$-KLH and elicits immune response even in the otherwise poorly responding mice line. The mechanism of stimulation of immune response by this conjugate is discussed.

CK 201 DETECTION OF CELLULAR PROTEINS BY SEQUENCE-SPECIFIC ANTISERA, Valery V.Antonenko[1], Igor A. Prudchenko[2], Inessa I. Mikhaleva[2], Natalya N. Mazurenko[3], and Fedor L. Kisseljoff[3], (1) Branch of the Shemyakin Institute of Bioorganic Chemistry, Pushchino, Moscow region, USSR; (2) Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Moscow, USSR; (3) All Union Cancer Research Center, USSR Academy of Medical Sciences, Moscow, USSR

The *c-fos* gene is the cellular homolog of the oncogene *(v-fos)* carried by two murine osteogenic sarcoma viruses (FBJ-MSV and FBR-MSV), it plays a key role in the processes of cell proliferation. It encodes a nuclear phospho-protein that is associated with chromatin and that displays a sequence-specific DNA binding activity as part of a complex with at least one other cellular protein *(p39/c-jun)*. With a view to investigate its functional activity we have synthesized in solution a set of peptides corresponding to N- and C-terminal sequences of the oncoprotein. Polyclonal antibodies to these peptides were raised using conventional methods. Antibodies to N-terminal peptides recognized a "traditional" *c-fos* gene product with molecular weight 55 kD whereas antibodies to the C-terminal peptides detected antigenes of 33, 35-36, 46, 62, 75, and 90 kD. Obviously proteins with molecular weights 35 and 46 kD are the products of *fos B* and *fra I* genes. We suggest that the rest proteins are the genes' products antigenically related to *fos* protein. These proteins may be responsible for coupling short-term signals at the cell membrane to long-term cellular adaptive responses by altering patterns of gene expression. We are currently investigating the biological functions of detected proteins.

CK 202 INDUCTION OF A PROTECTIVE IMMUNITY TOWARD SCHISTOSOMIASIS WITH A MULTIPLE ANTIGENIC PEPTIDE, Claude Auriault*, Isabelle Wolowczuk*, Hélène Gras-Masse†, Denis Boulanger*, Marc Bossus†, André Tartar† and André Capron*, * Centre d'Immunologie et de Biologie Parasitaire, Unité Mixte INSERM U 167 - CNRS 624, Institut Pasteur, 1, rue du Prof. Calmette, 59019 LILLE Cédex(France), † Service de Chimie des Biomolécules, URA 1309 CNRS, Institut Pasteur, 1, rue du Prof. Calmette, 59019 LILLE Cédex (France). Schistosomiasis is a chronic, debilitating parasitic disease that affects over 200 million people world wide. The P28 antigen is one of the major antigen of *Schistosoma mansoni*, transitory present at the larvae surface and clearly implied in the protective immunity toward this parasite in various experimental models. Synthetic peptides, derived from the primary sequence of the recombinant P28 were synthetized and the presence of B and T cells has been examined in the rat, mouse, baboon experimental models and in human. One of these peptides comprising amino-acids 115-131 has been shown to contain major T and B cell epitopes. A multiple antigenic 115-131 peptide (octopus) has been prepared and used as immunogen in rats mice and baboons. In all these experimental models the 115-131 octopus induced both humoral and cellular responses toward either the recombinant P28 or the native molecule. Moreover a previous immunization with the 115-131 octopus before a challenge with the recombinant P28 led to a 4 to 10 fold increase of the T cell proliferation and the antibody production. The active immunization of rats with this synthetic construction before infection allowed a significant protection (between 40 and 50 %).

CK 203 TAILORING AND ANTIGEN PRESENTATION OF SYNTHETIC PEPTIDES FOR THE INDUCTION OF DETERMINANT SPECIFIC HUMORAL IMMUNE RESPONSES. Boersma W.J.A., Deen C., Zegers N.D., Gerritse K. & Claassen E.. Dept. Immunology, TNO Medical Biological Laboratory, POB 45, 2280AA Rijswijk, The Netherlands. Selected amino acid sequences containing crucial primary structure differences can be synthesized and applied for the purpose of induction of determinant specific humoral immune responses. We have induced specific humoral responses to sequences derived from various (human) proteins. The human IgG2 is on of four homologous proteins (huIgG1-4). Only few diagnostically useful specific reagents could be raised with standard procedures. Therefore the synthetic peptide approach was chosen. The selection of specific sequences was based on the secondary structure parameters described according to predictive algorythms. In our experience short peptides have a low probability to mimic putative antigenic sites of a protein. Therefore peptides of 15 and more amino acids were used for immunization. Part of these peptides proved to be immunogenic such that in the presence of a proper adjuvans, they could be used for immunization without prior attachment to an immunogenic carrier protein. This enabled us to generate various highly specific Mabs to human IgG2. Short synthetic peptides in general represent single determinants and as such provide useful tools for epitope specific analysis of the mechanism of humoral responses. The immunogenic peptide SP29 includes two determinants: one mimicking a part of the hinge of huIgG2 and one peptide specific determinant. To each determinant a specific MAb was raised. Immune-complexes of MAb anti-huIgG2 and SP29 showed an enhanced ability to induce anti-huIgG2 antibodies. This observation indicates that immune complexes on a single determinant level greatly influence the regulation of humoral immune responses. Enhanced responses to specific antibody covered selected determinants in complexes may be of great importance in application of peptide based vaccins.

CK 204 DESIGN AND CHARACTERIZATION OF A POTENT SYNTHETIC IMMUNOGEN AGAINST HIV. Pele Chong, Dwo Y. Sia, Joel Haynes and Michel Klein. Connaught Centre for Biotechnology Research, 1755 Steeles Ave. West, Willowdale, Ontario, Canada. M2R 3T4.

The core proteins of some enveloped viruses, such as Influenzae and Hepatitis viruses had been shown to function as potent T-helper determinants in the induction of antibodies responses against their corresponding envelope proteins. A panel of peptides of HIV1 gag gene was selected according to conventional structure prediction algorithms for T-cell and B-cell epitopes. In particular, an unconjugated peptide HIV1-p24 was injected into BALB/C (H-2d) mice in incomplete Freund's adjuvant and elicited a strong antibody response against p24 and its precursor(p55) as judged by immunoblotting. By testing a series of trauncated peptide analogs of HIV1-p24, the precise T-helper determinant ia mapped. A T-B tandem chimeric peptide containing the T-cell epitope of HIV1-p24 and a B-cell neutralization epitope of HIV1 gp41 is synthesized and found to induce a strong gp41 antibody response in immunized mice. The molecular characterization of a potent HIV1-p24 T cell epitope which is capable of serving as a carrier for both autologous and heterologous B-cell epitopes represents a first step towards rational design of a synthetic vaccine against AIDS.

CK 205 THE USE OF POLYMER CONTAINING A REPEATING PEPTIDE SEQUENCE TO STIMULATE T-CELL INDEPENDENT ANTIBODY PRODUCTION

Kathleen Hilman+, Robert Blackburn+, Orit Shapiro-Nabor*, and Hana Golding+; + Division of Virology,FDA-CBER, and * The Laboratory of Cellular Development and Oncology, NIDR, Bethesda,MD 20892.

The immunogenicity of a CD4 peptide sequence 302-314, was determined for two forms of the peptide: a polymer synthesized in a head to tail configuration (according to the method of Linder and Robey), and the traditional conjugate consisting of the peptide linked to CSA. Two in vivo systems were used; the T-cell competent BALB/C mouse, and a T-cell deficient nude mouse line. The response obtained in the T-cell competent BALB/c mice was ten fold greater for the conjugate than for the polymer. However, the reverse was true for the nude mice, where the polymer produced a two to three fold better response than was observed for its conjugated counterpart. Furthermore, the nude mice immunized with polymer produced predominately IgG 2a and b. The BALB/c mice, immunized with polymer produced similar subclasses, and sera from both groups were able to react with the CD4 molecule on Western blots. These results suggest that repeating epitopes on the same polymer may provide cross-linking for antigen specific receptors on B-cells resulting in a relatively T-cell independent immunogen which can induce isotype switching.

