FRONTIERS OF NMR IN MOLECULAR BIOLOGY - II

Organizers: Peter Wright, John Markley and Masatsune Kainosho

April 8-14, 1991

Plenary Sessions Page

Poster Sessions

Frontiers of NMR in Molecular Biology-II

Keynote Address

CG 001 PROGRESS IN MULTIDIMENSIONAL NMR WITH APPLICATION TO MOLECULAR BIOLOGY, R.R.Ernst, S. Boentges, M. Blackledge, J. Briand, M. Ernst, C. Griesinger, T. Levante, Z.L. Mádi, T. Schulte-Herbrüggen, S. Smith, and O.W. Sørensen, Laboratorium für Physikalische Chemie, ETH-Zentrum, 8092 Zürich, Switzerland.
The potential of NMR for addressing questions of biological relevance will be analyzed. The determination of the molecular structure of biomolecules in solution by two- and three-dimensional NMR has become an almost routine procedure, at least for medium-size molecules with a well defined, sufficiently rigid structure. In many cases of more mobile molecules, dynamics and structure can not be separated, and structural studies have also to face the problem of molecular dynamics. Potential and limitations of NMR techniques for the characterization of molecular dynamics will be discussed. Experimental results for various types of dynamics in cyclic peptides are presented.

Structural Analysis of Proteins and Folding Intermediates
(joint with Protein Folding meeting)

CG 002 STRUCTURAL AND GENETIC ANALYSIS OF THE INTERACTIONS THAT STABILIZE PROTEINS. Sun Dao-pin, Hale Nicholson, Xue-Jun Zhang, Walt A. Baase, Michael Blaber, Dirk W. Heinz and Brian W. Matthews, Institute of Molecular Biology, Howard Hughes Medical Institute, University of Oregon, Eugene, OR 97403
The lysozyme from bacteriophage T4 is being used as a model system to determine the roles of individual amino acids in the folding and stability of a typical globular protein. Such studies can provide quantitative information on the contributions made by different types of interactions including H-bonds, hydrophobic interactions, salt bridges and disulfide bridges. The emphasis of the talk will be on two topics, first, the contributions of electrostatic interactions to protein stability, and, second, the influence of amino acid replacements within α-helices on protein stability.

Studies of mutant lysozymes suggest that engineered salt bridges between solvent-exposed residues on the surface of a protein contribute little to stability. In contrast, however, engineered electrostatic interactions with so-called "α-helix dipoles" consistently enhance the stability of the protein. Possible reasons for this difference will be discussed.

In order to determine the importance of "α-helix propensity" in protein stability, different replacements have been made within α-helical segments of the T4 lysozyme. Several such substitutions of the form Xaa → Ala increase the stability of the protein, supporting the idea that alanine is a strongly helix-favoring amino acid.

CG 003 ELUCIDATION OF PROTEIN FOLDING PATHWAYS BY NMR AND AMIDE PROTON EXCHANGE. Heinrich Roder and Gülnur A. Elöve, Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA 19104-6059.

An important aspect of the protein folding problem concerns the structural characterization of intermediate states on the folding pathway. The fact that folding intermediates are generally unstable and short-lived limits the direct application of structural tools such as NMR. Nevertheless, it is possible to obtain detailed structural information on early events in folding by combining NMR spectroscopy with NH exchange pulse labeling and rapid mixing methods. The formation of hydrogen bonded structure during folding is probed by exposing the protein to a proton labeling pulse at different refolding times, followed by 2D NMR analysis of the patterns of amide protons trapped in the refolded protein. Earlier pulse labeling studies on cytochrome c [Roder, Elöve & Englander (1988) Nature 335, 700] revealed a 20 millisecond folding event in which amide sites in two α-helices near the chain termini become protected from exchange while amide sites in other helical segments and those involved in irregular H-bonds remain largely exposed out to about 100 ms. The fact that the N- and C-terminal helices form a tight contact in the native structure suggests that association of the two helices is an important early event in cytochrome c folding. Further evidence for such a helix pairing reaction was obtained by pulse labeling experiments involving systematic variation of the labeling conditions which show that amide protons on either helix exhibit the same degree of protection, indicating that the helices are stabilized by mutual contacts. The observation that the formation of stable helical structure is accompanied by docking of two helices illustrates the importance of key tertiary interactions in directing the pathway of folding. The pulse variation results also provide evidence for multiple parallel folding pathways. The role of the axial heme ligands in cytochrome c folding is addressed by stopped-flow and pulse labeling experiments under a variety of refolding conditions. The results indicate that His 18 remains ligated under most conditions, but the Met 80 ligand is replaced by His 26 or His 33 in a major population of unfolded molecules. This non-native ligand becomes trapped in the partially folded early intermediate and is replaced by the native methionine ligand in a subsequent folding phase on the 100 millisecond time scale.

