SYNTHETIC PEPTIDES: APPROACHES TO BIOLOGICAL PROBLEMS

Organizers: James Tam and Thomas Kaiser

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Synthetic Peptides: Approaches to Biological Problems

Peptide Synthesis

V 001 COGNITIVE FEATURES OF CONTINUOUS ANTIGENIC DETERMINANTS, H. Mario Geysen, Tom J. Mason and Stuart J. Rodda, Department of Molecular Immunology, Commonwealth Serum Laboratories, Parkville, Victoria 3052, Australia.

Antigen-antibody interactions represent a diverse set of recognition events between large molecules. The minimum repertoire of antibodies necessary to achieve specific binding depends on the cognitive features of the sites of interaction of both the antigen and the antibody. If low stringency in the requirements for successful antigen-antibody binding were a cognitive feature of epitopes, then a small minimum set of antibodies would be required in the repertoire. However, this would increase the likelihood of an induced antibody population recognizing (cross-reacting with) a self molecule. An understanding of the features of an antigen which determine recognition would be of great value to the understanding of protein-protein interactions in general, and to the design of peptides with specific biological effects.

Recent advances in methods for the simultaneous synthesis of large numbers of peptides have made possible the systematic assessment of the effects of amino acid substitution on recognition between peptide epitopes and the corresponding antibody. The ability of antibodies to bind to peptide analogues of the epitopes recognized by those antibodies was studied. This form of analysis identifies the allowed substitution pattern for each residue. A total of 103 epitopes within 63 well-defined antigenic peptides homologous with the relevant antigen sequence were identified, and the contribution of each amino acid residue to the antibody binding activity of each epitope was investigated. For each residue in the epitope, complete sets of peptide analogues containing single amino acid replacements were used to determine the alternative amino acids for which antibody binding activity was retained. The data are summarized in a replaceability matrix which indicates relationships between amino acids in terms of recognition by antibodies. In addition, the average number of residues with limited replaceability in continuous epitopes, and the frequency with which each amino acid is found in those epitopes, was determined. Finally, the potential for cross-reactivity between different antigens and a given antibody is discussed.

V 002 **MULTIPLE ANTIGEN PEPTIDE SYSTEM: A NOVEL DESIGN FOR VACCINE,** **James P. Tam, The Rockefeller University, 1230 York Ave., New York, N.Y. 10021.**

A convenient and versatile approach to the synthesis of a peptide-antigen carrier suitable for generating antibodies and vaccines is described. The approach known as the "multiple antigen peptide" system (MAP) ultilizes a simple "scaffolding" of a low number of sequential levels (n) of a trifunctional amino acid as the core carrier and 2^n peptide antigens to form a macromolecule with a high density of peptide antigens. A MAP model chosen for study was an octabranching MAP consisted of a core-matrix made up of three levels of lysine and eight amino ends for anchoring peptide antigens. The MAP containing both and the carrier and antigenic peptides was synthesized as a single manipulation by the solid-phase method. Five such octabranching MAPs with peptide antigens of nine to sixteen residues were tested in rabbits and mice. All elicited good immunogenic responses of high titered-antibodies and four of the five were reactive with their native proteins. Thus, the MAP provided a general but chemically unambiguous approach for the preparation of antibodies of predetermined structure and may be suitable for generating vaccines.

Structure Determination

V 003 MASS SPECTROMETRIC ANALYSIS OF SYNTHETIC PEPTIDES, Brian T.Chait, Rockefeller University, New York, N.Y. 10021.

The utility of the newer bombardment mass spectrometric methods (^{252}Cf fission fragment mass spectrometry, fast atom bombardment mass spectrometry, tandem mass spectrometry) for the analysis of synthetic peptides is discussed. It is shown that mass spectrometry comprises one of the most useful, rapid and definitive methods for confirming the accuracy of peptide synthesis, for determining the purity of the final product, and for providing information concerning errors in the synthesis. Examples are given successful mass spectrometric analyses of both protected and unprotected polypeptides with molecular weights up to 16,000 daltons. The use of mass spectrometry for the identification and quantification of byproducts to allow rapid, rational improvement and optimization of the chemistry of peptide synthesis is also discussed.

V 004 INSERTION OF A WATER MOLECULE INTO AN α-HELIX BACKBONE AND OTHER MODES OF HYDRATION: X-RAY STRUCTURE ANALYSIS. I. L. Karle, Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, D.C. 20375-5000.

Single crystal x-ray diffraction structure analyses have identified the manner in which water molecules are essential to creating mini-polar areas on apolar helices. Amphiphilic helical structures have a sequence of residues such that the polar side chains occur on one side of the helix (with affinity for water and ions) and the apolar side chains occur on the other side of the helix (with hydrophobic characteristics). Completely apolar peptides, such as membrane-active peptides, can acquire amphiphilic character by at least two different means, for example: (I) Curving of the helix by the presence of Pro residues. The helix of Boc-Trp-Ile-Ala-Aib-Ile^{5}-Val-Aib-Leu-Aib-Pro^{10}-Ala-Aib-Pro-Aib-Pro^{15}-Phe-OMe is bent by ~30° at Pro^{10}. The bend in the helix causes carbonyl oxygens O(7), O(9), O(10) and O(12) to be exposed to the exterior. Except for O(9), these atoms do not participate in any intrahelical hydrogen bonds, but are available for hydrogen bonding (or perhaps ligand formation) with external moieties. In the crystal, additional water molecules cocrystallize in the vicinity of the protruding C=O groups and form hydrogen bonds with them. (II) Insertion of a water molecule into the helical backbone of Boc-Aib-Ala-Leu-Aib-Ala-Leu-Aib-Ala-Leu-Aib-OMe. The C-terminal half assumes an α-helix conformation, whereas the N-terminal half is distorted by an insertion of a water molecule W(1) between N(Ala^{5}) and O(Ala^{2}), forming hydrogen bonds N(5)H···W(1) and W(1)···O(2). The insertion of the water molecule causes changes in the torsional angles only at $C^{\alpha}(3)$. The remaining ϕ and ψ angles are quite normal for a 3_{10}- or α-helix. The distortion of the helix exposes C=O(Aib^{1}) and C=O(Aib^{4}) to the outside environment with the consequence of attracting additional water molecules. The solvent molecules form polar channels between the polar sides of neighboring peptide molecules in the crystal. The other side of the helical molecule is completely hydrophobic with the leucyl side chains from neighboring molecules interdigitating. The undulating polar channels in the molecular aggregates of this model apolar peptide suggest a mode of ion transport through membranes by largely hydrophobic peptides.

Synthetic Peptides: Approaches to Biological Problems

V 005 IDENTIFICATION OF FOLDED STRUCTURES IN PEPTIDE FRAGMENTS OF PROTEINS BY 2D NMR SPECTROSCOPY, Peter E. Wright, H. Jane Dyson, Satoshi Ebina, Mark Rance, Richard A. Houghten and Richard A. Lerner, Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037.
State-of-the-art two-dimensional NMR techniques provide an important experimental approach to elucidation of transient structures formed by peptide fragments of proteins in water solution. Most of our studies to date have concerned immunogenic peptides (or their derivatives) which are able to induce protein-reactive anti-peptide antibodies. Two classes of secondary structure have been identified, reverse turns and nascent helix. The factors which stabilize and modify the β-turn structure have been investigated by systematic substitution of amino acids at key positions. For the peptides of sequence YPXDV, the β-turn populations in water correlate with the β-turn probabilities determined from protein crystal structures. This shows that short-range interactions determined by the local amino acid sequence are potentially able to dictate secondary structure in the absence of the medium- and long-range interactions found in folded proteins. The observation of structure in linear peptide fragments of proteins in water solution has important implications for both immunological recognition and for initiation of protein folding.

Protein Engineering

V 006 DESIGN AND CONSTRUCTION OF BIOLOGICALLY ACTIVE PEPTIDES, E.T. Kaiser, Laboratory of Bioorganic Chemistry and Biochemistry, The Rockefeller University, New York NY 10021-6399.
Starting from the design and construction of peptides containing amphiphilic secondary structural regions, we have recently undertaken the preparation of small proteins and their structural analogs by peptide segment synthesis-condensation. An example of such a protein is the enzyme ribonuclease T_1. This enzyme contains an amphiphilic helix which lies in proximity to a series of β-strands. The active site residues of the enzyme are removed significantly from the helix. As the target for our first structural analog, we have chosen to replace the naturally-occurring helix by a designed helix which has a quite different sequence but appears to be likely to pack properly against the other portions of the enzyme molecule. The construction of the native sequence and progress towards the preparation of the mutated enzyme containing the α-helix replacement will be described.

V 007 THE DE NOVO DESIGN OF FOUR-HELIX BUNDLE PROTEINS, Lynne Regan, William DeGrado, Zelda Wasserman, Cand Siew Ho, C.R.&D. The Experimental Station, E.I. Du Pont De Nemours and Co. Wilmington DE 19898.

We have adopted a synthetic approach to understanding the structural basis for protein function. In order to test ideas on interations which are believed to be important in protein folding and stability, we are attempting to design simple proteins which should fold into predetermined three-dimensional structures. Our designs have focussed on an idealized version of a four helix bundle protein. This folding pattern is found in a variety of naturally occurring proteins, such as myohemerythrin, cytochrome C', apoferritin and growth hormone. One design approach was to simplify the model by designing a bundle formed of four identical helices, then to optimise the sequence of the helix to give stable packing interations. Subsequently, different loop sequences, by which to connect the helices, were designed and tested. The sucess of this step-wise approach allowed us to acheive a stable design for the full-lenght, 73 amino acid protein. Using recombinant DNA techniques we were able to synthesise the complete protein, which is indeed able to fold into a stable helical structure as we designed.

V 008 SOME DESIGN CONSIDERATIONS FOR PROTEIN SECONDARY STRUCTURE, Leonard G. Presta and George D. Rose, Department of Biological Chemistry, Pennsylvania State University, Hershey Medical Center, Hershey, PA 17033.

Recent efforts in protein engineering have focused on changing the specificity or enhancing the stability of natural proteins as well as designing elements of secondary structure or even entire proteins *de novo*. We have been analyzing segments of secondary structure in X-ray elucidated molecules in order to extract general principles that can be used in protein design. Elements of secondary structure can be classified as ***repetitive*** and ***non-repetitive***. Both helix and sheet are repetitive structures because their residues have repeating mainchain torsion angles, (ϕ and ψ), and their backbone N-H and C=O groups are arranged in a periodic pattern of hydrogen bonding. Non-repetitive regions, those with non-repeating mainchain angles, include reverse turns and Ω-loops. Turns are sites where the polypeptide chain changes its overall direction, and their frequent occurrence is responsible for the globularity of globular proteins. Loops are chain segments that trace a "loop-shaped" path in space, with small end-to-end distance between their segment termini. The segment mainchain of an idealized loop resembles a Greek omega (Ω). Useful generalizations from both repetitive and non-repetitive categories will be discussed, with particular emphasis on α-helices and Ω-loops.