CK 206 SYNTHETIC PEPTIDES OF THE E2 GLYCOPROTEIN OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS PROTECT MICE FROM VIRUS CHALLENGE, Ann R. Hunt, A. Jane Johnson, and John T. Roehrig, Centers for Disease Control, Ft. Collins, CO 80522. Thirteen peptides representing 62 percent of the extra-membranal domain of the Venezuelan equine encephalitis (VEE) virus E2 glycoprotein were synthesized and analyzed for their antigenic, immunogenic, and protective capacities. Twelve of 13 peptides elicited antibody reactive with the homologous peptide. Thirteen peptides elicited antiviral antibody which recognized either the Trinidad (TRD) strain of VEE virus or the TC-83 derivative, or both. Six peptides protected 25-75 percent of Balb/c mice challenged with TRD virus. The TRD amino-terminal peptide protected 100 percent of NIH Swiss mice from challenge. The amino-terminal peptides of TRD and TC-83 viruses were the most reactive, both as protective immunogens and as antigens recognized by antiviral antibody. Although these 2 peptides differ in sequence by only one amino acid, their respective antisera showed significantly different binding patterns with the 2 peptides as well as with TC-83 and TRD viruses. Both antisera reacted by ELISA with other VEE subtype viruses, but not with other alphaviruses. The protection afforded by the TRD amino-terminal peptide could be passively transferred by high-titered antipeptide antiserum. Antisera to the amino-terminal peptides did not bind or neutralize infectious virus, but did bind to virus-infected cells.

CK 207 SYNTHETIC PEPTIDE VACCINE APPROACH TO DENGUE VIRUSES, Alison J. Johnson, Ann R. Hunt, James H. Mathews, and John T. Roehrig, DVBID, Centers for Disease Control, Fort Collins, CO 80522.
Computer modeling was applied to locate possible antigenic regions on the extramembranal portion of the envelope (E) glycoprotein of dengue 2 (D2) virus (Jamaica strain). Based on this analysis 18 synthetic peptides were assembled by conventional t-Boc solid phase chemistry. After immunization mouse antipeptide sera were reviewed by ELISA, neutralization, and immunofluorescence. A comparison of peptide immunogenicity in NIH-Swiss and BALB/c mice revealed possible genetic restriction in BALB/c animals. Previous analyses with Murray Valley encephalitis (MVE) virus identified an active, non-restricted T-helper cell epitope within the amino acid sequence 352-368 (peptide 17). Using this observation we investigated the ability of this peptide to serve as a source of T-cell help when mixed with those peptides demonstrating a genetically restricted immunogenicity. While both heterologous (MVE) sequences or homologous (D2) sequences provided T-cell help resulting in an elevated antipeptide response in BALB/c animals for several peptides, homologous (D2) sequences were more efficient, eliciting higher antipeptide titers. Details of the peptide sequences and their antigenicity will be presented.

CK 208 MODULATION OF FUNCTIONAL ACTIVITY OF NEUTROPHYLS *IN VITRO* BY SYNTHETIC FRAGMENTS OF HIV PROTEINS, Alexander T. Kozhich, Dmitry I. Gabrilovich*, Vadim S. Ivanov, Sergey A. Moshnikov, Leonid D. Tchikin, Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Moscow, USSR; *Central Institute of Epidemiology, USSR Academy of Medical Sciences, Moscow, USSR
Neutrophyls are essential element of immune response against bacterial and viral infections. The impairment of their function during HIV-infection has been reported in literature. To study the action of viral antigens on functional activity of neutrophyls from blood of persons infected with HIV we used several synthetic peptides: CRIKQIINNWQE (423-434 env), NMWQEVGKANWAPP (430-443 env), MKVVKIEPLGVAPTKAKRRVVQREKRA (492-517 env), SGIVQQQNNLLRA (551-563 env), RAIEAQQHLLQLTVWG (557-573 env), IWGCSGKLICTTAVPWNAS (600-618 env), IEIKDTKEALDKIEEE (92-107 gag), DRLIERAEDSG (43-53 vpu), VEMCHAPWDVDDL (68-81 vpu).
Using NBT-test (reduction of nitroblue tetrazolium) it was shown that all these peptides reduced the activity of neutrophyls from HIV-positive blood to various extent at ARC-stage only. Peptide 600-618 env reduce the activity of neutrophyls up to 90%, the similar supression is produced by whole viral lysate. Supression of activity is inversely proportional to the concentration of HIV-antibodies in patients blood. Upon treatment of pure granulocyte fraction this action is weaker.

CK 209 EPITOPE MAPPING OF T CELL RECOGNITION OF PHOSPHOLIPASE A_2, Mei-chang Kuo,Ph.D., Annette M. Lussier, Richard Koury , Julian F. Bond, Ph.D. and Julia L. Greenstein, Ph.D. ImmuLogic Pharmaceutical Corporation, One Kendall Square, Building 600, Cambridge, MA 02139.
The murine immune reponse to the major honey bee allergen, phospholipase A_2 (PLA_2), has been investigated. To determine the dominant epitopes involved in the T cell recognition of PLA_2, a panel of murine T cell hybridomas has been characterized. These hybridomas are derived from mouse strains with distinct Major Histocompatibility Complex antigens and are specific for PLA_2. The comparison of the T cell epitopes used by different strains will determine if T cells from mice expressing H-2^d, H-2^b, H-2^k and H-2^a gene products can recognize similar or distinct T cell epitopes. The mapping studies have been designed to examine the recognition of purified PLA_2 compared to enzymatically and chemically cleaved fragments, biochemically modified protein and protein fragments, and synthetic peptides. T cell recognition is measured by the production of interleukin-2 following activation of each T cell hybridoma with antigen and antigen presenting cells. The result suggests that the dominant T cell epitopes are dependent on the tertiary conformation of PLA_2.

CK 210 EPITOPE ANALYSIS OF A PHOSPHOLIPASE A2 NEUROTOXIN USING SYNTHETIC PEPTIDES, John L. Middlebrook and Bradley G. Stiles, Dept. of Toxinology, Pathology Division, U.S. Army Med. Res. Inst. of Infect. Dis., Frederick, MD 21701-5011
Pseudexin is a weak phospholipase A2 neurotoxin found in the venom of the red-bellied black snake, *Pseudechis porphyriacus*. Recent work (Schmidt and, termed pseudexins A, B and C. Immmunological studies with rabbit antisera against pseudexin have shown that there are extensive ELISA cross-reactions with many other phospholipase A2 neurotoxins from snake venoms (Middlebrook and Kaiser, 1989). Monoclonal antibodies were produced against the pseudexins and these antibodies react in an ELISA with the pseudexins and many other phospholipase A2 neurotoxins. In order to better characterize the epitopes against which the monoclonal antibodies are directed, we synthesized short overlapping segments of pseudexin B corresponding to the entire length of the toxin. These peptides were used as the capture antigen in an ELISA. Of a total of 15 monoclonal antibodies against pseudexin, 4 reacted with the peptides from a screen consisting of 9 residues, "walking" through the sequence shifting 1 amino acid at a time. Rabbit polyclonal antisera gave only weak, non-specific reactions with the same peptides. Analogous peptides from other phospholipase A2 neurotoxins, corresponding to the immunoreactive sites of pseudexin, also were recognized by the monoclonal antibodies.

Schmidt, J.J. and Middlebrook, J.L. 1989 Toxicon 27, 805-818.
Middlebrook, J. L. and Kaiser, I.I. 1989 Toxicon 27, 965-978.

CK 211 Natural antibodies and cellular immunity against four distinct and defined antigens of P. falciparum infected individuals.

Rao, D.N. Department of Biochemistry, All India Institute of Medical Sciences, New Delhi-110 029, India.