CG 004 NMR, PROTEIN STRUCTURES IN SOLUTION AND THE PROTEIN FOLDING PROBLEM. K. Wüthrich, Institut für Molekularbiologie und Biophysik, ETH-Hönggerberg, CH-8093 Zürich, Switzerland.

On a level of general availability and practicability, the NMR approach for the determination of three-dimensional protein structures in solution[1] has by now been in use for a period of about five years[2]. This lecture will emphasize features of NMR structures of proteins that have been shown during this period to be complementary to data that can be obtained from X-diffraction in protein crystals. A central theme will be the use of NMR for studies of protein hydration in solution[3].

References:

1. K. Wüthrich, "NMR Proteins and Nucleic Acids" (Wiley, New York, 1986).
2. K. Wüthrich, Acc. Chem. Res. 22, 36-44 (1989). The Development of Nuclear Magnetic Resonance Spectroscopy as a Technique for Protein Structure Determination.
3. G. Otting and K. Wüthrich, J. Amer. Chem. Soc. 111, 1871-1875 (1989). Studies of Protein Hydration in Aqueous Solution by Direct NMR Observation of Individual Protein-Bound Water Molecules.

NMR Methods

CG 005 NMR METHODS FOR STUDYING ISOTOPICALLY LABELED ENZYME/INHIBITOR COMPLEXES, Stephen W. Fesik, Gerd Gemmecker, Robert T. Gampe, Jr., Edward T. Olejniczak, Hugh L. Eaton, Andrew M. Petros, Placido Neri, and Erik R.P. Zuiderweg, Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL 60064

NMR studies on enzyme/inhibitor complexes can potentially provide structural information that is useful for designing improved inhibitors. However, one must obtain the structural information *rapidly* in order to provide insights at an early stage in drug design and to enable new inhibitors to be studied when bound to the enzyme in a timely fashion. In addition, since the design of analogs of lead inhibitors requires detailed structural information, it is important to obtain the three-dimensional structures as precisely as possible. In this presentation, approaches will be described for rapidly and accurately determining the three-dimensional structures of enzyme/inhibitor complexes. The methods involve the use of isotopically labeled ligands to determine the enzyme-bound conformation of the inhibitor and to quickly identify those portions of the ligand that interact with the enzyme. In addition, multi-dimensional NMR methods will be presented which involve the use of isotopically labeled enzymes to study the entire three-dimensional structure of enzyme/inhibitor complexes and define the intermolecular interactions that stabilize the complex. The utility of these methods for providing detailed structural information on enzyme/inhibitor complexes will be illustrated in the study of cyclosporin A (a potent immunosuppressant) bound to its putative target protein, cyclophilin (peptidyl-prolyl cis-trans isomerase).

CG 006 ROTATIONAL RESONANCE IN DIPOLAR COUPLED SPIN SYSTEMS: DISTANCE MEASUREMENTS IN LARGE PROTEINS, A. E. McDermott[1,2], F. Creuzet[1,3], L.K. Thompson[1,3], R.G.S. Spencer[1,3], M. Auger[1], K. Halverson[2], B. Gebhard[3], I. van der Hoef[3], J. Lugtenburg[3], M.H. Levitt[1], P. Lansbury[2], J. Herzfeld[4], and R.G. Griffin[1,2], [1]Francis Bitter National Magnet Laboratory and [2]Department of Chemistry, MIT, Cambridge, MA, USA; [3]Department of Chemistry, Leiden University, Leiden, The Netherlands; [4]Department of Chemistry, Brandeis University, Waltham, MA, USA