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Synthetic Peptides: Approaches to Biological Problems

V 009 The Conformational Restriction Of Peptidyl Immunogens With Covalent Replacements For The Hydrogen Bond,
Arnold Satterthwait,[1] Thomas Arrhenius,[1] Robert Hagopian,[1] Lin Chiang,[1] Fidel Zavala,[2] Victor Nussenzweig,[2] and Richard Lerner,[1] [1]Research Institute of Scripps Clinic, La Jolla, CA and [2]New York University Medical Center, New York, N.Y.

A major goal of peptide chemistry is the development of synthetic techniques for restricting peptide conformations to biologically active forms. Our approach is to replace putative amide-amide hydrogen bonds with covalent hydrogen bond mimics. Covalent substitutes for the hydrogen bond could be employed in the syntheses of a wide variety of regular and irregular peptidyl structures. Model studies indicate that a N-amino-methylamidinium link (N-CH2-NH"CR"NH+) and hydrazone-hydrocarbon links (N-N=CH-CHR-CH2, N-N=CH-CR=CH) can serve as replacements for the amide-amide hydrogen bond (N-H•••O=C-NH). Both mimics are stable at neutral pH and ambient temperatures. The hydrazone-ethane link has been substituted for an (i + 4)→i hydrogen bond in a series of pentapeptides with the potential for forming single turns of an alpha helix.

Chemically shaped peptides could serve as enhanced immunogens. To evaluate this strategy, a potential synthetic vaccine for malaria, (Asn-Ala-Asn-Pro)$_n$-NH_2, was conformationally restricted. Chou-Fasman analysis indicates a preference of the repeating tetrapeptide, Asn-Pro-Asn-Ala, for a reverse turn conformation which brings asparagine side chains into close proximity for hydrogen bonding. A site-directed method for replacing putative hydrogen bonds between asparagine side chains was developed. Using solid-phase synthesis, ethylene bridges were inserted between carboxamide side chains in a site-directed manner. Two dodecamers with asparagine side chains covalently linked with ethylene bridges either around proline (acetyl-(NPNA)$_3$-NH_2, peptide A) or around alanine (acetyl-(NANP)$_3$$NH_2$, peptide B) were synthesized. Antibodies to peptide A show a strong cross-reaction with P. falciparum sporozoites, a form of malaria which afflicts hundreds of millions of people each year.

Bioactive Conformation

V 010 THE ROLES OF THE SIGNAL SEQUENCE IN PROTEIN SECRETION.
Lila M. Gierasch[a], C. James McKnight[a], Maria Rafalski[a], Martha S. Briggs[a*], L. Chen[b], and P. C. Tai[b], a) Department of Chemistry, University of Delaware, Newark, DE 19716 and b) Boston Biomedical Research Institute, 20 Staniford Street, Boston, MA. (*Present address: Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104).
Targeting of nascent proteins to the export pathway and their translocation across either the cytoplasmic membrane (in prokaryotes) or the endoplasmic reticulum membrane (in eukaryotes) require a signal sequence. Yet, the mechanisms by which these relatively short (15 to 30 residues) and highly variable sequences facilitate these processes are still poorly understood. We have been applying biophysical methods to genetically-defined signal sequences from bacterial export proteins in order to characterize the properties that are correlated with ability of signal sequences to function in vivo. The capacity to adopt an alpha helical structure appears to be required of functional signal sequences. Furthermore, those sequences that are phenotypically export-competent in vivo show a high affinity for organized lipid assemblies when examined as isolated signal peptides, while export-defective sequences show reduced lipid affinity. Spectroscopy of signal peptide/lipid monolayers suggests that a surface-associated beta structure can occur when signal peptides are not inserted into the monolayer (at high lipid surface pressure), but that insertion is accompanied by a transition to an alpha helix.[1] Fluorescence studies of tryptophan-containing signal peptides show that the peptides insert well into the hydrophobic core of a lipid bilayer in a vesicle and that kinetics of insertion are rapid. These results will be discussed in terms of a model for the interactions of signal peptides with lipid systems and the importance of the intrinsic properties of signal sequences. The implications of this model for signal sequence function in vivo will be evaluated; recent evidence will be presented from reconstitution of bacterial protein export in vitro demonstrating that signal peptides interact with a proteinaceous species.[2]

This work was supported in part by grants from the NIH and the NSF.

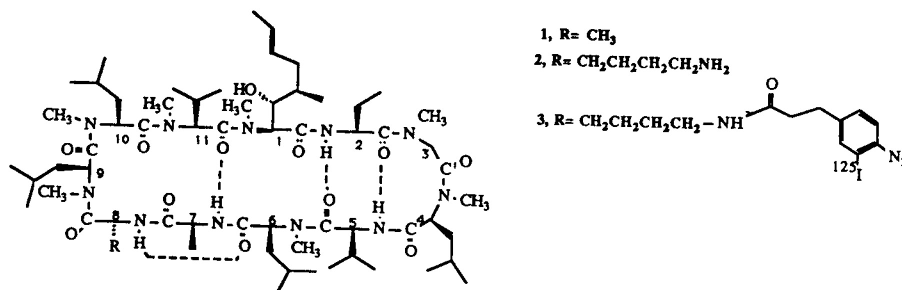
1. M. S. Briggs, L. M. Gierasch, A. Zlotnick, J. Lear and W. F. DeGrado, Science, 228. 1096-1099 (1985)
2. L. Chen, P. C. Tai, M. S. Briggs and L. M. Gierasch, Journal of Biological Chemistry, 262. 1427-1429 (1987)

Synthetic Peptides: Approaches to Biological Problems

V 011 SYNTHESIS AND ACTIVITY OF PHOTOAFFINITY LABELING AND CONFORMATIONALLY CONSTRAINED ANALOGS OF CYCLOSPORINE,

Daniel H. Rich, Roger D. Tung, J. Aebi, Brian Dunlap, William Mellon and A. Ruoho, School of Pharmacy, University of Wisconsin-Madison, Madison WI 53706.

Cyclosporine A (CsA, 1) is an orally active, cyclic undecapeptide which displays potent immunosuppressive and antiparasitic activities, and which is used widely to suppress rejection of transplanted human organs. The conformation of CsA in chloroform solution is known but the bioactive conformation of CsA at its receptor(s) is not known nor has the identity of the biologically relevant CsA receptor been established. We have developed new, simplified chemical methods for synthesizing the major fragments of the CsA ring (the MeBmt, the 8-11 tetrapeptide and the 2-7 hexapeptide portions). With these key fragments now readily available to us, we have begun to develop compounds which might be of use in studying the biology of the cyclosporines. We describe in this lecture the chemical synthesis of new, conformationally constrained analogs of CsA, and the synthesis of a new, biologically active photoaffinity labeling analog of CsA. The synthesis of a carrier free ^{125}I labeled AIPPS photoaffinity ligand attached to the ε-NH_2 of D-Lys^8-CsA has been accomplished. This analog, which strongly inhibits Con-A stimulated thymocyte proliferation, has been found to label isolated, purified cyclophilin when photolyzed in thymocyte cytoplasm.



V 012 APPLICATIONS OF THE CONSTRAINED ANALOG APPROACH TO BIOLOGICALLY ACTIVE PEPTIDES.

D. F. Veber, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486, U.S.A.

In recent years peptide modifications have been developed which allow the design of metabolically stable analogs which retain high biological potency. Application of these approaches to a variety of bioactive peptides (e.g. somatostain analogs and renin inhibitors) has revealed the importance of biliary excretion as a controlling factor for duration of action and transport as a limiting factor in oral availability. In contrast to the well understood metabolism of peptides, the structural factors which define excretion and transport are only poorly characterized.

An alternate approach to obtaining metabolically stable peptide analogs has been in the screening of microbial fermentations for natural products that interact with peptide receptors. In this way the CCK antagonist asperlicin was discovered. In a medicinal chemistry program directed at introducing oral activity into this lead, it was found that certain 3-substituted benzodiazepines are selective, orally effective CCK antagonists. Analogs having potencies in the nanomolar range have been developed. The structure-activity relationship for CCK antagonism and flunitrazepam receptor binding are seen to diverge significantly. Within this general class of drugs some compounds are recognized which also interact with opiate receptors as agonists. Thus the benzodiazepine nucleus is seen to be a template for receptor binding to the receptors for three distinct peptides of widely differing biological activity. Attempts to define the relationship of the benzodiazepines to the active form of the peptide have been undertaken. As a first step toward this end, cyclic analogs of CCK have been designed which are weak antagonists but of comparable binding potency to the known peptide antagonists.

Using these studies, a geometric size parameter can be proposed for predicting when a non-peptide analog is a possibility for a given peptide.

Synthetic Peptides:
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Vaccines Based on Synthetic Peptides

V 013 THE NEXT GENERATION OF FOOT-AND-MOUTH DISEASE VACCINES, F. Brown, Wellcome Biotech, Langley, Court, Beckenham, Kent BR3 3BS, U.K.

A peptide corresponding to the major immunogenic site of the protein VP1 of foot-and-mouth disease virus (FMDV) will elicit a protective neutralizing antibody response in guinea pigs, cattle and pigs. The response is much greater when the peptide is presented as a linear dimer or tetramer fused to β-galactosidase and pigs receiving as little as 40μg of the tetramer are protected against challenge infection. A response approaching that obtained with virus particles is obtained when the peptide is presented as part of the core protein of hepatitis B virus. Moreover, responsiveness to the peptide can be enhanced by the presence of a covalently linked T cell epitope. These results indicate that a molecular vaccine based on peptides is feasible for foot-and-mouth disease.

V 014 STUDIES ON THE DEVELOPMENT OF A SYNTHETIC PEPTIDE VACCINE TO FELINE LEUKEMIA VIRUS, John H. Elder, Department of Molecular Biology, Research Foundation of Scripps Clinic, La Jolla, CA 92037.