The Magnitude of the antibody response to four distinct and defined antigens of Plasmodium falciparum was assessed in 800 inhabitants of age group between 1-5 years in Gaziabad District (Uttar Pradesh, India), a region where malaria is endemic. Antibodies against repeat sequence of CS and erythrocytic stages of the parasite were detected by ELISA. The test antigens were $(NANP)_4$, $(EENV)_2$, K (DDEHVEEPTVA) and K $(EENVEHDA)_2$ were chemically synthesized by us. The relationship between the age, the presence of specific antibodies, the sero-positivity and celluler immunity for each antigen will be discussed.

CK 212 TYPE-SPECIFIC AND CROSS-REACTIVE ANTIGENIC DETERMINANTS OF THREE HUMAN RETROVIRUSES, Ale Närvänen, Mirja Korkolainen and Marja-Liisa Huhtala, Labsystems Research Laboratories, Pulttitie 8,00880 Helsinki, Finland
We have synthetized overlapping peptides from the transmembrane protein gp41 of HIV-1, from the transmembrane protein gp34 of HIV-2 and from the both envelope proteins of HTLV I by using solid phase synthesis on the rods as described by Geysen (J.Immunol.Meth.,102:259-274,1987). These peptides, 14 amino acids in length, have been tested in conventional EIA using human sera from patients infected with HIV-1, HIV-2 and HTLV I. The immunoreactivity and the number of reactive epitopes varies between individuals infected with same virus. However some of the epitopes are reactive with all studied sera. These immunodominant epitopes can be devided to type-specific determinats, which react only with corresponding anti-viral antibodies, and cross-reactive determinants, which react also with sera from patients infected with another retrovirus. According to our result HIV-1 and HIV-2 have type-specific determinants but share also common antigenic determinants whereas HTLV I is immunologically much more distant.

CK 213 DETERMINATION OF SERUM IgG-CLASS ANTIBODIES BY A HUMAN PAPILLOMAVIRUS DERIVED SYNTHETIC PEPTIDE
Päivi Parkkonen, Harri Luoto, Jorma Paavonen* and Matti Lehtinen, Inst.of Biomed.Sci., Univ.of Tampere; Dept. of Obst.& Gynecol.,Univ.Central Hospital, Helsinki, Finland
We have used a human papillomavirus, HPV specific peptide for IgG-class antibody analysis in ELISA. It corresponds to a sequence of HPV type 16 E2 protein (aminoacids 318-336) originally described by Dillner et al (PNAS 86:3838-3841,1989). Alltogether 150 paired sera were drawn from a cohort of women referred to the hospital for koilocytotic atypia.HPV strains in the infected cervix were classified by in situ-hybridization into three categories: HPV 16,18 and others. Our results can be summarized as follows:
Thirty-six to 50% of women were positive for the peptide antibodies. Seroconversions were seen in 7 patients. All the 5 HPV 18 DNA-positive patients showed only low or no antibodies to the peptide.
We conclude that the HPV 16 E2 peptide: H-K-S-A-I-V-T-L-T-Y-D-S-E-W-Q-R-D-Q-C is suitable for HPV IgG-class antibody analysis in cervical HPV infections, except for infections caused by HPV 18.

CK 214 IDENTIFICATION OF CONTACT REGIONS IN gp120 AND gp41 OF HIV-1 USING SYNTHETIC PEPTIDE ANALOGS, Natalie C. Pavuk, Dale A. McPhee, Nancy L. Haigwood and Bruce E. Kemp, Special Unit for AIDS Virology, Macfarlane Burnet Centre for Medical Research, St. Vincent's Institute of Medical Research, Melbourne, AUSTRALIA and Chiron Corporation, Emeryville, U.S.A.
The gp160 precursor env protein appears to exist as a tetramer. The gp41 transmembrane product, responsible for anchoring gp120, maintains the tetrameric structure of identical subunits (Schawaller *et al*, 1989). Non-covalent binding between gp120 and gp41 occurs within the N-terminal half of both proteins. Our studies have focussed on potential interaction sites between gp120 and gp41 using synthetic peptide analogs to compete with native protein for binding. We identified two potential contact regions, one in gp120(105-117) and a proposed complementary region in gp41(572-591). Reduction of virus infectivity with either peptide indicated a direct effect on infectious virus particles, presumably by disruption of gp120/gp41 interactions. The gp41(572-591) peptide bound specifically to native recombinant gp120(31-509) immobilized on sepharose, consistent with a binding site for this region on gp120. Although the gp120 and gp41 regions are highly conserved and complementary we cannot exclude other mechanisms of gp120/gp41 disruption such as gp41 sub-unit destabilization. We are at present testing the effect of the peptide analogs on purified ^{3}H-mannose labelled gp41 tetrameric complexes. These results may clarify our hypothesis of gp41/gp120 non-covalent interactions.

CK 215 SURFACE LOOPS AND COILS AS A POSSIBLE BASIS FOR CROSS-REACTIONS BETWEEN ANTIGENS OF THE HUMAN MALARIA PARASITE PLASMODIUM FALCIPARUM, Ranjan Ramasamy, Institute of Fundamental Studies, Kandy, Sri Lanka. An IgM monoclonal antibody that inhibits parasite growth in vitro was shown to recognise several previously characterised asexual blood stage antigens of Plasmodium falciparum as well as new antigens. Among the reactive antigens identified were FIRA, GYMSSA, RESA and the S-antigen. Analysis of the cross- reactions between FIRA and GYMSSA by epitope scanning was performed. The most reactive peptides in GYMSSA had the common sequence STNS. The cross-reactive epitopes in FIRA could in many cases be explained by a replacement net analysis performed on the STNS epitope. It is proposed that the cross-reactive epitopes, which in several cases have no obvious linear homology, may be present as loops or coils on the surface of the molecules. The many repeat as well as unique regions of parasite antigens may be involved in such cross-reactions. The results imply that there are constraints on the development of malaria vaccines based on synthetic peptides.

CK 216 MONOCLONAL ANTIBODIES AGAINST AN MHC-DERIVED SYNTHETIC PEPTIDE REACT WITH AN EPITOPE PRESENT ON BACTERIAL PROTEINS, Richard B. Raybourne and Kristina M. Williams, Immunobiology Section, Division of Microbiology, Food and Drug Administration, Washington, DC 20204. Molecular mimicry between bacterial antigens and the human MHC class I protein HLA-B27 has been postulated to play a role in the spondyloarthropathies. To investigate this hypothesis on a molecular level, with respect to the epitopes involved, monoclonal antibodies were produced against a synthetic peptide, the sequence of which was derived from a polymorphic region of the HLA-B27 molecule (amino acids 63-83). Two antibodies (J7F2 and H2B6) were selected for study on the basis of their ability to react with bacterial envelope proteins (ELISA) and B27-positive cells (immunofluorescence). J7F2 had preferential reactivity with B27-positive cells and neither antibody reacted with MHC class I negative cells. Based on SDS-PAGE blot analysis of bacterial envelope proteins, both antibodies had the same pattern of reactivity (against 36 and 19 kd proteins) as monoclonal antibodies produced against bacterial envelope and reactive with B27-positive cells. This apparent epitope similarity was investigated by utilizing synthetic peptides to inhibit binding of the monoclonals. When coupled to BSA, the B27 synthetic peptide, and a smaller peptide derived from it, were efficient inhibitors of anti-peptide and anti-bacterial antibody binding to bacterial antigens, and to B27-positive cells. These studies utilize synthetic peptides to provide insight into the molecular basis of cross-reactivity between bacterial proteins and MHC class I molecules.

CK 217 A PEPTIDE BASED ASSAY FOR INHIBITORS OF THE HIV-1 PROTEASE, Paul R. Sleath, Roy A. Black, Ronald C. Hendrickson, Charles T. Rauch and Carl J. March, Department of Protein Chemistry, Immunex Research and Development Co., 51 University St., Seattle, WA 98101. An assay has been designed to screen for inhibitors of the HIV-1 protease based on the cleavage of a synthetic peptide substrate. The substrate corresponds to a natural cleavage site of the protease and is labelled on the N-terminus with fluorescein isothiocyanate (FITC). Following incubation with the protease, labelled substrate peptide can be separated form the labelled product peptide on the basis of differential charge of the two species, with the substrate peptide binding to cation exchange resin while the product remains in solution. Thus, measuring the fluorescence of the supernatant after adding cation exchange resin to the digestion mixture gives a quantitative measure of the amount of substrate peptide that has been cleaved. The assay can be conducted in 96 well plates with excellent reproducibility allowing the rapid screening of large numbers of compounds.