The past few years have witnessed the development of several methods for performing high resolution NMR in solids, which in turn permit spectroscopy of large biological systems. Magic angle spinning (MAS) is perhaps the most widely applicable of these, and is now routinely employed to record chemical shift spectra of large proteins (MW=100 kD). To date, however, there has not existed an approach to determine "interesting" internuclear distances in the range 3-6 Å such as are found in proteins, nucleic acids, membranes, etc. In this talk we describe an approach to this problem termed rotational resonance (R2). Specifically, when homonuclear dipolar-coupled spin pairs are introduced into solids, R2 occurs when the spinning speed is adjusted so that the condition $\delta = n\omega_r$ is satisfied. Here, δ is the isotropic shift difference, ω_r is the spinning speed, and n is an integer which determines the order of the resonance. Under these conditions the normally sharp resonance lines broaden and split. In addition, a rapid oscillatory exchange of Zeeman order between the dipolar coupled spins is observed. The spectral lineshapes and the time dependence of the exchange can be simulated numerically to obtain information on internuclear distances. Experimental data will be presented which demonstrate that the technique will be useful for measuring ^{13}C-^{13}C distances up to 5 Å in the membrane protein bacteriorhodopsin, and in an insoluble peptide related to Alzheimer's disease.

Three-Dimensional Structure Determination

CG 007 NEW METHODS FOR REFINEMENT OF SOLUTION STRUCTURES FROM NMR DATA, David A. Case, Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037

My talk will deal with methods for determination of high-resolution solution structures from nmr data, and for estimating the level of uncertainty in the results. Three aspects will be emphasized. The first involves the use of "back-calculation" of NOESY intensities from predicted structures as a refinement tool. I will describe the models we are currently using to model internal and rotational motions, and illustrate the expected level of agreement with experiement through comparisons with observed NOESY intensities from plastocyanin. A second area arises from a re-analysis of proton chemical shift data. A comparison of over 4000 observed shifts with those predicted from various empirical models and the corresponding X-ray structures shows that useful structural information can be extracted, at least for protons bonded to carbon. I will present our experience with refinement methods based on these ideas. Finally, I will discuss progress on the construction of realistic simulated spectra from molecular dynamics simulations, and their use in the evaluation of refinement techniques.

CG 008 SOME EFFORTS TOWARDS DETERMINING HIGH-RESOLUTION DNA AND PROTEIN STRUCTURES IN SOLUTION. Thomas L. James, Miriam Gochin, Deborah J. Kerwood, Ingmar Sethson, Uli Schmitz and Paul D. Thomas, Departments of Pharmaceutical Chemistry and Radiology, University of California, San Francisco, CA 94143.

The structure of any molecule can be determined with a sufficient number of structural constraints. Problems addressed with DNA structure and with protein structure studies are often of a different nature. In general, we are interested in fairly subtle structural changes in the DNA helix which are sequence-dependent and, consequently, guide protein, mutagen or drug recognition. These subtle variations demand detailed knowledge of the structure and, therefore, accurate internuclear distance and perhaps torsion angle constraints. But one can define a protein tertiary structure with moderate accuracy using distance geometry or restrained molecular dynamics calculations without accurately determining interproton distances; a qualitative assessment of 2D NOE intensities is often sufficient. However, even for proteins, more accurate structures are obtained with more structural constraints *and* with more accurate structural constraints. To obtain the best possible structures, one should utilize as many structural constraints as possible and determine these constraints as accurately as possible – certainly we wish to avoid systematically biased structural constraints. Use of an iterative complete relaxation matrix approach using the MARDIGRAS algorithm enables us to ascertain relatively accurate interproton distances from 2D NOE peak intensities with little or no *a priori* knowledge about the molecular structure. In addition to the distances, MARDIGRAS yields an estimate of upper and lower bounds for each observable proton pair, which may be used with either distance geometry or restrained molecular dynamics calculations. The distance information from the 2D NOE analysis can be augmented by limited torsion angle information derived from coupling constants obtained from double-quantum-filtered COSY (2QF-COSY) and exclusive COSY (ECOSY) spectra. Broad lines prevented direct analysis of coupling constants, so we used simulation of 2QF-COSY cross-peaks using the programs SPHINX and LINSHA; this enables us to extract vicinal coupling constants and subsequently torsion angle constraints. But not all constraints are equally important in the determination of structure. We have been examining this question in particular in the context of restrained molecular dynamics calculations for a few oligonucleotide duplex sequences.