We have prepared synthetic peptides corresponding to the predicted amino acid sequences of the envelope gene products of several variants of feline leukemia virus. Antisera were prepared to these peptides conjugated to carrier proteins and tested for ability to elicit in vitro neutralizing responses. A number of sites were identified which may serve to neutralize virus infectivity, including epitopes in both the major envelope glycoprotein, gp70, as well as the small membrane-spanning envelope protein, p15E. We next tested the antipeptide antisera for ability to cross-neutralize a panel of FeLV variants, including members of all three major subtypes of FeLV. We found that in certain instances, the ability of the antisera to neutralize the variant viruses correlated directly with sequence conservation or diversity at a given epitope; i.e., conserved epitopes served as broadly cross-neutralizing sites whereas epitopes which varied in amino acid sequence facilitated only type-specific neutralization. However, we also found that certain conserved sites, located in the C-terminal portion of gp70 and within p15E, did not serve as broadly cross-neutralizing epitopes. The implication from this observation is that the exposure of certain epitopes varies from one FeLV variant to the next (generally along major subtype lines), either by differences in folding of the molecule or via differential glycosylation. Another possibility is that localized changes in the conformation of the epitope results in lowered affinity of antibody binding in certain variants, resulting in failure to neutralize the virus. We have found an example of the latter in a detailed analysis of one particular conserved neutralizing epitope. Using a monoclonal antibody prepared to intact virus, but reactive with one of our peptides, we were able to precisely map a five amino acid epitope required for antibody binding. A variant which is not neutralized at the site contains this five amino acid epitope, but also has a single amino acid change (leucine for proline) three amino acids N-terminal to the epitope. Synthetic peptides were prepared corresponding to the region of both the susceptible and resistant FeLV isolates and used to compete for antibody binding to whole virus. The results indicate that this single amino acid change causes a ten-fold diminution in the ability of the peptide to compete for antibody binding. We postulate that the leucine for proline substitution alters the beta turn potential of the antibody binding epitope, resulting in lower antibody binding affinity and failure to neutralize the variant virus.

V 015 SYNTHETIC HEPATITIS B IMMUNOGENS, A. Robert Neurath[1], Stephen B.H. Kent[2], Nathan Strick[1], Karen Parker[2], Linda Martin[2], [1]The New York Blood Center, 310 E. 67th Street, New York, 10021; [2]California Institute of Technology, Pasadena, CA 91125.
Mapping of epitopes on the hepatitis B virus (HBV) envelope (env) protein(s) involved in neutralization of infectivity and in protection against infection is difficult, since virus infectivity titrations can be carried out only in chimpanzees. Nevertheless, it was possible to identify such epitopes in distinct regions of the HBV env protein using synthetic peptides and monoclonal antibodies. The HBV env consists of three distinct, but related protein species: S-protein, M-protein (S + preS2) and L-protein (S + preS2 + preS1). Studies with synthetic peptides derived from the S, preS2 and preS1 regions of the HBV env protein have shown that each of these regions contains an epitope(s) involved in the process of virus-neutralization. In considering the development of an immunogen with potential for vaccination, one has to consider virus-neutralization epitopes on each of these three regions of the HBV env. Therefore, delineation of dominant epitopes on the S, preS2 and preS1 region is required. The dominant B-cell epitopes on the preS1 and preS2 region appear to be contiguous and are mimicked with relative ease by synthetic peptides. Antigenic determinants of the S-region appear to be non-contiguous and are dependent on the maintenance of disulfide bonds. Results of delineation of contiguous determinants essential for biological functions of the preS1 + preS2 sequence of the HBV env protein and attempts to mimic with synthetic peptides S-protein epitopes will be described. The design of fully synthetic immunogens containing essential portions of each the S, preS1 and preS2 sequence will be discussed.

The preS2 sequence was reported to react with gluteraldehyde-polymerized human serum albumin. It was postulated, without any direct evidence, that immunization with preS2 sequences may result in auto-immune responses to human serum albumin and to human hepatocyte cell membranes. Experimental evidence disproving this postulate and supporting the safety of vaccines containing preS sequences will be presented.

V 016 SYNTHETIC CONSTRUCTIONS OF A *PLASMODIUM FALCIPARUM* SURFACE ANTIGEN STIMULATE BOOSTABLE IMMUNITY IN LOWER ANIMALS AS WELL AS MONKEYS, Robert T. Reese, The Agouron Institute, La Jolla, CA 92037.
The extracellular asexual blood form of the malarial parasite is the merozoite. Its surface is composed of a series of different molecules many of which are likely to play a role in its attachment to its host cell the erythrocyte. One of these is a 75 kDa molecule which by its amino acid sequence places it within the 70 kDa group of heat shock proteins. Because it is a very major highly conserved *Plasmodium falciparum* protein, it is considered a potential vaccine candidate. Constructions modeling various portions of this molecule have been made using recombinant bacteria and synthetic peptide chemistry. In addition to the large construction from the recombinant organisms, the smaller synthetic peptides were capable of stimulating boostable IgG antibody responses in lower animals as well as in monkeys. The peptide constructions were extremely useful in defining those epitopes seen by rabbits, monkeys, and man.

Prediction of Peptide/Protein Structure

V 017 INFLUENCE OF MEMBRANE ENVIRONMENTS ON PEPTIDE AND PROTEIN STRUCTURE,
Charles M. Deber, Research Institute, Hospital for Sick Children, Toronto M5G 1X8; and Department of Biochemistry, University of Toronto, Toronto M5S 1A8, Ontario, Canada

Lipid interactions with proteins and peptides, whether involving membrane binding and penetration of extracellular ligands, or the production and maintenance of the functional conformation(s) of integral membrane proteins, exert a broad influence on molecular events at, and within, cellular membranes. Experimental and predictive aspects of these phenomena will be examined: (1) Lipid-induced secondary structure in proteins and peptides - We will describe the complexes formed between phospholipid (e.g., lysophosphatidylcholine) micelles and amphiphilic bioactive peptides such as substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2), an undecapeptide neuromodulator involved in the transmission of pain information. Experimental results from NMR, CD, and other physical techniques demonstrate that membrane binding and penetration induces significant secondary structure in substance P and related tachykinin peptides. These studies also allow the identification of specific membrane-interactive residues of the peptide. Recent CD studies confirm that parallel effects occur for substance P complexes with the bilayers of phospholipid vesicles as well as the monolayers of micelles. Such membrane-induced reduction in the neuropeptide conformational ensemble is discussed as a possible requirement for a receptor-cognitive conformation. In a related investigation, the D-glucose-dependent increase in the secondary structure of the hexose transport protein ("band 4.5") of human erythrocytes is interpreted in terms of substrate-induced membrane penetration of a protein segment. (2) Transmembrane (TM) segments of receptor proteins - With the goal of identifying characteristic residues in receptor protein TM regions which may participate in signal transduction, we have analyzed the membrane-occurring residues in 37 integral membrane proteins categorized by function [11 receptors, 10 transport proteins, 16 membrane-anchored proteins (MAP's) (i.e., those with a functional external domain attached to the cellular membrane)]. We found that amino acid compositions of receptor TM regions could not be distinguished statistically from corresponding regions in MAP's (although both differed statistically from transport protein TM regions in several residues). TM regions in receptor proteins are thus predicted to function mainly to anchor (and position) receptors in their cellular membranes. The preponderance of membrane-adjacent Arg and Lys residues at the cytoplasmic side of receptor and (MAP) TM regions further supports the "anchor" hypothesis, both from electrostatic and helix dipole considerations. The possibility will be discussed that some residues in receptor aqueous domains may participate in signal transduction, conceivably by membrane penetration of hydrophobic "epitopes" which ultimately contact cytoplasmic domains. Membrane penetration of non-polar segments of the amphiphilic aqueous domains of receptor proteins seems plausible in view of the experimental demonstration (in (1) above) of such properties in segments of neuropeptides.

V 018 MIMICS OF SECONDARY STRUCTURAL ELEMENTS OF PEPTIDES AND PROTEINS, William F. Huffman and James F. Callahan, Smith Kline & French Laboratories, P.O. Box 1539, King of Prussia, PA 19406-0939.
Peptide backbone chain reversals, or reverse turns, are postulated to be important features of the biologically-active conformations of peptides and proteins. A detailed chemical description of the role of secondary structural elements in the presentation of peptide and protein pharmacophores is essential for the understanding of these biological events at the molecular level. Conformationally-restricted mimics of reverse turns limit the possible conformational array and thereby allow one to better define the role of reverse turns in pharmacophores. To be successful, a mimic must restrict the peptide backbone to a known set of conformations in the region of the proposed turn and allow for the incorporation of side-chains which are important to receptor recognition and activation. The design and synthesis of a conformationally-restricted gamma-turn (C-7 turn) will be presented. It will be shown that the mimic restricts the torsion angles of the i+1 residue to a conformation consistent with a gamma-turn and that all of the requisite sidechains can be introduced in a straight-forward manner. The incorporation of a series of gamma-turn mimics into linear and cyclic peptides will also be discussed.

V 019 CONFORMATIONS OF THREE PEPTIDES DEDUCED FROM EXPERIMENTS AND MOLECULAR ENERGETICS, Vincent Madison, Ziva Berkovitch-Yellin, David Fry, David Greeley, and Voldemar Toome, Roche Research Center, Hoffmann-La Roche Inc., Nutley, New Jersey 07110.

We have utilized molecular graphics in conjunction with energy minimization and molecular dynamics modules of the CHARMM program package (1) to optimize conformations of peptides subject to constraints from experimental results. This methodology will illustrated by means of structural results obtained for synthetic peptide analogs in three systems. Oiki, Danho and Montal have shown that a 22-residue peptide forms trans-membrane channels which conduct sodium ions in vitro. Based on functional and energetic considerations, we have developed a model for the ion-pore formed by this peptide. For two poypeptide hormones, conformational families have been derived based on molecular dynamics and energy minimization constrained by interproton distances determined from nuclear magnetic resonance spectra. A vasoactive intestinal peptide (VIP) analog, [N1-Ac, Lys12, Lys14, Nle17, Val26, Thr28] VIP, and analogs of growth hormone releasing factor residues 1-29 (GRF1-29) have been investigated. For the VIP analog, there are a variety of backbone conformations which are all consistent with the experimental interproton distances. In the absence of solvent and counter ions, calculations on the peptide required screening of formal charges on acidic and basic side chains to prevent collapse of secondary structure due to formation of intramolecular ion pairs. GRF1-29 has considerable alpha helix in aqueous-alcohol solutions. Replacement of Gly15 by Ala15 in GRF1-29 produces spectral and conformational changes distant from the site of substitution. these changes seem to be caused by increased rigidity of the helical segment.

1. B.R. Brooks, R.E. Bruccoleri, B.D. Olafson, D.J. States, S. Swaminathan, and M. Karplus, J. Comp. Chem 4, 187-217 (1983).

V 020 WEAK INTERACTIONS IN PROTEINS, S.K. Burley+, G.A. Petsko* and D. Ringe*, Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139, +Department of Medicine, Brigham and Women's Hospital, Boston, MA 02115.