CK 218 IMMUNOGENICITY OF SYNTHETIC VP1 PROTEIN FRAGMENTS AND FMDV-VACCINE DESIGN, Olga M. Volpina, Andrey Yu. Surovoy, Vasily M. Gelfanov, Larysa A. Grechaninova, Aleksey V. Yarov, Vadim T. Ivanov, Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, 117418 Moscow, USSR Immunogenic and antigenic properties were compared among a large number of synthetic fragments of the VP1 protein of foot-and-mouth-desease virus A22 strain. Free peptides with 39-70, 131-159, 175-189 & 197-213 sequences of VP1 were able to induce antibody response. However, only two regions (131-159 & 197-213) seem to induce antiviral response. Peptides from 39-70 & 175-189 regions were found essential for activation of T-helper cells during the antiviral immune response. We also found that the immunodominant region 131-159 of FMDV is composed of a set of T- & B-epitopes each of which is specific for different animal species. In order to overcome the Ir-gene control and to broaden the species selectivity we prepared the peptide 136-159 which includes the above mentioned set of T-epitopes. This peptide induced virus neutralizing antibodies and 100% protection of cattle and sheep. Further studies are in progress to determine the optimal construction of future vaccine including all immunocompetent VP1 regions.

CK 219 N-ACETYL MURAMYL DIPEPTIDE BINDS N-ACETYL NEURAMINIC ACID: POSSIBLE IMPORTANCE TO ALLERGIC ENCEPHALOMYELITIS. Fred Westall, Institute for Disease Research, Alta Loma, CA 91701-8293 and California State Polytechnic University, Pomona, CA 91768-4032.
N-acetyl muramyl dipeptide (MDP) is an immunoactive component of Freund's complete adjuvant. It is capable of initiating several autoimmune diseases, eg. experimental allergic encephalomyelitis (EAE). When the sequence phe-ser-trp-gly-ala-glu-gly-gln-arg (the trp peptide of myelin basic protein) and MDP in an oil emulsion are injected into guinea pigs EAE is produced. The trp peptide and MDP form a complex (J.Int.Ded.Biol 2:1,1986) which is important for disease induction. The trp peptide is also capable of binding serotonin (Brain Res.Bull.12:425,1983). Along with producing neurological effects, serotonin can participate in immune activation(BBRC 131:1161,1985). N-acetyl neuraminic acid has been implicated in both serotonin(PNAS 53:959, 1965) and immune receptors(Int. J.Cancer 38:915, 1982). Thus it is not surprising that both MDP and serotonin can bind to n-acetyl neuraminic acid. During EAE and MS, serotonin metabolism is greatly affected; much more than would be expected from neurological lesions. From our binding studies acetyl neuraminic acid possesses the same binding pattern as trp peptide. Therefore trp peptide activated T cells should also recognize n-acetyl neuraminic sites. This immunological activity can explain the excess alteration of serotonin metabolism and the relapsing nature unique to both EAE and MS.

Peptide Hormones and Growth Factors

CK 300 STRUCTURE-ANTIAGGREGATORY ACTIVITY STUDIES OF CYCLIC PEPTIDES RELATED TO RGD AND FIBRINOGEN (Fg) γ-CHAIN PEPTIDES
Fadia El-Fehail Ali[1], R. Calvo[1], T. Romoff[1], E. Sorenson[1], J. Samanen[1], A. Nichols[2], J. Vasko[2], D. Powers[2], J. Stadel[2], [1]Peptide Chemistry Department, [2]Pharmacology Department, Smith Kline & French Laboratories, King of Prussia, PA 19406-0939

Structure-activity (a/b)[3] studies of antiaggregatory agents based on Ac-RGDS-NH_2 1 (91/4.2), revealed that incorporation of the RGDS sequence into cyclic hexa or pentapeptide disulfides provided analogs 2 Ac-cyclo(S,S)-CRGDSC-NH_2 (33/5.3) and 3 Ac-cyclo(S,S)-CRGD-Pen-NH_2 (4.0/--) with enhanced potency. Replacing Arg^2 by (N^{α}-MeArg) provided 4 Ac-cyclo(S,S)-C(N^{α}-MeR)GD-Pen-NH_2 (0.39/0.0024), which displayed surprisingly high potency and affinity. Incorporating the cyclic structure of 3 into the Fg γ-peptide 5[4] HHLGGAKQRGDV (--14.5)[4] gave peptide 6 Ac-HHLGGAK-cyclo(S,S)-CRDGPen-NH_2 (9.6/0.074), which displayed much greater affinity than 5. These data suggest the optimization of conformation in RGD and Fg-γ analogs with the cyclic pentapeptide disulfide structure. The potential benefits of such peptides are being explored in thrombosis eg. adjunctive therapy in thrombolysis. 3.a) IC_{50} (μM) inhibition of canine platelet aggregation: ADP activated platelet rich plasma (PRP). b) K_i (μM) binding of purified human receptor. 4. Ruggeri, Z.M., et. al. PNAS 83, 1986, 5708-5712.

CK 301 NOVEL METHOD FOR CLEAVAGE OF T-BOC SYNTHESIZED PEPTIDES BY USE OF A POLYMERIC ACID, Mark A. Bobko and Henry R. Wolfe, Dept. of Analytical Biochemistry, Sterling Drug Inc., 25 Great Valley Pkwy, Malvern, PA 19355.

The current method(s) for cleavage of t-boc synthesized peptides involves the use of a strong acid (either anhydrous HF or TFMSA). Because both acids are highly corrosive, the user must exercise extreme care; and in the case of HF, requires the use of a special apparatus. However, we have developed a method of cleavage which involves the use of a polymeric acid resin (Amberlyst XN-1010), which offers a safe and effective alternative to the use of HF or TFMSA.

CK 302 PROPERTIES OF ANTI-PEPTIDE ANTIBODIES RAISED AGAINST THE SINTHETIC PEPTIDE 20-36 OF NERVE GROWTH FACTOR. Giuseppe Corona, Emilio Bigon, Lanfranco Callegaro, Claudio Grandi*, Daniele Dalzoppo*, Carlo Moretto* and Angelo Fontana*. Department of Molecular Biochemistry, Fidia Research Laboratories, Via Ponte della Fabbrica 3/a, 35031 Abano Terme, Italy; *Department of Organic Chemistry, University of Padua, Via Marzolo 1, 35131 Padova, Italy.

Nerve growth factor (NGF) is a protein which regulates the development and normal function of sensory and sympathetic neurons. The comparative analysis of the primary structure of this protein revealed a high degree of homology between NGFs from various sources (rat, human, bovine and chicken). The sequences of the NGF molecules so far known reveals the presence of an invariant region characterized by a prominent hydrophilicity (and thus likely surface exposure), suggesting that this region of the NGF molecule could play an important role for the immunogenicity. Thus a peptide corresponding to the invariant sequence 20-36 (VWVGDKTTATDIKGKEV) was synthesized by solid-phase methods and characterized by amino acid analysis, HPLC, fingerprinting upon chemical and enzymatic digestion, as well as FAB-mass spectrometry. Anti-peptide (20-36) antibodies were elicited in rabbits, purified by affinity chromatography using the peptide as ligand and used in turn to prepare a Sepharose immunoaffinity column for a rapid and efficient purification of NGF from bovine seminal plasma. This antipeptide antibodies were found unable to inhibit nerve growth factor, suggesting that the chain segment 20-36 of NGF likely does not contain critical residues for activity.

CK 303 THE ANALYSIS OF THE TOPOGRAPHY AND DYNAMICS OF LIGAND-RECEPTOR INTERACTIONS USING FLUORESCENT PEPTIDE PROBES, S.P. Fay, H. Mueller, W. Swann, Z. Oades, R.J. Freer*, and L.A. Sklar, Scripps Clinic and Research Foundation, La Jolla, CA 92037, *Medical College of Va., Richmond, VA 23298.