Protein Structure

CG 009 Protein modules: structure and function

Iain D. Campbell, *Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK*

An important feature of modern biochemistry is the rapid increase in the amino-acid sequence database. Analysis of the sequences of proteins from multicellular organisms shows that many proteins are constructed from various "modules". These modules are usually autonomously folding units of 40-100 amino-acids. We have determined the structure of three such modules by NMR, including those of epidermal growth factor (G), fibronectin type 1 (F1) and the complement control protein (C). Structural studies of several other modules are well advanced, including an immunoglobulin module (I) from the cell-surface receptor CD2 and a fibronectin type 3 module (F3) which has an RGD sequence that binds to integrin. We have also developed a strategy for studying such modular proteins [1]. This includes determination of individual module structure, the definition of functional patches on the module surface and modelling the structure of intact protein from information about different module pairs.

1. Baron, M., Norman, D.G. and Campbell, I.D. *Trends in Biochemical Sciences* January 1991.

CG 010 Determination of the Three-Dimensional Structure of Interleukin-1β in Solution at High Resolution: Applications of Three and Four Dimensional Heteronuclear NMR. G. Marius Clore and Angela M. Gronenborn, Laboratory of Chemical Physics, Building 2, NIDDK, National Institutes of Health, Bethesda, MD 20892.

This talk will outline the current state of the art with regard to NMR structure determination of larger proteins (15-25 kDa), and illustrate this with regard to interleukin-1β (IL-1β), a protein of 153 residues and 17.4 kDa which plays a central role in the immune and inflammatory responses. In terms of number of residues, IL-1β is about one and a half times larger than the largest protein structures determined to date by NMR, namely those of *E. coli* and human thiroedoxin (108 and 105 residues, respectively). Because of its large size, a solution structure determination of IL-1β by NMR presents a formidable problem, and, indeed, conventional 2D NMR methods could not be applied successfully owing to extensive spectral overlap, as well as problems associated with increased linewidths commonly found for proteins in the 15-25 kDa range. In this talk, particular emphasis will be placed on a range of 3D ^{15}N and ^{13}C heteronuclear double and triple resonance NMR experiments to obtain complete ^{1}H, ^{15}N and ^{13}C resonance assignments, and on 4D $^{13}C/^{15}N$ and $^{13}C/^{13}C$-edited NOESY experiments to resolve all ambiguities involved in the assignments of ^{1}H-^{1}H NOEs. In addition, 3D NMR methods combined with conformational grid searches for obtaining stereospecific assignments and ϕ, ψ and χ_1 torion angle restraints will be discussed. Finally, the high resolution three-dimensional structure of IL-1β, based on 3146 experimental restraints comprising 2780 distance and 366 torsion angle restraints, will be presented. A total of 32 structures have been calculated using the hybrid distance geometry-dynamical simulated annealing method, and the atomic rms distribution about the mean coordinate positions is 0.4 Å for the backbone atoms, 0.8 Å for all atoms, and 0.5 Å for all atoms of residues with surface accessibilities ≤40%. The implications of the structure for the mechanism of receptor binding will be discussed in the light of mutational data.

Nucleic Acid Structure

CG 011 ANALYSIS OF THE STRUCTURE AND DYNAMICS OF SYNTHETIC 32 BASE-PAIR MODELS OF THE HOLLIDAY JUNCTION INTERMEDIATE IN DNA RECOMBINATION. Walter J. Chazin & Shiow-Meei Chen, Department of Molecular Biology (MB2), Research Institute of Scripps Clinic, 10666 North Torrey Pines Road, La Jolla, CA 92037.