A study of the atomic environments of aromatic side chains in protein crystal structures has identified a group of weakly-polar, enthalpically favoured interactions which contribute to the stability of a protein. A search for these types of interactions in protein-ligand structures shows that they are equally important in stabilizing these complexes. These interactions are weakly polar and include edge-to-face interactions between aromatic groups (side chains or ligands) which bring a partially positive hydrogen atom of one aromatic ring near the partially negative II-electron cloud of another aromatic ring, and the interactions of electronegative atoms such as oxygen, nitrogen, sulfur, and fluorine with aromatic rings. These interactions occur frequently throughout protein structures and the distribution of observed geometries for each pair of weakly-polar interactions differs substantially from random. Nor do they arise from packing constraints. Calculations suggest that these interactions make enthalpic contributions to the stabilization of protein structures and protein-ligand complexes. The enthalpy of such an interaction is close to that of a hydrogen bond. Therefore, such an interaction could change the way in which the ligand interacts with a protein.

S.K. Burley, G.A. Petsko, "Weakly Polar Interactions in Proteins", Advances in Protein Chemistry, in press.

Biologically Active Peptides

V 021 GASTRIN RELEASING PEPTIDE: A SYNTHETIC APPROACH TO RECEPTOR RECOGNITION AND MITOGENIC POTENTIAL. David C. Heimbrook, Mark E. Boyer, Victor M. Garsky, Nancy L. Balishin, David M. Kiefer, Allen Oliff, & Mark W. Riemen. Department of Cancer Biology, Merck, Sharp & Dohme Research Laboratories, West Point, PA 19486.
Gastrin Releasing Peptide (GRP) serves a variety of physiological functions, and has been implicated in the pathophysiology of small cell lung cancer. Previous work has demonstrated that the modified C-terminus of GRP, acetyl GRP 20-27, exerts full agonist activity in a variety of assay systems. However, no systematic comparison of binding of GRP fragments to its receptor and mitogenic potency has been reported. To investigate whether smaller GRP fragments could bind to the GRP receptor without stimulating mitogenesis, we performed binding inhibition and thymidine uptake assays in Swiss 3T3 fibroblasts. These studies were facilitated by the development of two GRP-based radioligands: [125I-Tyr15, Nle27] GRP 15-27 and [3H-Phe15] GRP 15-27. [125I-Tyr15, Nle27] GRP 15-27 displayed superior characteristics when compared to iodinated methionine-containing GRP sequences. However, [3H-Phe15] GRP 15-27 exhibited enhanced chemical stability compared to iodinated GRP-derivatives and thus was used in subsequent studies. We examined a series of C-terminal GRP fragments, from the pentapeptide to the octapeptide, with both N-acetyl and free amine moieties at the N-terminus. N-acetylated derivatives were more potent than their primary amine counterparts in both assays. Deletion of N-terminal residues from GRP 20-27 resulted in significant loss of potency in both assays: the EC_{50}'s of N-acetyl GRP 21-27 were 100-fold higher than N-acetyl 20-27, those of N-acetyl GRP 22-27 were 10,000-fold higher, and N-acetyl GRP 23-27 showed minimal activity at concentrations below 100 uM. These results suggest that : 1) both His20 and Trp21 play an important role in binding of GRP to the receptor, and 2) for this series of N-terminal deletions, binding to the receptor and mitogenic activity are tightly coupled.

V 022 SYNTHESIS AND BIOLOGICAL EVALUATION OF GROWTH HORMONE RELEASING FACTOR, STRUCTURAL LINEAR AND CYCLIC ANALOGS. E.P. Heimer, A. Felix, M. Ahmad, T. Lambros, T. McGarty, C.-T. Wang, T. Mowles*, and D. Davidovich**, Exploratory Research, *Animal Science Research and **Toxicology Department, Hoffmann-La Roche Inc., Nutley, N.J. 07110.
Growth hormone-releasing factor, GRF(1-44)-NH_2, was first isolated from a human pancreatic tumor and its sequence published in late 1982. It is now well documented that it stimulates the specific release of growth hormone in humans as well as other animal species. We have been actively engaged in the development of GRF(1-44)-NH_2 for human clinical use. Clinical preparations of GRF(1-44)-NH_2 have been carried out on gram-scale by solid phase peptide synthesis and the product fully characterized. Structure-activity studies have shown that only the first 29-amino acid residues are required for full intrinsic biological activity and we have pursued an analog program of GRF(1-29)-NH_2 with the aim of developing a variant with enhanced prolonged activity to be used for performance enhancement of livestock (porcine, bovine). Analogs of GRF(1-29)-NH_2 in which Gly^{15} was replaced by other hydrophobic amino acids (e.g. Ala) have been shown to have increased potency. Modification at the NH_2-terminus has been found to enhance stability to degradation by diaminopeptidase. These observations have been used to develop a novel proprietary analog which is currently in field trials and will be used for performance enhancement in livestock. Cyclic analogs of GRF which retain the bioactive conformation have also been prepared and will be described.

V 023 MOLECULAR MECHANISMS OF CELL-MATRIX INTERACTIONS, Erkki Ruoslahti, La Jolla Cancer Research Foundation, 10901 North Torrey Pines Road, La Jolla, CA 92037.
Extracellular matrix provides cells with anchorage, traction for movement and positional recognition. This is accomplished through adhesive interactions between cell surfaces and individual extracellular matrix proteins. Fibronectin is a prototype adhesive protein of extracellular matrices. In fibronectin, only three of its 2,500 amino acids--an Arg-Gly-Asp (RGD) tripeptide--are crucial for the interaction of this protein with cells. Synthetic peptides containing the RGD sequence mimic the cell attachment-promoting activity of fibronectin when coated onto a surface, whereas soluble peptides inhibit the attachment of cells to a surface coated with fibronectin or with the peptides themselves. The same amino acid triplet is the cell attachment site of several other adhesion proteins including vitronectin, collagens, osteopontin, von Willebrand factor, fibrinogen and possibly thrombospondin and laminin. The RGD sequences in the various adhesive proteins are recognized by cell surface receptors that are capable of distinguishing between the RGD sequences of different proteins. Four such receptors have been identified: those for fibronectin, vitronectin, and type I collagen and a receptor from platelets that recognizes several RGD proteins. Amino acid sequences derived from cloned cDNAs show that these receptors are related but distinct proteins. The RGD receptors belong to a larger superfamily of proteins, integrins. Other members of this superfamily include the leukocyte adhesion proteins LFA-1, Mac-1, and p150,95, as well as cell surface antigens designated as VLA. Whether these proteins also recognize an RGD sequence is not known. Variability of the conformation of the RGD sequences may be what allows the individual recognition of adhesion proteins by the RGD receptors. Thus, synthetic peptides containing the RGD sequence in different conformations display distinct receptor specificities. Such peptides provide useful probes for the analysis of the biological roles of the receptors.

Late Addition

V 024 PRODUCTION AND CHARACTERIZATION OF BIOSYNTHETIC HUMAN INSULIN-LIKE GROWTH FACTOR I, R.D. DiMarchi, H.B. Long, B.E. Schoner and R. Belagaje, Biochemistry, Eli Lilly Research Labs, Lilly Corporate Center, Indianapolis, IN 46285.
A synthetic gene capable of coding for the production of insulin-like growth factor I (IGF-I) was prepared, cloned, and expressed in Escherichia coli as a fusion protein. The synthetic gene was designed to provide for the positioning of a single tryptophan residue NH_2-terminal to the natural IGF-I sequence. A novel chemical procedure for selectively cleaving the biosynthetic IGF-I fusion protein at a position CO_2H-terminal to the single tryptophan residue was developed. The IGF-I produced through this chemical cleavage was purified to homogeneity as its S-sulfonate derivative. Disulfide-interchange of the IGF-I sulfonate to its natural structure was performed in a single step through the presence of excess reducing agent. The IGF-I is recovered from this reaction in a yield of approximately 50%. A particular disulfide isomer is also observed to form in a lesser, but appreciable yield, to that of the properly paired hormone. Chemical, immunological, and biological analysis of the biosynthetic IGF-I has revealed it to be indistinguishable from naturally isolated IGF-I. Application of this selective chemical cleavage methodology to other peptide hormone has also been completed.

Synthetic Peptides: Approaches to Biological Problems

Peptide Synthesis and Design

V 100 SCALE UP OF THE CONTINUOUS LOW PRESSURE FLOW SYNTHESIS OF ATRIOPEPTIN III (RAT),
Eric Atherton, B. Helen Matthews, and John R. Richards, Cambridge Research Biochemicals, Cambridge, England.

The low pressure continuous flow method of peptide synthesis using kieselguhr supported polydimethylacrylamide resin and employing the mild combination of N -Fmoc and t-butyl side chain protection is gaining in popularity. The method has been routinely used on a small scale but lends itself particularly well to scale up, primarily because the system is simple. It involves pumping solvents and reagents, according to a pre-set program, through a stationary column and has the advantage that the reactions can be readily monitored by passing the column eluate through a UV recorder. This technique has been applied to the scale up of the assembly of atriopeptin III,(rat), a twenty four residue peptide containing three arginine residues and a disulfide bridge, on a 7.5 mmol. scale using a support with a loading of 0.5 $mmol.g^{-1}$. Acylations were accomplished using Fmoc-amino acid pentafluorophenyl esters in the presence of hydroxybenzotriazole without the need for any repeat couplings. Cleavage, cyclisation and purification of the peptide will be illustrated. In terms of the efficiency, economy and ease of operation the method offers considerable potential mechanical advantages over discontinuous batchwise procedures for large scale peptide synthesis.

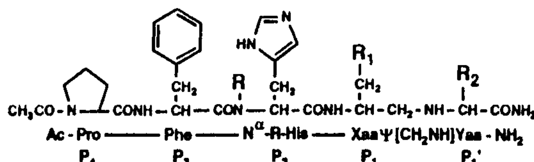
V 102 MOLECULAR MECHANICS AND COMPUTER GRAPHICS STUDIES ON COMFORMATIONALLY CONSTRAINED PEPTIDE ANALOGS, V.N. Balaji, S. Profeta, Jr., and A. Mobasser, Discovery Research, Allergan, Inc., 2525 Dupont Drive, Irvine, CA 92715. Modification of naturally occuring oligopeptide drugs can significantly alter their aqueous stabilities, conformational properties, and susceptibility to metabolic degradation. In order to gain a better understanding of structure-activity relationships for such modifications, we have explored a number strategies for introducing structural constraints into oligopeptides. In this paper we present the results of our studies (molecular mechanics and computer graphics molecular modeling studies) on introducing conformational restrictions into dipeptides and polypeptides through peptide mimics (e.g., thiopeptide and N-methyl peptide units) and side chain modifications (e.g., Aib side chain, substituted and unsubstituted cycloalkyl side chains). The utilization of such constraints to bias the oligopeptide confimation toward different types of β-turns and specific Φ,Ψ values is described. The ability to possibly influence the formation of regular helical structures (e.g., α-helix, threefold and fourfold helical structures, collagen-type helical structures) is also illustrated.