In order to study the receptor binding pocket size and dynamics of interaction of ligand and G-protein with human neutrophil formyl peptide receptor, we have employed a family of fluorescent formyl peptides containing 4(CHO-met-leu-phe-lys-fluorescein), 5(CHO-met-leu-phe-phe-lys-fluorescein), or 6(CHO-met-leu-phe-phe-phe-lys-fluorescein) amino acids. Upon binding to the receptor, the fluorescent peptides containing 4 or 5 amino acids were quenched. Though not quenched, the bound hexapeptide was the only fluorescent peptide to be accessible to antibodies to fluorescein, suggesting that while the carboxy terminus of the tetra- and pentapeptides were protected in the receptor binding pocket, the fluorescein on the carboxy terminus of the hexapeptide was sufficiently exposed to be immunologically recognizable. This indicates that the receptor binding pocket accommodates at least 5 but no more than 6 amino acids. Equilibrium and dynamic binding studies with pentapeptide in guanine nucleotide sensitive, permeabilized human neutrophils showed that when G-protein was dissociated from the ligand-receptor complex by excess GTP[S], the affinity of the receptor for ligand decreased approximately two orders of magnitude. Kinetically, this change in affinity was due to a corresponding increase in the off-rate for ligand binding, while regardless of the presence or absence of GTP[S], the association rate constant of $2x10^7$/msec for ligand was essentially diffusion limited.

CK 304 ACTIVITY AND STRUCTURE OF BACTENECINS, ANTIMICROBIAL PEPTIDES FROM BOVINE NEUTROPHILS. R. Gennaro, M. Zanetti*, B. Skerlavaj*, L. Litteri*, D. Romeo* and R. Frank°. Inst. of Biology, Univ. of Udine, Udine, Italy; *Dept. of Biochemistry, Univ. of Trieste, Trieste, Italy; EMBL, °Heidelberg, FRG. In bovine neutrophils a potent cellular defense system against pathogens is associated with a population of large granules, absent in human cells. From granule extracts we have purified highly cationic peptides, named bactenecins (Bac), which are able to exert toxic effects on a variety of bacteria as well as on human herpes simplex virus (HSV1). The smallest bactenecin, Bac1.6, is active at 1-30 μg/ml against both *E. coli* and *S. aureus*. Bac1.6 is a dodecapeptide whose sequence RLCRIVVIRVCR is maintained in a cyclic structure by a disulfide bond. Computer modeling results in a conformation in which the chain adopts an antiparallel extended structure forming a γ-turn at residue 7. Two other peptides, Bac5 and Bac7, from their mass of approx. 5 and 7 kDa, kill gram-negative bacteria, with Bac7 being also able to directly inactivate HSV1. Both peptides are characterized by a high content of Pro (>45%) and Arg (>20%), while the remaining residues are mainly hydrophobic (Ile, Leu, Phe). Determination of their sequence has shown that Bac5 and Bac7 have repeated RPP and PRP sequences, respectively, usually spaced by a hydrophobic residue. Bac5 and Bac7 are stored in the granules of intact neutrophils as probactenecins of about 15 and 20 kDa, respectively. These precursors, devoid of antibacterial activity, are released by the neutrophil, during the defense reaction, into the phagocytic vacuole, where active Bac5 and Bac7 are generated by cleavage of the proforms catalyzed by a neutral serine protease(s).

CK 305 **N-ALKYL Ψ[CH_2NR] PSEUDOPEPTIDE ANALOGS OF NEUROKININ A_{4-10},** Scott L. Harbeson, Stephen H. Buck and Scott A. Shatzer, Merrell Dow Research Institute, Cincinnati, OH 45215. Our efforts have been directed toward the preparation of antagonists of Neurokinin A (NKA, HKTDSFVGLM#) by introduction of the Ψ[CH_2NR] moiety into the backbone of [Leu^{10}]-NKA_{4-10}. MDL 28,564 [Ψ(CH_2NH)^9, Leu^{10}]-NKA_{4-10}, was shown to bind to the NK-2 receptor (hamster urinary bladder) with significant affinity (Ki=200 nM) but was a partial agonist (15% of NKA) in an NKA mediated phosphatidylinositol (PI) turnover assay. Conversion of the Ψ[CH_2NH] pseudopeptide bond of MDL 28,564 to the tertiary amine Ψ[CH_2NCH_3] pseudopeptide bond of MDL 29,916, [Ψ(CH_2NCH_3)^9, Leu^{10}]-NKA_{4-10}, significantly reduced partial agonist activity in the PI turnover assay (5% of NKA) with no effect on receptor affinity (K_i=200 nM). To our knowledge, this is the first report of the introduction of N-alkyl Ψ[CH_2NR] bonds into peptides. The methodology which has been developed for introduction of N-alkyl Ψ[CH_2NR] pseudopeptide bonds into the peptide backbone using the solid phase method will be presented. The effects of these substitutions on peptide conformation and antagonist activity will also be presented.

CK 306 CHEMICAL AND RECOMBINANT DNA SYNTHESIS OF GROWTH HORMONE RELEASING FACTOR (1-40)-OH ANALOGS AND THEIR BIOLOGICAL EVALUATION, Edgar P. Heimer, Arthur M. Felix, Robert S. Siegel,[1] Gregory P. Thiel,[1] Ching-Tso Wang, Robert Campbell, Geneva R. Davies[1] and Theodore Lambros, Roche Research Center, Hoffmann-La Roche Inc., Nutley, N.J. 07110 and [1]The Salk Institute Biotechnology/Industrial Associates, Inc., La Jolla, CA. Human growth hormone releasing factor (1-40)-OH, GRF(1-40)-OH, was one of the first GRF fragments to be described and found to possess the full biological activity of the parent compound, GRF(1-44)-NH_2. Reports from our laboratories have shown that analogs of GRF in which the Gly^{15} residue is replaced by Ala resulted in derivatives which were significantly more potent both *in vitro* and *in vivo*. This increase in biological activity has been postulated to result from increased α-helicity and maximization of amphiphilic structure. We have also shown that Met^{27} can be substituted by Leu which circumvents the potential problem of Met(O) formation. In addition Asn^{28} has been reported to occur naturally in several animal species in place of Ser^{28} (human). We now report on the solid phase peptide synthesis of a series of GRF(1-40)-OH analogs incorporating Ala^{15}, Leu^{27} and Asn^{28}. In parallel a similar series was prepared by recombinant DNA technology using an S. cerevisiae α-mating factor system in continuous fermentation. Details of the chemical synthesis and the recombinant preparation, as well as biological evaluation of these novel analogs, will be described.

CK 307 SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF CONANTOKIN G ANALOGS, J-F Hernandez, B M. Olivera*, L. J. Cruz*, R. Myers, J. Abbott* and J. Rivier, The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA 92037, and *Department of Biology, The University of Utah, Salt Lake City, UT 84112.

The γ-carboxyglutamate (γ)-containing conantokin G (GEγγLQγNQγLIRγKSN-NH_2) (also called "sleeper" peptide), a neurotoxin isolated from the venom of a fish-hunting cone snail (*Conus geographus*) (McIntosh et al. J. Biol. Chem. 259 (23), 14343, 1984) is one of three known and related toxins (all from the genus *Conus*) which share several structural features, including a common N-terminal tetrapeptide. Synthetic conantokin G was shown to induce, when administered *i.c.v.* in mice, a sleep-like state in animals under 2 weeks old and a marked hyperactivity in older mice (J. Rivier et al. Biochemistry 26, 8508, 1987). Recently, we have shown that conantokin G inhibited N-methyl-D-aspartate receptor-mediated elevation of cGMP in neonatal rat cerebellar slices *in vitro* (E. Mena et al. Private communication) suggesting a molecular mechanism for the observed behavioral activity. In order to further clarify this mechanism of action, we synthesized: 1) N-terminally-substituted and deleted analogs and 2) an iodinatable probe which would be used in binding experiments and/or autoradiography, to identify the target of the natural toxin in the mammalian central nervous system and/or in the natural prey. Attempts at correlating structure and activity, after *i.c.v.* administration in mice, are presently being carried out. The acid-sensitive peptide-amides were synthesized on a 2, 4 DMBHA resin, using the strategy and a TFA cleavage, purified by RP-HPLC and characterized by optical rotation, AAA and MS.