A specific DNA structure, the Holliday junction (HJ) is an intermediate in all recombination and many repair processes in the cell (1). The resolution of HJs appears to play an important role in determining the outcome of these genetic events. The HJ is an inherently mobile structure (migration of the junction is required to generate recombinant product) that would be extremely difficult to study by physical methods. To gain at least some insight into the molecular details of HJs, Seeman and Kallenbach proposed the study of synthetic immobile junctions (2,3). Two 32 base-pair, immobile HJs have been prepared and studied by 2D ^{1}H NMR. Although the relatively large (by ^{1}H NMR standards) size of the molecules (M_r~20 kDa) presents a rather formidable challenge, complete ^{1}H resonance assignments have been obtained. The experimental techniques and the assignment protocol that proved successful for the study of these HJs will be described, and the most useful connectivitities in the spectra identified. We will also discuss our progress towards understanding the conformation and dynamics of these unusual DNA structures.

References:
1. R. Holliday (1964) *Genet. Res.* 5, 282-304.
2. N. Seeman (1982) *J. Theor. Biol.* 99, 237-247.
3. N. Seeman & N.R. Kallenbach (1983) *Biophys. J.* 44, 201-209.

CG 012 STRUCTURES AND SEQUENCE SPECIFICITY OF INTRAMOLECULAR DNA TRIPLEXES, Juli Feigon, Vladimír Sklenář, Román Macaya, Edmond Wang, Peter Schultze and Dara Gilbert, Department of Chemistry and Biochemistry and the Molecular Biology Institute, University of California, Los Angeles, CA 90024

Homopurine:homopyrimidine DNA sequences readily form triple-stranded structures under appropriate conditions. Such sequences are over represented in the eukaryotic genome and may play a role in genetic regulation and recombination. It has been proposed that these sequences can form a structure termed H-DNA, in which a mirror repeat sequence folds back on itself to form a three-stranded structure and a single (usually purine) strand. We have been studying the conformation of intramolecular triplexes formed from folding of a single DNA strand. These molecules are an excellent model system for the structure of the triple-strand part of H-DNA. Information on the structure of these intramolecular triplexes obtained from ^{1}H NMR studies will be presented. In addition to their interest because of possible roles in *vivo*, DNA triplexes are important because of their potential use as therapeutic agents, through targeting of a modified DNA oligonucleotide to a specific control sequence. An understanding of the sequence requirements and stabilities of triplexes is essential for such applications. We have substituted various base triplets into our intramolecular triplexes. Information on the formation, stability, and base pairing schemes of these alternative base triplets in the substituted intramolecular triplexes will be presented.

Interactions of Protein and Drugs with DNA

CG 013 LOOP ARCHITECTURE IN DNA HAIRPINS, S.S. Wijmenga*, M.M.W. Mooren*, D.E. Pulleyblank**, M.J.J. Blommers#, G.A. v.d. Marel##, J.H. van Boom## and C.W. Hilbers*, *Faculty of Science University of Nijmegen, Laboratory of Biophysical Chemistry, Toernooiveld, 6525 ED Nijmegen, The Netherlands, **University of Utrecht, Laboratory of Organic Chemistry, Dept. of NMR Spectroscopy, Padualaan 8, 3584 CH Utrecht, The Netherlands, #Department of Biochemistry, University of Toronto, Toronto, Ontario M5S 1A8, Canada, ##Gorlaeus Laboratories, State University Leiden, P.O. Box 9502, 2300 RA Leiden, The Netherlands.

In recent years we have studied the loop architecture of synthetic DNA hairpins with four residues in the loop region. It was shown that for some sequences a basepair can be formed by the terminal bases of the loop sequence [1]. Particularly surprizing was the example in which a Hoogsteen AT basepair was found to be formed.
The presence or absence of such a basepair clearly influences the melting temperature of the hairpin and therefore the experiments were extended to hairpin molecules with loop sequences -GAAA- and -GTTA-, which exhibit an unusual stability.