V 103 PROPOSED STRUCTURAL BASIS FOR TOXICITY OF PARDAXIN, A PEPTIDE SECRETED BY THE RED SEA MOSES SOLE, Jay W. Fox, David C. Benjamin, Philip Lazarovic, and Naftali Primor, Univ of Virginia Med. School, Charlottesville, VA 22908 (JWF, DCB), NICHD, Bethesda, MD (PL), and Osborn Lab. Marine Sci., New York, NY (NP). Pardachirus marmoratus secretes a peptide into the environment which is toxic to fish. Pardaxin-1 (PX-1) is isolated from this secretion as a tetramer with each identical subunit comprised of 33 amino acid residues. PX-1 is capable of binding liposomes of various phospholipid compositions. Unilamellar liposomes when subjcted PX-1 become hyperpolarized and nonspecifically permeablized with PX-1 forming a cation-specific pore in the membrane. The toxin is cytolytic but only very weakly bactericidal. By designing and synthesizing various structural analogs of PX-1 we are attempting to determine the structural foundation for the toxicity of this peptide as well as its specificity for various cell types.

V 104 PHOTOCHEMICAL APPROACHES TO THE PREPARATION OF LHRH BY FRAGMENT CONDENSATION. Jean Gauthier, Suman Rakhit, François Bruderlein, Monique Baillet, Serge Valois, and Yves Bousquet. Department of Chemistry, Bio-Méga Inc., Laval (P.Q.) Canada H7S 2G5
Several photochemical approaches in peptide synthesis are illustrated by the synthesis of luteinizing hormone releasing hormone. Strategies leading to the preparation of this hormone will be discussed. The generation of protected peptide segments from solid phase supports by photolysis has been achieved. Several protected segments have been condensed in solution or on solid supports. The optical purity of the compounds has been evaluated and results will be compared.

V 105 BIMANE AND PROTEIN CONJUGATED SV-40 LARGE T-ANTIGEN NUCLEAR SIGNAL ANALOGS: CHEMICAL SYNTHESIS AND STUDIES OF SUBCELLULAR DISTRIBUTION
Thomas J. Lobl, Mark A. Mitchell+, Linda L. Maggiora and Robert E. Lanford*. Biopolymer Chemistry and +Cancer and Viral Diseases Research, The Upjohn Company, Kalamazoo, MI 49001, and *Department of Virology and Immunology, Southwestern Foundation for Biomedical Research, San Antonio, TX 78284
The signal sequence of a nuclear directed protein encodes the necessary information for targeting the attached proteins to the cell nucleus. We were interested in exploring the sequence/structural requirements for a functional transport signal and in learning if signal sequences would also target a small molecule such as a fluorescent tag to the nucleus. We chemically synthesized the large T-antigen nuclear signal 126-134 (PKKKRKVED, wild type) with an amino terminal Cys to facilitate coupling the peptide to proteins and fluorescent tags. When this peptide was conjugated to Bovine Serum Albumin (BSA) with m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) and microinjected into the cytoplasm of green monkey kidney cells (TC-7) it was found to be transported to the nucleus. The same peptide fluorescently tagged with monobromobimane showed peptide distributed throughout the cell. Peptides with Asn, Orn or Gln, substituted for Lys128, were synthesized and conjugated similarly to BSA. None of the BSA conjugates of these signal analogs transported as effectively as did the conjugate of the wild type. In addition the protein conjugated reverse signal (DEVKRKKKPC) did not transport. We conclude that the nuclear signal requires a specific sequence for proper function and small molecules attached to a functioning nuclear signal may not be retained in the nucleus even if they are successfully transported.

V 106 AN ANALYSIS OF LIGAND-ENZYME BINDING INTERACTIONS OF HUMAN RENIN,
L.L. Maggiora, B. Mao, D.J. Staples, A.E. deVaux, R. Poorman, J. Hinzmann, D. Pals and T.K. Sawyer, The Upjohn Company, Kalamazoo, MI 49001
Some important components of inhibitor/substrate-enzyme binding interactions of the aspartic acid protease, renin, have been studied by structure-activity relationships and molecular modeling. A structure-activity relationship has been established for a series of substrate-based inhibitors that have been chemically modified at the scissile amide bond to a $-CH_2NH-$ moiety (see figure). Analysis of the results of these studies has been augmented by molecular modeling involving molecular dynamics simulations and energy minimizations of selected ligands bound to the active site of a model of human renin. The modeling has allowed us to predict likely bound-ligand conformations, as well as to identify specific hydrophobic and electrostatic interactions that might contribute to the binding energy. In addition, kinetic measurements have been performed to compare the K_i values of selected inhibitors with the K_m and K_{cat} values of the corresponding P_1-P_1' modified substrates.



V 107 HELIX PREDICTION IN SYNTHETIC PEPTIDES, Gene Merutka and Earle Stellwagen, Department of Biochemistry, University of Iowa, Iowa City, IA 52242.

Two analogs of the S-peptide of ribonuclease, acetylAETAAAKFLRQHMamide and acetylAETSSSRYL-RQHMamide, were designed to examine helicity prediction in small synthetic peptides. The peptides were prepared by solid phase synthesis, purified by reverse phase HPLC, and examined by far ultraviolet circular dichroism. The pH dependence of the dichroic spectrum of each peptide at 2° is characteristic for a helix/coil transition with an isosbestic point at 203 nm and maximal helical content at pH 5. In order to decrease the freezing point of the solvent, the thermal dependence of the helix/coil transition was observed in 4.7 M $NaClO_4$ at pH 5. Using the change in ellipticity accompanying complexation of S-peptide with S-protein observed at 222 nm, -29,000 deg cm^2/dmole, as a limit, the thermal transition of peptide acetylAETAAAKFLRQHMamide has a Tm of -5°. By contrast, the thermal transition for peptide acetylAETSSSRYLRQHMamide has a Tm of about +15° and a change in ellipticity only half as large. Such comparisons suggest that the amino acid replacements in the latter peptide shorten the helix formed as predicted by the Chou-Fasman procedure. Supported by PHS program project grant HE14388.

V 108 SYNTHESIS AND BIOLOGICAL PROPERTIES OF ANTIGENIC FRAGMENTS OF $p28^{sis}$ AND $p55^{fos}$ ONCOPROTEINS, Inessa I. Mikhaleva[1], Valery V. Antonenko[1], Igor A. Prudchenko[1], Vadim T. Ivanov[1], Natalya N. Mazurenko[2] and Fedor L. Kiselev[2]. (1) Shemyakin Institute of Bioorganic Chemistry USSR Academy of Sciences, Moscow, USSR; (2) Institute of Cancerogenesis All Union Cancer Research Center USSR Academy of Medical Sciences, Moscow, USSR

With the aim of investigating the role of some oncoproteins and their cellular analogs in cancerogenesis we have prepared polyclonal antibodies specific to oncoproteins $p28^{sis}$ and $p55^{fos}$. Antisera have been obtained using potential antigenic protein fragments which were conjugated with protein carriers. Selection of antigenic sites has been carried out on the base of known procedures of hydrophilicity estimates and β-turn prediction.

Using conventional solution methods we have synthesized decapeptide corresponding to the sequence 201-210 of $p28^{sis}$, peptides 1-15 and 6-15 of viral protein $p55^{v-fos}$ and C-terminal decapeptide (residues 371-380) fragment of cellular $p55^{c-fos}$ as well as a number of shortened peptides. The antibodies to these peptides were tested by immunoblotting and radioimmuno-precipitation in cells expressing sis and fos oncogenes. We found that antibodies specifically recognized oncogene protein products ($p28^{sis}$, $p56^{sis}$, $p55^{fos}$, $p64^{fos}$, $p75^{fos}$) and may be usefull as sensitive tools in human tumor analysis.

Synthetic Peptides: Approaches to Biological Problems

V 109 STUDY OF THE ANTIGENIC STRUCTURE OF PERTUSSIS AND CHOLERA TOXIN USING A SYNTHETIC PEPTIDE FRAGMENT

Perin F., Presentini R., Ancilli G., Bartoloni A., Rappuoli R. & Antoni G. Sclavo Research centre, Siena, Italy.

A significant degree of homology exists between subunit 1 of pertussis toxin (PT-S1) and subunit A of cholera toxin (CT-A). In particular the sequence 8-18 of PT-S1 (Tyr-Arg-Tyr-Asp-Ser-Arg-Pro-Pro-Glu-Asp-Val) is very similar to the sequence 6-16 of CT-A (Tyr-Arg-Ala-Asp-Ser-Arg-Pro-Pro--Asp-Glu-Ile). The peptide 8-18 of PT-S1 was synthesized and antipepide antibodies were produced in rabbits using a peptide-KLH conjugate. ELISA and immunoblot analyses demonstrated that antipeptide antibodies recognised the immunizing peptide and PT-S1.

Furthermore antipertussis antibodies recognized both PT-S1 and the 8-18 peptide; therefore the sequence 8-18 of PT-S1 represents at least a portion of a natural antigenic determinant of pertussis toxin. A positive interaction of antipertussis and anti 8-18 antibodies with cholera toxin was also evidentiated due to the high degree of homoloy existing between the two proteins at least in this region. However anticholera toxin antibodies do not interact neither with PT-S1 nor with the 8-18 peptide, which demonstrates that antibodies produced by cholera toxin are mainly directed either against portions with low homology between the two molecules, or they present a fine specificity able to evidentiate also small structural differences in highly homologous primary structures.

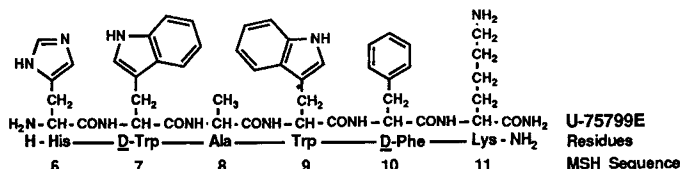
V 110 STRUCTURE/FUNCTION STUDIES ON HUMAN TGF-ALPHA, USING SYNTHETIC AND RECOMBINANT APPROACHES. Deborah DeFeo-Jones, Joseph Y. Tai, Ronald J. Wegrzyn, Gerald A. Vuocolo, Audrey E. Baker, Linda S. Payne, Victor M. Garsky, Allen Oliff, & Mark W. Riemen. Department of Cancer Biology, Merck, Sharp & Dohme Research Laboratories, West Point, PA 19486.