CK 308 SYNTHETIC PEPTIDES AS MOLECULAR PROBES OF THROMBIN'S INTERACTIONS WITH SUBSTRATES AND INHIBITORS. Glen L. Hortin, Barbara M. Benutto, and Beverly L. Trimpe, Dept. of Pediatrics, Washington Univ. Sch. Med., St. Louis, MO 63110

Many physiological substrates or inhibitors of thrombin contain tyrosine sulfate residues at potential sites of contact with the protease. Here, interaction of thrombin with sites of sulfation in several proteins-- hirudin (Hir), heparin cofactor II (HCII), and human fibrinogen γ'-chain (γ')-- was examined using synthetic peptides. Clotting of purified fibrinogen by thrombin was inhibited by peptides, where the residues of corresponding protein sequence are indicated in parentheses, at the IC_{50} values: sulfato-Hir(54-65), 0.17 μM; Hir(54-65), 1.3 μM; HCII(49-75), 28 μM; HC II(54-75), 38 μM; γ'(410-427), 130 μM. Control peptides-- sulfato-cholecystokinin octapeptide and α_2-antiplasmin(434-452)-- at 250 μM inhibited clotting less than 50%. Anticlotting peptides did not block thrombin's active site. Hydrolysis of chromogenic substrates S-2366 and Chromozyme TH was unhindered. In fact, the peptides allosterically increased thrombin's affinity for substrates. All of the anticlotting peptides decreased K_m for these substrates up to 50% without changing V_{max}. Peptide concentrations inducing this effect paralleled those required for anticlotting activity. The results suggest that the peptides bind to a common site and exert the same allosteric effect. Thrombin's anion-binding exosite, proposed site of interaction with hirudin peptides, apparently has broad binding specificity. Peptides serve as useful reagents to examine allosteric modulation of thrombin and interactions with natural substrates and inhibitors.

CK 309 THE DESIGN AND STUDY OF ALKALINE PHOSPHATASE/SOMATOSTATIN HYBRID PROTEINS AS PROBES FOR SOMATOSTATIN-RECEPTOR INTERACTIONS

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The utility of inserting peptide hormones into the surface loops of globular proteins in order to probe hormone-receptor interactions is being investigated. A highly active cyclic analogue of somatostatin (Veber et al. (1981) Nature 292, 55-58) indicates a requirement for a β turn centered around Trp and Lys for potent receptor interactions. Computer graphic analysis indicated that a partial somatostatin sequence could be constrained in the same bioactive conformation by insertion into an Ω loop on the surface of alkaline phosphatase. The amino acids 92 - 94 of the Ω loop-like structure were replaced by the following somatostatin sequence in one step by oligonucleotide directed in vitro mutagenesis: Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser. The fusion protein was purified by the standard procedure for isolation of the wild type alkaline phosphatase. The phosphatase activity of the mutant protein is about 20% lower than that of the wild type protein. Trypsin cleaves the hybrid protein specifically at the somatostatin loop without loss of phosphatase activity. A commercial available polyclonal antibody raised against somatostatin recognizes the hybrid protein but not the wild type alkaline phosphatase. The IC_{50} for binding to membrane fractions of the rat cortex is about 1 μM for both the fusion protein and the wild type alkaline phosphatase. The IC_{50} for Somatostatin is about 1 nM.These results indicate that the requirements for specific interactions with the somatostatin loop on the surface of the hybrid protein are more stringent for the brain membrane receptor than they are for trypsin or somatostatin antibodies. Additional mutants are under investigation.

CK 310 THE CHEMICAL SYNTHESIS OF HUMAN INSULIN. John P. Mayer, Gerald S. Brooke, Harlan B. Long, and Richard D. DiMarchi, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, Indiana 46285

The total chemical synthesis of human insulin A and B chains was achieved by automated solid phase methodology. Double coupling was performed at each residue with either the symmetrical anhydride or HOBt ester where appropriate. The protecting group scheme utilized was as follows: Glu(OcHex), Arg(Tos), His(pi-BOM), Cys(4-MeBzl), Lys(2, Cl-Z), Tyr(2, Br-Z), Ser(Bzl), Thr(Bzl). Emphasis was placed on optimizing assembly efficiency, HF cleavage, solubilization, and purification as well as evaluating the extent of side reactions especially those related to cysteine. The assembled peptide-resins were cleaved under high HF conditions (90/5/5 HF: p-thiocresol: m-cresol), converted to their respective S-sulfonates and assayed by reversed-phase chromatography against external standards. After desalting, the crude peptides were purified by preparative reversed-phase chromatography (>95% purity) and characterized by HPLC, FAB-MS, amino acid analysis and partial preview sequencing. Disulfide pairing of the A- and B-Chain S-sulfonates was performed by co-reduction with DTT in an oxygen containing atmosphere. The methodology optimized above was used to prepare several IGF-I/insulin hybrids whose biological activities will be presented.

CK 311 NEOKYOTORPHIN FROM THE BRAIN OF HIBERNATING GROUND SQUIRRELS AND ITS SPECIFIC BIOLOGICAL ACTIVITY, Inessa I. Mikhaleva*, Vladimir I. Svieryaev*, Boris V. Vaskovsky*, Rustam H. Ziganshin*, Vadim T. Ivanov*, Yuri M. Kokoz**, Alexander A. Povzun** and Stella G. Kolaeva**, *Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Moscow, USSR, **Institute of Biological Physics, USSR Academy of Sciences, Moscow Region, USSR

To study unknown peptide factors responsible for regulation of hibernation state of animals the extract of whole brain of hibernating ground squirrels *Citellus undulatus* was subjected to ultrafiltration, gel-filtration and HPLC separation. All fractions obtained were tested on hypothermic, antimetabolic and cardiotropic activity. The latter test was based on differences between the hearts of hibernators and non-hibernators that in electrophysiological terms might be attributed to changes in electrogenesis regulation (absence of the plateau phase and delayed stage of repolarization in heart action potential). The RP-HPLC fractions were tested for their ability to influence Ca^{2+}- and K^{+}-currents in atrial frog preparations by patch-clamp technics. Components able to increase or inhibit Ca^{2+}- and K^{+}-currents were localized. One of them appeared to be neokyotorphin - an analgetic pentapeptide isolated earlier from bovine brain. We found that synthetic neokyotorphin in 0.5 μM concentration induces a twofold increase of voltage-dependent Ca^{2+}-current in the atrial fibres, strongly inhibits K^{+}-current through normal rectifier K^{+}-channels and increases intracellular Ca^{2+}-concentration in rat myocardial cells. It also sharply accelerates heart rate and arousal of hibernating animals. Therefore neokyotorphin might be considered as a factor responsible for transition of hibernating animals to active state.

CK 312 STRUCTURE-ACTIVITY STUDIES ON INHIBITORS OF RECOMBINANT HIV-1 PROTEASE Michael L. Moore*, William M. Bryan, Stephen A. Fakhoury and William F. Huffman, Department of Peptide Chemistry, and Thomas J. Carr, Arthur C. Chandler III, Brian D. Dayton, Thomas D. Meek, Brian W. Metcalf, Thaddeus A. Tomaszek, Jr. and Geoffrey B. Dreyer, Department of Medicinal Chemistry, Smith Kline & French Laboratories, King of Prussia, PA 19406.

Using structure-activity data based on peptide substrates, a series of inhibitors of the HIV-1 protease was prepared in which the scissile dipeptide was replaced by a variety of non-hydrolyzable dipeptide isosteres. Inhibitors containing the hydroxyethylene isostere were considerably more potent than those containing reduced peptide bond, phosphinate or hydroxymethylene isosteres, behaving as competitive inhibitors with K_i values in the 10–100 nM range. Structure-activity relationships of hydroxyethylene isostere inhibitors generally agreed with those of substrates with respect to peptide length and amino- or carboxy-terminal blocking groups.

CK 313 A SERUM FACTOR ENHANCING THE GROWTH OF LIVER TUMOR IN HEPATECTOMIZED MICE, Hideki Morimoto, Yoshinori Nio, Shiro Imai, Takahiro Shiraishi, Michihiko Tsubono, Chen-Chiu Tseng and Takayoshi Tobe, First Department of Surgery, Kyoto University Faculty of Medicine, Kyoto 606, Japan

DS mice and their syngeneic SC 42 carcinoma were employed in the present study. Hepatectomy significantly accelerated the growth of tumor inoculated in the rest liver. This tumor growth-enhancing effect depended on the volume of removed liver. Liver tumors grew three times in weight in 40% hepatectomized mice as much as those in sham-operated mice on day 16 after inoculation. Moreover, an in vitro DNA synthesis (^{3}H-thymidine incorporation) of the tumor cells increased by addition of sera of hepatectomized mice. The sera on day 3 after hepatectomy showed the maximal enhancing effect of DNA synthesis, and this effect was not shown by addition of sera on day 49 after hepatectomy. (Liver completely regenerates to the original volume by day 28 after 40% hepatectomy in this model.) An analysis using electrophoresis suggested that the factor, which enhanced tumor growth and closely related with liver regeneration in this model, may appear in sera early after hepatectomy. We are studying whether or not this factor in mouse is same as Hepatogrowth Factor (HGF) in human and rat.

CK 314 TRIALKYLSILANES AS SCAVENGERS IN THE ACIDIC DEBLOCKING OF PROTECTING GROUPS IN PEPTIDE SYNTHESIS, Daniel A. Pearson, Mary Blanchette, Mary Lou Baker, Cathy A. Guindon, Peptide Research Lab, Immunetech Pharmaceuticals, 11045 Roselle St., San Diego, CA 92121. Triethylsilane and triisopropylsilane were used as scavenging agents in peptide synthesis in the acidic deblocking of protecting groups. The efficiency of scavenging ability was compared with anisole and ethanedithiol in a kinetic experiment. It was found that triethylsilane/TFA was a particularly effective scavenger in the removal of the cysteine S-trityl group in peptides containing many cysteines. It was also found that triethylsilane/TFA reduces the indole ring of tryptophan. When triisopropylsilane is used instead this reduction is markedly retarded.

CK 315 EFFECT OF EXOGENOUS LIPOCORTIN-1 (LC1) AND PLA_2 INHIBITORY PEPTIDE (ANTIFLAMMIN) ON THE RESPONSE OF Th2 CELLS TO IL-1.

M.Rocio Sierra-Honigmann and Patrick A. Murphy, Department of Medicine, Division of Infectious Diseases, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The lipocortins (LC) were originally described as a family of phospholipase-A_2 (PLA_2) inhibitory proteins whose synthesis is up-regulated by corticosteroids. Exudates from steroid treated animals are a rich source of LC. One of these proteins, LC1, inhibits PLA_2 *in vitro* when purified to homogeneity. A nine amino acid synthetic peptide based on the sequence of LC1 has been shown to possess PLA_2 inhibitory activity both *in vivo* and *in vitro*, as well as a potent anti-inflammatory action (*Nature*, 335:726,1988). This peptide and some of its variants are known as antiflammins. Because IL-1 is known to activate PLA_2 in many cell systems, we decided to investigate whether purified LC1 or synthetic antiflammin could suppress IL-1 action on T cells. Both substances inhibited the response of the D10.G4.1 clone to IL-1 plus 3D3 (α-T cell receptor, clonotypic mAb), but did not inhibit the response to IL-2 plus 3D3. This suggests that the action of IL-1 on these cells requires the activity of PLA_2.

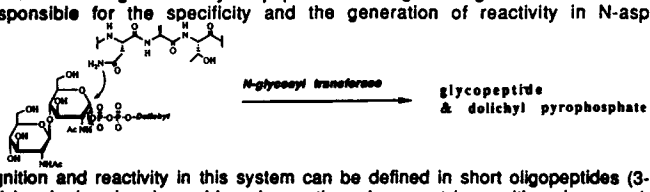
CK 316 HUMAN IMMUNODEFICIENCY VIRUS (HIV) ASPARTYL PROTEASE-TARGETED INHIBITORS: DESIGN, SPECIFICITY AND STRUCTURE-ACTIVITY RELATIONSHIPS, Tomi K. Sawyer, Alfredo G. Tomasselli, Charles S. Craik*, Roger A. Poorman, Ben M. Dunn**, W. Gary Tarpley, John O. Hui, Dianne L. DeCamp*, Paula E. Scarborough**, Tom J. McQuade, Douglas J. Staples, Li Liu, V. Susan Bradford, Carol A. Bannow, John H. Kinner, Jessica Hinzmann, and Robert L. Heinrikson, Biopolymer Chemistry and Infectious Diseases Units, Discovery Research Division, The Upjohn Company, Kalamazoo, MI 49001, *Departments of Biochemistry/Biophysics & Pharmaceutical Chemistry, University of California, San Francisco, CA 94143, and **Department of Biochemistry, University of Florida, Gainesville, FL 32610

HIV GAG/POL peptides having systematically modified P_1-P_1' dipeptide substitutions were characterized by their substrate/inhibitor properties against recombinant HIV-1 and HIV-2 aspartyl proteases. The specificities of selected HIV aspartyl protease inhibitors were also determined based on their effects on human renin, pepsin, cathepsin-D, cathepsin-E, and gastricsin. The design of a potent HIV aspartyl protease inhibitor, H-Val-Ser-Gln-Asn-Leuψ[CH(OH)CH_2]Val-Ile-Val-OH (U-85548E), and the structure-activity relationships of U-85548E derivatives will be described. Such compounds provide prototype leads for the development of anti-HIV therapeutic agents which effect inhibition of viral replication/maturation/infectivity by targeting HIV protease within host cells (*e.g.*, lymphocytes, macrophages, monocytes).

CK 317 EVIDENCE FOR A DIRECT INVOLVEMENT OF THE CARBOXYL TERMINUS OF HUMAN GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR IN RECEPTOR BINDING, Gail F. Seelig, Winifred W. Prosise, Julie E. Scheffler, Tattanahalli L. Nagabhushan and Paul P. Trotta, Schering-Plough Research, U.S.A., Bloomfield, N.J. 07003

Rabbit polyclonal antiserum (345-6) was raised against a synthetic peptide corresponding to the linear segment 110-127 of the C-terminal sequence of GM-CSF. Purified IgG from Ab 345-6 recognized both peptide 110-127 and purified E. coli-derived recombinant GM-CSF (rh-GM-CSF) in a direct ELISA. Ab 345-6 antagonized the binding of 125-I-rh-GM-CSF to the KG-1 cell line and inhibited rh-GM-CSF-dependent proliferation of the AML-193 cell line. Rabbit polyclonal antiserum raised to purified rh-GM-CSF, Ab 349-6, bound to peptide 110-127 in an ELISA and antagonized binding of 125-I-rh-GM-CSF and proliferation of the KG-1 cell line. Peptide 110-127 did not inhibit binding or cellular proliferation at concentrations up to 1,000 ng/ml, nor did it exhibit agonist activity on KG-1 or AML-193 cells. Anti-idiotypic antiserum was prepared by immunizing sheep with the purified IgG fraction. The resulting sheep antiserum, Ab 1418, recognized Ab 345-6 on a direct ELISA but did not recognize rh-GM-CSF or the peptide 110-127 to which Ab 345-6 was raised. Antiserum as well as a purified IgG fraction inhibited 125-I-rh-GM-CSF receptor-binding and cell proliferation. Pre-immune serum had no effect on these assays. Purified Ab 1418 did not inhibit either the ability of interleukin 3 to stimulate the proliferation of the AML-193 cell line, or binding of 125-I-interleukin 4 to Daudi cells. These data directly support that at least a portion of the 110-127 sequence of GM-CSF is directly involved in receptor interaction.