Structural analogs of human Transforming Growth Factor-alpha (TGF-alpha) were prepared, either by chemical synthesis or by recombinant DNA methodology. The synthetic derivatives encompassed defined substructures of the complete molecule. The recombinant molecules represented both the complete 50 amino acid polypeptide and various mutant forms of the parent structure. Various types of mutant peptides were represented: amino- and carboxy-terminal deletions; amino-terminal substitutions; point mutations; and radical changes dealing with the highly conserved disulfide structure. None of the synthetic peptides exhibited any biologic effects. However, the recombinant parent molecule was fully active in both binding and mitogenesis assays. Further, though some of the recombinant TGF-mutants showed no activity, several of the modified molecules produced interesting biologic effects. The mitogenic response of the active mutants paralleled their ability to bind to the EGF/TGF receptor. The data from the synthetic and recombinant peptides support the hypothesis that sequences from various regions of the molecule are required for biologic activity.

V 111 SYNTHESIS OF THE P10 SINGLE-STRANDED NUCLEIC ACID BINDING PROTEIN FROM MURINE LEUKEMIA VIRUS, William J. Roberts, James I. Elliott and Kenneth R. Williams, Yale University, New Haven, Ct. 06510. The p10 murine leukemia virus (MuLV) protein is a basic single-stranded nucleic acid binding protein encoded by the extreme 3' region of the gag gene of MuLV type C. It contains the Cys-X_2-Cys-X_4-His-X_4-Cys sequence shared by all retroviral gag polyproteins. A similar sequence is found in the gene 32 single-stranded DNA binding protein of bacteriophage T4 and is believed to be the zinc binding region of the protein. Solid phase synthesis of the protein was performed based on the known primary structure of the native protein while incorporating the acetamidomethyl (ACM) derivative of cysteine at all three cysteine positions. The Chou and Fasman predicted secondary structure based on the primary sequence alone predicts less than 10% beta structure, little if any helix and the remainder random coil. Circular dichroism experiments carried out on the ACM derivatized peptide were in good agreement with these predictions revealing a structure which is apprximatley 70% random coil, less than 30% beta structure and less than 10% helix. With a K_a of greater than 10^8 M^{-1} for single-stranded RNA, the synthetic protein binds as tightly as the p10 protein does when isolated directly from infected HTG-2 cells. We have successfully removed the ACM groups from the synthetic p10 protein by the use of mercuric acetate followed by treatment with dithiothreitol to sequester the mercuric ion. After ACM removal and subsequent carboxyamido-methylation of the peptide, amino acid analysis and gas phase sequencing confirmed that all three cysteines had been successfully deblocked. Because the p10 protein of MuLV type C contains only 56 residues and can be synthetically produced in large quantities, it provides an excellent model for understanding nucleic acid and zinc binding properties of proteins.

V 112 DISCOVERY OF A SYNTHETIC GROWTH HORMONE SECRETAGOGUE WHICH EFFECTS COMPETITIVE ANTAGONISM TO α-MELANOTROPIN, D.J. Staples, L.L. Maggiora, A.M. Castrucci†, M.E. Hadley† and T.K. Sawyer, The Upjohn Company, Kalamazoo, MI 49001; †University of Arizona, Tucson, AZ 85721.

α-Melanotropin is a tridecapeptide hormone, Ac-Ser[1]-Tyr[2]-Ser[3]-Met[4]-Glu[5]-His[6]-Phe[7]-Arg[8]-Trp[9]-Gly[10]-Lys[11]-Pro[12]-Val[13]-NH_2 (α-MSH), which is physiologically-important for melanogenesis (melanin-based pigmentation). In a search to identify α-MSH inhibitors, we have discovered that H-[D-Trp[7], Ala[8], D-Phe[10]]-α-MSH_{6-11}-NH_2 (U-75799E), a known weakly potent secretagogue of growth hormone *in vitro / in vivo*, provided a lead molecule in the design of competitive antagonists to α-MSH. The structure-activity relationships of U-75799E will be described. Finally, the structure-conformation relationships of U-75799E and related α-MSH derivatives explored by molecular modeling strategies will be addressed.



V 113 SUBSTANCE P AND-GMDP DERIVATIVES: SYNTHESIS AND BINDING TO RAT BRAIN MEMBRANES. Yuri N.Utkin, Elena M.Lazakovich, Alexey A.Kaydalov, Tatyana M.Andronova, Victor I.Tsetlin and Vadim T.Ivanov, Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Moscow, USSR.

Substance P(SP) and muramylpeptides specifically affect the mammalian central nervous system. The present work is aimed at characterization of the SP and detection of specific binding sites in rat brain for one of the muramylpeptides, N-acetyl-β-glucosaminyl(1→4)-N-acetylmuramyl-L-alanyl-D-isoglutamine (GMDP). Using Bolton-Hunter reagent, hydroxyphenyl derivatives of SP and GMDP, containing additional C-terminal Lys, were synthezied. After their isolation, characterization, iodination ($Na^{125}I$) and HPLC purification homogeneous radioactive SP and-GMDP derivatives of high specific activity (≥ 2000 Ci/mmol) were obtained. By reductive alkylation of $[^{125}I]$ SP and HPLC purification, a homogeneous photoactivable SP analogue was obtained. Both SP and its photoactivable derivative specifically bind to rat brain membranes with K_d 0.7 nM and B_{max} 60 fmol/mg of protein. Photosensitive SP analogue was used for labelling the receptor component in rat brain. Radioactive GMDP derivative in saturable manner specifically binds to rat brain membranes with K_d 3 nM and B_{max} 11 fmol/mg of protein. Both nonradoiactive iodinated GMDP derivative and nonmodified GMDP inhibit this binding.

V 114 CORRELATION BETWEEN PEPTIDE SYNTHESIS AND SITE-DIRECTED MUTAGENESIS IN DEFINING ACTIVE SITES OF HUMAN GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (GM-CSF). M.A. Vadas[a], L. Madley[a], M.F. Shannon[a], I. Clark-Lewis[b], J. Schrader[b], A.F. Lopez[a]
[a] The Institute of Medical and Veterinary Science, Adelaide, Australia.
[b] The Biomedical Research Centre, The University of British Colombia, Vancouver, Canada.

GM-CSF is a127 amino-acid glycoprotein with two disulphide bonds responsible for the stimulation of proliferation and differentiation of hemopoietic progenitor cells and for the activation of mature cells. GM-CSF was chemically-synthesized and found to have all the biological functions of the native molecule but was 100-300 fold less potent. Synthesis of shorter peptides revealed that peptide 14-127 was fully active, 19-127 was less potent and 24-127 was totally inactive.
Site-directed mutagenesis substantially correlated the above findings with mutant protein produced in a rabbit reticulocyte system or by transfection of COS cells. Notably, deletion of amino-acids 14-18 partially reduced and a 14-24 deletion abolished activity. The 18-24 amino-acid region of GM-CSF is predicted to be a hydrophilic α helix. The function of single amino-acid deletions and substitutions predicted to alter these properties will be discussed.
The techniques of chemical synthesis and site-directed mutagenesis are complementary tools in defining biologically active sites of growth factors.

Synthetic Peptides: Approaches to Biological Problems

V 115 A MULTI-DIRECTIONAL APPROACH TO SYNTHESIS AND PURIFICATION OF SULFATED PEPTIDES, R. M. Wagner, USDA/ARS/VTERL, College Station, TX 77841.
Synthesis of biologically-active sulfated peptides is rarely a trivial matter because of incompatibility of blocking/deblocking and sulfation procedures. Synthesis protocols were designed to maximize purity and minimize side-reactions. Synthesis and purification of these peptides enables further characterization of their biochemical and physiological properties.

V 116 **MAGAININ PEPTIDE STRUCTURE GOES FROM UNFOLDED TO HELIX UPON BINDING TO MEMBRANE SURFACE.** Robert W. Williams*, Michael Zasloff^, and David Covell ,
*Department of Biochemistry, Uniformed Services University of the Health Sciences, Bethesda, MD 20814, ~Department of Mathematical Biology, and ^Human Genetics Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892
Magainins are 23-residue peptides isolated from the skin of Xenopus laevis. They protect the frog from infection under the most adverse circumstances and exhibit a broad-spectrum antimicrobial activity in vitro [Zasloff (1987) Proc. Natl. Acad. Sci. USA 84, 5449]. We have examined the mechanism of this activity by correlating properties of peptide sequence with structural changes that occur when synthetic magainin and other peptides bind to membranes. Raman spectroscopy of magainin 2 shows that it is 95% unfolded in physiological saline, and that it forms a 71% alpha-helix 14% turn structure upon binding to lipid vesicles. In comparison, melittin is 80% helix in solution and 83% helix bound to lipid. To quantitate the disruption of membrane bilayer by excess peptide, we have measured a lipid melting equilibrium constant, Kmelt = [I1080(+peptide) - I1080(pure unmelted vesicles)] / [I1062(+peptide) - I1062(pure melted vesicles)], from Raman bands corresponding to C-C stretching modes for cis and gauche conformers at 1062 and 1080 wavenumbers respectively. For magainin Kmelt = 0.11, while for melittin Kmelt = 4.2, indicating that magainin does not spontaneously span DPPG membranes as does melittin. However, model studies show that magainin may form a trans-membrane pore in the presence of a membrane potential. (Supported by USUHS GM7160)

Bioactive Peptides

V 200 LOCATION, SEQUENCE AND ANTIGEN PRESENTATION OF T CELL EPITOPES OF THE M. LEPRAE 65K Da PROTEIN STUDIED USING SYNTHETIC PEPTIDES. D.C. Anderson, Michael E. Barry and Rene R.P. deVries*. University of Washington, Seattle, WA 98195 and *University Hospital of Leiden, Leiden, The Netherlands.
The 65K Da protein, homologous in sequence to an E.coli heat shock protein, is an important T and B cell antigen of this intracellular parasite, which causes leprosy. Activation of cellular immunity is critical for a successful immune response against M. leprae. We have used synthetic peptides to locate T cell stimulating regions of this protein, and to precisely define some of their sequences. The epitope for the HLA-DR3 restricted species-specific clone 2F10 is defined as the sequence LQAAPALDKL, just N terminal to the binding site for a species-specific antibody. The critical residues in this sequence have been mapped, and peptide variants constructed in an attempt to correlate T cell stimulation with peptide secondary structure induced in solution, and with the calculated peptide hydrophobic moment. Peptides representing hypervariable regions of the beta chain of this clone have also been checked for their effect on antigen presentation of this T cell epitope.