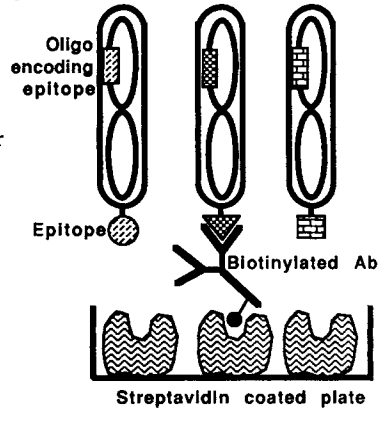
CK 318 PEPTIDE CONFORMATION AS A DETERMINANT IN PROTEIN PROCESSING REACTIONS, Karen L. Shannon and Barbara Imperiali, Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125. The synthesis of novel conformationally restricted amino acids continues to play a vital role in elucidating the relationship between structure and biological activity of peptides. Our general goal has been to understand the mechanism responsible for the specificity and the generation of reactivity in N-asparagine-linked glycosylation.



Biological recognition and reactivity in this system can be defined in short oligopeptides (3-12 amino acid residues) in which a hydroxyl amino acid, serine or threonine, must be positioned one residue away from the reactive asparagine. By restricting the conformational space available to the oligopeptide through the use of α,α-disubstituted amino acids, D-amino acids, cyclic imino acids (such as proline and pipecolic derivitives), and by correlating these results with kinetic behavior in the glycosylation assay, we aim to elucidate key mechanistic details in asparagine glycosylation. The peptides assayed to date fall into three distinct categories, glycosylation substrates, glycosylation inhibitors, and nonbinders. This poster will aim to define the peptide secondary structure which is consistent with our experimental results.

CK 319 AN EPITOPE LIBRARY, George Smith*, Jamie Scott* and Bill Dower#
*Division of Biological Sciences, University of Missouri, Columbia, MO 65211; & #Affymax Research Institute, 3180 Porter Drive, Palo Alto, CA 94304

We are developing a way of surveying vast numbers of short peptides for tight binding to an antibody, receptor or other binding protein. The centerpiece of the technique will be an "epitope library": a mixture of 100 million filamentous phage clones, each displaying a small peptide epitope on the surface. The epitopes will be encoded by oligonucleotides spliced into a coat-protein gene. Those phage in the library whose displayed epitope binds tightly to a given antibody (or other binding protein) can be affinity-purified (see diagram), propagated in bacteria, and their displayed peptides sequenced at the DNA level. Results of surveying small model libraries with antibodies against myohemerythrin will be presented.



CK 320 NEW APPROACHES TO THE DESIGN OF PROTEIN KINASE C INHIBITORS.
A.Tartar, A.Ricouart and C.Sergheraert. Chimie des Biomolécules, URA CNRS 1309, Institut Pasteur, 1 rue du Prof. Calmette, 59019 LILLE Cédex (France)
Protein kinase C (PKC) is recognized as a major regulatory enzyme implicated in the control of a wide variety of physiological processes. Using the consensus recognition pattern of PKC substrates, it is possible to synthesize short peptides which, depending on the presence of a phosphorylable residue will be substrates or inhibitors of the enzyme. A major drawback of these peptides is their sensitivity to proteases. Using retro-inverso analogues, we have been able to obtain potent PKC inhibitors which remain totally unaffected even after prolonged treatment with various proteases. We have also tried to improve affinity and specificity through the design of bisubstrate inhibitors. To achieve phosphorylation, the catalytic domain of PKC must interact with both ATP and the peptidic substrate. By covalent association of mimics of these two components through a suitable linker, it is possible to take advantage not only of an increased binding energy but also of a significant entropic contribution. A series of constructs, using quinolinesulfonamide derivatives as ATP mimics linked to peptide moieties corresponding to the substrate consensus recognition pattern of PKC, was prepared. One of these compounds was 20 fold more active than H7, towards PKC while the corresponding separate components were almost inactive when used as a mixture.

CK 321 Synthesis and Deletional Studies on a Lysosomal Enzyme Activator Protein. Tomich, J.M., Weiler, S., Cai, B. Nelson, M. and Barranger, J.A. University of Southern California School of Medicine, Childrens Hospital of Los Angeles, Division of Medical Genetics.

Human Saposin C is an 82 residue sphingolipid activator protein that specifically increases the activity of the lysosomal enzymes glucocerebrosidase and galactocerebrosidase. The sequences of the Saposin C molecule as well as its precursor form, prosaposin, have been deduced from the cloned c-DNA (J. O'Brien et. al. *Science* 241, 1098-1101, 1988). We have chemically synthesized the saposin C (82 mer) using solid phase methods with t-boc amino acids. Upon cleavage and refolding we generate a fully active molecule - as compared to biologically produced material. The synthetic peptide also possesses an identical affinity constant for homogeneous glucocerebrosidase.

Through a series of deletional analyses we are able to assign the catalytic enhancement activity to an 18 residue fragment. This peptide possesses > 80% of wild type activity although with a significantly lower activation constant. Initial structural analyses suggest that this 18-mer has the ability to form an amphipathic helix. Studies are currently in progress to elucidate the enzyme binding site and nature of the protein-protein interaction.

CK 322 BOVINE TRANSFORMING GROWTH FACTOR-α: SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY, *Jacob S. Tou, *Mike F. McGrath, #Mark E. Zupec, *John C. Byatt, *Bernie N. Violand, *Larry A. Kaempfe and *Billy D. Vineyard, *Animal Sciences Division and #Biological Sciences Division, Monsanto Company, Chesterfield, MO 63198

The bovine sequence of transforming growth factor-α (bTGF-α) was recently identified. It is highly homologous to the related human and rat sequences with 50 amino acid residues containing three disulfide linkages. Pure bTGF-α was synthesized via an automatic peptide synthesizer and purified to homogeneity in high yield. The integrity of this synthetic peptide was confirmed by HPLC, amino acid analysis, mass spectrometry, sequencing and peptide mapping. The disulfide linkages of this synthetic bTGF-α and a pure misfolded peptide (side product) were established by peptide mapping. In the bovine liver radioreceptor assay, bTGF-α competed with radiolabeled mEGF and had activity comparable to mEGF and hTGF-α. This synthetic peptide significantly increased bovine mammary cell proliferation. The response was greater than that observed by hEGF. The misfolded bTGF-α, however, showed very low activity in both the receptor binding and the cell proliferation assays.

CK 323 STRUCTURAL CORRELATES FOR ATRIAL NATRIURETIC FACTOR (ANF) ANTAGONISM, Thomas W. von Geldern, Diane M. Beno, Gerald P. Budzik, Terry P. Dillon, William H. Holleman, Terry J. Opgenorth, Todd W. Rockway, Alford M. Thomas, and Siobhan B. Yeh, Cardiovascular Research Division, Abbott Laboratories, Abbott Park, IL 60064.

ANF, a 28-amino acid peptide hormone secreted by atrial cardiocytes, is involved in the maintenance and regulation of fluid volume homeostasis. Several distinct ANF receptors exist, and the hormone has a variety of physiological and pharmacological effects, including the stimulation of cyclic-GMP production, inhibition of ACTH-induced aldosterone biosynthesis, and opposition of the renin-angiotensin system. Sorting out these individual receptor-effector systems is a difficult problem which would be simplified by the availability of selective ANF antagonists. Such antagonists would also help elucidate the involvement of ANF in specific disease states.

Structure-activity studies of ANF analogues by our group have revealed a set of residues which seem to be responsible for transduction of the ANF binding "signal". Nonisofunctional replacement of these residues leads to compounds showing ANF antagonist properties. While a single substitution is often sufficient to confer antagonist activity, better antagonists result from wholesale replacement of the critical residues. We have found this residue replacement strategy to be generally effective, and have succeeded in creating antagonists from several families of smaller ANF agonists. Antagonism of ANF binding, functional responses and *in vivo* activity will be discussed.