Synthetic Peptides: Approaches to Biological Problems

V 201 ANALYSIS OF TOPOGRAPHIC ANTIGENIC DETERMINANTS ON HUMAN CHORIOGONADOTROPIN USING SYNTHETIC PEPTIDES, Jean-Michel Bidart, Frédéric Troalen, Claude Bohuon and Dominique H. Bellet, Département de Biologie Clinique, Institut Gustave Roussy, 94805 Villejuif Cedex, France.
Human choriogonadotropin (hCG) consists of non covalently linked α and β subunits encoded by separate genes, and is structurally related to the pituitary glycoprotein hormones. We have characterized two antigenic determinants recognized by monoclonal antibodies using competitive inhibition with synthetic peptides, mapping with monoclonal anti-peptide antibodies and recombination experiments with subunits from different species. A first epitope is present on the free β subunit (not present on the α/β dimer) and is localized to the core of the protein (residues 1-112). A synthetic peptide inclusive of the NH2-terminal region (portion β1-7) was found to inhibit the antibody-β subunit interaction, indicating that this region is part of the epitope which might be located nearby or in the hCG-β portion interacting with the α-subunit (J.M. Bidart et al, J. Biol. Chem., 262, 8551-8556, 1987). The second antigenic determinant is specifically associated to the α/β dimer of hCG. The topographic antigenic region is constituted by a limited number of residues located on hCG-β near to Asp112, and most likely the sequence Asp-Asp-Pro (111-112-113), and by highly conserved residues far apart in the primary structure of the α subunit. Our findings constitute a new approach in the evaluation of the three-dimensional structure of hCG.

V 202 **IMMUNOGENICITY OF SYNTHETIC PEPTIDE VARIANTS OF A VACCINE AGAINST P. FALCIPARUM SPOROZOITES,** **Howard M. Etlinger, Hugues M. Matile, Arnold Trzeciak and Dieter Gillessen, Central Research Units, F. Hoffmann-La Roche & CO, Ltd., Basel, Switzerland.**
A synthetic peptide-based vaccine against sporozoites is currently undergoing clinical trials. This vaccine contains Ac-Cys-$(NANP)_3$ conjugated to Tetanus toxoid (TT). Since the antibody responses to $(NANP)_3$ and sporozoites of human volunteers were generally low, TT-conjugates containing variant peptides ($(NANP)_3$-Cys, Ac-Cys-$(ANPN)_3$, Ac-Cys-$(NPNA)_3$, Ac-Cys-PN$(ANPN)_2$, Ac-Cys-NA$(NPNA)_2$, Ac-Cys-$(PNAN)_3$, and Ac-Cys-AN$(PNAN)_2$) were evaluated in mice and monkeys for their potentially greater immunogenicity. Each conjugate initiated the production of similarly high levels of anti-sporozoite antibody. However, the fine specificty of anti-peptide antibody elicited by each conjugate was different. It remains to be determined whether such fine specificity differences correspond to differences in the ability of the antibodies to prevent infection by sporozoites.

V 203 THE USE OF SEQUENCE SPECIFIC ANTIBODIES TO IDENTIFY A SECONDARY BINDING SITE IN THROMBIN, Jan Hofsteenge, Gudrun Noé, Giorgio Rovelli and Stuart R. Stone, Friedrich Miescher-Institut, P.O.Box 2543, CH-4002.
The peptide comprising residues 62-73 of the B-chain of human α-thrombin has been synthesized, and polyclonal antibodies were raised against this peptide conjugated to keyhole limpet hemocyanin. These antibodies were found to bind to the synthetic peptide, a CNBr fragment containing this sequence, and the entire thrombin molecule. The antibodies had no effect on the hydrolysis by thrombin of D-Phe-Pipecolyl-Arg-paranitroanilide, and caused only a minimal decrease (20%) in the second order rate constant for inactivation by antithrombin III. On the other hand, the antibodies competed with hirudin for a binding site on thrombin, and a dissociation constant of 3.4 ± 0.5 nM was found for the binding of the antibodies. The release of fibrinopeptide A from the Aα-chain of fibrinogen by thrombin was competitively inhibited with an inhibition constant (K_I) of 11.7 ± 0.4 nM. Also the activation of protein C by thrombin in the presence of thrombomodulin was inhibited by the antibodies ($K_I'=10.7 \pm 1.5$ nM). In contrast, the antibodies had no effect on the activation of protein C in the absence of thrombomodulin. These results are discussed in relation to data obtained recently on the interaction of proteolytic derivatives of human α-thrombin with the ligands described above.

V 204 GLYCOSYLATION OF SYNTHETIC PEPTIDES BY LARVAL ARTEMIA MICROSOMES,

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Studies on chitin synthesis in Artemia larvae indicate that a lipid intermediate, dolichol phosphate, is glycosylated by the addition of N-acetyl-D-glucosamine (GlcNAc) residues to yield a oligosaccharides up to 8 units in size. These oligosaccharides are transferred en bloc to a protein acceptor forming a glycoprotein which serves as a primer for chitin synthetase. Using an in vitro assay procedure based upon the methods of Welply et al. (J. Biol. Chem. 258, 11856-11863;1983), two ^{3}H-acetylated tripeptide acceptors were found to serve as substrates for the oligosaccharyl transferase which transfers an endogenous chitin oligosaccharide from the lipid intermediate to appropriate acceptors. The sequence of these synthetic peptide acceptors is Ac-Asn-Tyr-Thr-NH_2 and Ac-Asn-Leu-Thr-NH_2. The assay is linear with added microsomal protein and time of incubation.The enzyme appears to require Mg^{2+} for activity; transfer is blocked by 50 mM EDTA. The product of the reaction has been analyzed by gel permeation and paper chromatography; the product contains a short oligosaccharide (N=2 or 3) based upon Bio-Gel P-4 chromatography. Incubation of microsomes with ^{3}H peptide and UDP- ^{14}C GlcNAc yielded a double labeled glycopeptide product which was characterized by gel and paper chromatography. Supported by NIH Grant GM-30952.

V 205 THE USE OF SYNTHETIC PEPTIDES FOR THE ANALYSIS OF THE ENVELOPE OF HEPATITIS B VIRUS (HBV),

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The outer coat of HBV contains HBsAg determinants coded by the S and pre-S regions of the genome. We have developed a model of HBsAg structure using predictive algorithms. The presence of 4 membrane spanning helicies is predicted, together with an external loop between residues 111 and 156 where the major a , or protective, determinants are located. To test this model, a panel of synthetic peptide analogues to potentially exposed regions were prepared together with a peptide analogue of an internal loop structure. Reactivities of these peptides with human antibodies were then measured either by ELISA or by the assessment of affinity. Peptides mimicking external regions reacted with antibodies induced by native HBV. High levels of antibody capable of binding to a peptide mimicking carboxyl terminus residues 217 to 226 were detected. An analogue of the possible contact residues for antibody within the major a determinants was performed using animal antisera to related hepadnaviruses having amino acid replacements in the region 124 to 139. Affinity measurements showed common residues may be important for anti-HBs induction. Competitive inhibition experiments using pre-S peptides indicate the presence of similar conformational determinants on S and pre-S1 proteins.

V 206 MAPPING OF FUNCTIONAL AND ANTIGENIC DOMAINS OF THE $\alpha 4$ PROTEIN OF HERPES SIMPLEX 1.

Jeff Hubenthal-Voss#, Richard A. Houghten[$], Lenore Pereira[&], and Bernard Roizman[#*]

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Monoclonal antibodies to $\alpha 4$, the major regulatory protein of herpes simplex virus 1, have been shown to differ in their effects on the binding of the protein to its DNA binding site in the promoter-regulatory domain of an α gene (Kristie et al.1986.Proc.Natl.Acad. Sci.USA.83:4000-4007). To map the epitopes, we assayed the reactivity of the monoclonal antibodies with C terminal truncated $\alpha 4$ proteins produced from 3' truncated genes by transient expression in transfected BHK cells. All 10 monoclonal antibodies tested reacted with an N terminal 288 amino acid polypeptide. To map the epitopes more precisely, 29 fifteemer oligopeptides overlapping by 5 amino acids at each end were synthesized and reacted with the monoclonal antibodies. Nine monoclonal antibodies were found to be reactive and mapped to seven different oligopeptides. Of the two monoclonal antibodies which blocked the binding of $\alpha 4$ to DNA, one (H950) reacted with oligopeptide 3 near the N terminus of the protein whereas the second (H942) reacted with oligopeptide 23 near the C temnius of the 288 amino acid polypeptide. Moreover, oligopeptides 19 and 27 were found to retard DNA in a band shift assay. In contrast with these results, a truncated $\alpha 4$ polypeptide 825 amino acids long bound specifically to the promoter of the $\alpha 0$ gene whereas a shorter, 519 amino acid long truncated polypeptide did not. The 825 amino acid polypeptide was previously shown to induce the transient expression of a viral gene expressed late (γ_2) in infection (Mavromara-Nazos et al.1986.Virology.149:152-164).

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V 207 DEGRADATION OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) BY RAT SPINAL CORD HOMOGENATE, Frank Jordan, Guy F. Barbato and Barry R. Komisaruk, Dept. of Chemistry and Institute of Animal Behavior, Rutgers University, Newark, NJ 07102.
Incubation of several neuropeptides with rat spinal cord homogenate led to the degradation of only two peptides, substance P and vasoactive intestinal polypeptide (VIP). No opioid peptides tested were degraded by the homogenate, suggesting morphological specificity of the spinal cord with regard to enzyme localization. Substance P was cleaved following Phe^7 and Phe^8, as reported previously by others. VIP was specifically cleaved on the carboxy-side of Tyr^{10} and Tyr^{22}. Peptidolysis of VIP by spinal cord homogenate was completely inhibited by EDTA and 1,10-phenanthroline, suggesting the involvement of a metalloendopeptidase in the degradation of VIP.

V 208 IDENTIFICATION OF TWO HELPER T-CELL SPECIFIC EPITOPES ON HIV EXTERNAL ENVELOPE GLYCOPROTEIN. Kai Krohn[1,2], Annamari Ranki[3,2], Paolo Lusso[2], Larry Arthur[4], Bernie Moss[5] and Scott Putney[6]. [1]Laboratory of Tumor Cell Biology, NCI, Bethesda, Md,. [2]Institute of Biomedical Sciences, University of Tampere, Finland, [3]Department of Dermatology, University of Helsinki, Helsinki, Finland, [4]FCRF, NCI, Frederick, Maryland, [5]NIAID, Bethesda, Md., [6]Repligen Co., Cambridge, Mass.
An effective protective immune response towards HIV would require co-operation of helper/inducer T-cells, cytotoxic T-cells and neutralizing antibody producing B-cells. We have looked for helper T-cell response to native or recombinant HIV envelope proteins and to corresponding synthetic peptides in HIV infected gibbon apes and in immunized primates. A strong group specific T-cell response was seen in chimpanzees immunized with native gp120 or with recombinant envelope protein PB1 (aa 295-474), in rhesus monkeys immunized with a recombinant vaccinia virus expressing HIV gp160 and in HIV infected gibbon apes. Two of the 76 overlapping synthetic peptides representing the entire sequence of gp160 were recognized by helper T-cells. A broad group specific response, directed against all isolates tested, were seen only when these two peptides, located in conserved sequencies of gp120 were simultaneously recognized. Identification of all T- and B-cell epitopes involved in a protective anti-HIV response may be required for generation of a subunit HIV vaccine.

V 209 SYNTHESIS AND ANALYSIS OF PEPTIDES TO MURINE CD4 MOLECULES, Georgina J. Clark, Kerrie Reynolds, Margaret M. Henning, Nicholas J. Deacon and Ian F.C. McKenzie, Research Centre for Cancer and Transplantation, Department of Pathology, University of Melbourne, Parkville, Victoria, 3052, Australia.

Our laboratory has determined the partial amino acid sequence of murine CD4, constructed oligonucleotides and isolated a cDNA clone encoding murine CD4; the clone is identical to those isolated using the human CD4 cDNA clone. To determine which site(s) on CD4 molecules is(are) involved in Class II restricted T cell interactions and also which might be involved in HIV infections (although human and mouse are clearly different in this regard). Nine peptides were made, which cover most of the extracellular region of the CD4 molecule, and rabbit antibodies to these.

The antibodies were characterised by RIA; reaction with intact cells (FACS); cell surface chemistry and Western blotting:- one antisera gave variable reactions but, surprisingly, the antibody to the NH-2 terminal peptide did not react with intact molecules. These peptides and antibodies are currently being tested in vitro and in vivo for their ability to block CD4/substrate interaction (presumably CD4/Class II) but thus far none have any remarkable activity and, in particular, the CD4 peptides do not bind directly to Class II antigens. While the analysis will continue with new combinations of overlapping peptides, the lack of any effect thus far may indicate that the tertiary structure of the CD4 molecules is important in cellular interactions.

V 210 SPECIFICITY AT THE LEVEL OF SINGLE AMINOACIDS OF ANTI-WHOLE FOOT-AND-MOUTH-DISEASE VIRUS SUBPOPULATIONS PRESENT IN POLYCLONAL ANTI-PEPTIDE SERA, Rob H. Meloen, Wouter C. Puyk, Hanneke Lankhof, Adri Thomas, Wim M.M. Schaaper, Central Veterinary Institute, P.O. Box 65, 8200 AB Lelystad, The Netherlands. Polyclonal anti-peptide sera raised against small synthetic peptides corresponding with an imunodominant site on the surface of Foot-and-Mouth-Disease Virus appear to consist of multiple antibody subpopulations. Adsorption of these sera with whole virus removes part of the anti-peptide activity but all anti-virus activity. The fine specificities of the anti-peptide/anti-virus subpopulations could be determined at the level of single amino acids. They showed to be similar to fine specificities of monoclonal and polyclonal antibodies raised against whole virus reactive with the same peptides.

V 211 SITE-DIRECTED ELISA ASSAYS WHICH CAN DISTINGUISH BETWEEN HIV 1 AND HIV 2 SPECIFIC ANTIBODIES. D. Elliot Parks*, Philip R. Johnson[1], Erling Norrby+, Alice Whalley*, Jon Rosen* and Richard Smith*, *Johnson & Johnson Biotechnology Center, La Jolla, California, [1]Laboratory of Infectious Disease, NIAID, Bethesda, Maryland, +Department of Virology, Karolinska Institute, Stockholm, Sweden
Synthetic peptides representing highly antigenic, immunodominant regions of human and simian immunodeficiency virus transmembrane glycoprotein have been formulated into ELISA assays for the detection of antibody to these retroviruses. Site-directed serology utilizing these synthetic peptides allows the detection of type specific antibodies. A 32 amino acid peptide sequence representing a conserved region of the transmembrane protein of HIV 1 specifically detects antibody induced following exposure to HIV 1. The sensitivity of this synthetic peptide microwell ELISA assay is comparable to viral lysate based assays commercially available. Synthetic peptides have been synthesized from the conserved region of the SIV gp32 transmembrane protein which are analogous to the sequences from HIV 1. Peptides made in this region are highly immunoreactive with sera from SIV infected primates. This reactivity extends across four primates species and identify infection with at least two distinct isolates of SIV in experimental and naturally infected monkeys. These sequences can also be used to detect seropositive individuals exposed to this human retrovirus in West Africa. When used, in parallel, these two synthetic peptide assays can be employed to distinguish between individuals exposed to HIV 1 and HIV 2 in human immunodeficiency virus strains.

V 212 SYNTHETIC PEPTIDE AS ANTIGEN FOR THE DETECTION OF HUMORAL IMMUNITY TO P. FALCIPARUM SPOROZOITES, D.N. Rao and V. Anuradha, Department of Biochemisty, All India Institute of Medical Sciences, New Delhi - 110 029 (India). The detecton of antibodies to sporozoites in the sera of individuals living in malaria endemic areas is of epidemiological relevance, since antibody levels should correlate with the rates of disease transmission and immunity. Most antibodies to sporozoites are directed against a single, repetitive epitope of the circum sporozoite protein (CSP). A peptide consisting of 16 aa's of four tandem repeats of $(Asn\text{-}Ala\text{-}Asn\text{-}Pro)_4$ was made chemically first preparing a tetra then to octa and finally to hexadeca sequence by a classical solution technique. Its purity and homogeneity was checked by TLC, HPLC and aminoacid composition. The above synthetic peptide was used to detect the presence of antisporozoite antibodies in blood samples of humans living in endemic area i.e. six villages with different age groups were studied. The EIA results will be discussed in detail.

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V 213 FIBRONECTIN BINDING BY STAPHYLOCOCCUS AUREUS IS INHIBITED BY SYNTHETIC PEPTIDES AS RECEPTOR ANALOGS, Giuseppe Raucci and Magnus Hook, Department of Biochemistry, University of Alabama, Birmingham, AL 35294.

The binding of fibronectin to staphylococcal cells, which is a mechanism of host tissue adhesion,is mediated by a receptor protein inserted in the bacterial cell wall. The gene coding for the receptor has been cloned and the fibronectin binding site was identified to be a 120 amino acid sequence after subcloning in different expression systems (EMBO J., 1987, 6, p. 2351-2357). Sequence analysis demonstrates that this fibronectin binding region consists of three 38 amino acids homologous sequences. A synthetic peptide corresponding to one of these 38mers, blocks the binding of fibronectin to S. aureus. Furthermore, this peptide is immunogenic and polyclonal antibodies raised against it partially inhibit the binding of fibronectin to S. aureus. Additional peptides are presently being tested.

V 214 **Use of synthetic peptides in two different approaches to interfere with host cell penetration by *Trypanosoma cruzi*.**

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The trypomastigote stage of *Trypazonoma cruzi*, found in vertebrate bloodstream are capable of penetrating in host cells through fibronectin which acts as a bridge between parasite and host cell. A 85 KD glycoprotein, located at the surface of the trypomastigote has been shown to be involved in cellular adhesion mechanisms and necessary for penetration.

We have used synthetic peptides in two different strategies aimed at interfering with the invading phase.

-i- Having shown that fibronectin cell attachment domain (RGDS) mediates the adhesion of trypomastigotes, we have used a variety of peptides based on the RGDS sequence to study their inhibiting capacity in vitro and in vivo, (but also, for labelling different stages of the parasite)

-ii- A partial DNA sequence of the 85 KD protein (Peterson, Nature, 1986) revealed the existence of nonapeptide repeated sequence. An octadecapeptide copying this structure was used as immunogen and the antisera obtained immunoprecipitated three major proteins of molecular weights 68, 85 & 160 KD.

These two approaches proved to be able to achieve a partial but significant protection when tested in vivo.

V 215 **PREVENTION OF HIGH MOLECULAR WEIGHT AGGRETATES OF A SYNTHETIC MEMBRANE PROTEIN WITH ADDITION OF DETERGENT DURING HF CLEAVAGE,** **John M. Tomich[1,2], L. Wulf Carson[1], Katherine J. Kanes[2], Michael R. Emerling[2], John H. Richards[2], [1]Divisions of Medical Genetics, Childrens Hospital of Los Angeles, and [2]Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA.**

In our initial attempts to refold the chemically synthesized 73 amino acid membrane protein, M13-Procoat, we employed a series of treatments which, on occasion, produced a form of the protein which could be processed by the bacterial enzyme *leader peptidase*. In trying to determine the structure of the protein by NMR we encountered various degrees of aggregation from one sample perparation to the next. Aggregation was observed to occur during the initial phase of isolation - HF cleavage. Elimination of the organic precipitation and subsequent washes did not decrease the aggregation of the product. Inclusion of non-ionic detergents in HF-cleavage reactions displayed little ability in preventing aggregation. Cleavage in the presence of sodium laurylsulfate (SDS) however dramatically reduced the degree of aggregation even after the organic washing steps. The level of aggregation, as measured by ^{1}H-NMR, was reduced to the level of dimers and tetramers. The SDS is then quantitatively exchanged with 0.1% octylglucoside through use of a novel HPLC protocol and subsequently refolding in an aqueous buffer system for activity measurements and structural determination by 2D NMR.

V 216 **DEFINING THE NATURE AND HETEROGENEITY OF PEPTIDE ANTIBODY PREPARED TO A SHORT SEQUENCE FROM CALMODULIN: Determination of Calmodulin Crossreactive and Non-Crossreactive Specificities,** *Richard J. Wasley, Timothy A. Mietzner and Gary K. Schoolnik, Department of Medical Microbiology*

Immunization with predesigned synthetic peptides for the purposes of preparing site specific antibodies which crossreact with the homologous sequence in the native protein is a popular technique which has broad theoretical and practical utility in biomedicine. This study examines the nature of antibody elicited to a synthetic peptide corresponding to the region 80-92 of calmodulin. This peptide was conjugated to a carrier molecule through its amino terminus and used to immunize rabbits. The resultant serum had an average affinity of 5×10^7 *l/mol* for peptide 80-92. The C-terminal nine amino acid residues were absolutely required for efficient reactivity of this serum. Peptide sera crossreacted with calmodulin at an average affinity of only 2×10^5 *l/mol*. However, after affinity purification of peptide specific antibody over a calmodulin column, a subpopulation of antibodies which reacted with the native protein at a higher average affinity could be isolated. Further analysis of this affinity purified antibody by isoelectric focusing indicated that a discrete subset of antibodies was selected representing a quantitatively minor population of peptide-reactive immunoglobulins. These studies suggest that (i) the antibody response to a synthetic peptide conjugate is heterogeneic with respect to the peptide reactive antibodies produced and (ii) only a subset of these antibodies can crossreact with high affinity to the native protein.