So far, reasonably accurate structures have only become available for DNA hairpin loops consisting of four nucleotides. Therefore the experiments were extended to hairpins with an uneven number of loop residues.
A nucleotide hairpin which is part of the replication origin of the M13 phage was investigated. This hairpin has three nucleotides in the loop region. This is also the case for H-type DNA fragments studied by us. The
loop architecture in these hairpin structures will be discussed and related to that of four membered loops.
The derivation of an accurate loop structure requires the stereospecific assigment of as many resonances as possibble including the 5' and 5'' proton resonances. It will be shown that 3D experiments can be very helpful in achieving this.

[1] M.J.J. Blommers, J.A.L.I. Walters, C.A.G. Haasnoot, J.M.A. Aelen, G.A. van der Marel, J.H. van Boom and C.W. Hilbers, Biochemistry, 1989, 28, 7491.

Peptides and Oligosaccharides

CG 014 STRUCTURAL GLYCOBIOLOGY, Raymond A Dwek, Glycobiology Unit, Dept. of Biochemistry, University of Oxford, South Parks Road, Oxford, OX1 3QU, England.

At the molecular level information on the structure and dynamics of glycoproteins is essential to understand protein-oligosaccharide interactions and their role in affecting secondary or higher level structures. The IgM tailpiece (a flexible linker between IgM monomers); the prothrombin kringle (an important modular structure of mosaic proteins, like tPA) the Thy-1 domain (part of the immunoglobulin superfamily) and the Fc from IgG (of normal patients and those with rheumatoid arthritis) are part of our programme for structural glycobiology. This can be summarised in the following research questions:

1. How do different monosaccharides and their linkages determine the 3-D structure and dynamics of oligosaccharides? What general principles governing their structure emerge?

2. How do these principles apply when the oligosaccharide is covalently linked to a protein (or lipid)? How does the oligosaccharide affect the overall or local structure and dynamics of the protein (or lipid)? What surface does the glycoconjugate present that can interact with other molecules?

These will be discussed in relation to results on the three main glycosidic linkages that are found in proteins, *the N-linked oligosaccharides, the O-linked oligosaccharides and the glycosylphosphatidyl inositols (GPIs) anchors.*

CG 015 PROTEIN DISSECTION USING PEPTIDE MODELS

Peter S. Kim, Howard Hughes Medical Institute, Whitehead Institute for Biomedical Research, Department of Biology, M.I.T., Cambridge, MA 02142.

We use "protein dissection" as a way to study protein folding and macromolecular recognition.

We have made peptide models of intermediates in the folding of bovine pancreatic trypsin inhibitor (BPTI). These peptide models circumvent the problem of cooperativity and are highly soluble, permitting characterization of structures of folding intermediates using NMR (Oas & Kim, Nature 336: 42-48 [1988]). Two crucial one-disulfide intermediates, [30-51] and [5-55], each contain native-like, subdomain structure. Much of the folding pathway of BPTI can be explained in terms of native-like structure in these two early intermediates. This suggests that the protein folding problem can be reduced, in large part, to identifying and understanding subdomains of native proteins (Staley & Kim, Nature 344: 685-688 [1990]).

The leucine zipper motif, originally identified in Dr. S. McKnight's lab (Carnegie Institute) is important for mediating homodimer or specific heterodimer formation by several transcription activators. A synthetic peptide corresponding to the leucine zipper of the yeast protein, GCN4, has been studied using NMR (Oas et al., Biochemistry 29: 2891-2894 [1990]) and X-ray crystallography (with Prof. T. Alber's lab, Univ. of Utah). The results confirm our earlier proposal that leucine zippers are actually short coiled coils (O'Shea et al., Science 243: 538-542 [1989]). Remarkably, we find that the isolated leucine zipper regions from the nuclear oncogene products, Fos and Jun, are sufficient to mediate specific heterodimer formation (O'Shea et al., Science 245: 646-648 [1989]).

A region of GCN4 rich in basic amino acid residues, immediately adjacent to the leucine zipper, is involved in DNA recognition. We find that this basic region by itself, when dimerized via a flexible disulfide linker in place of the leucine zipper, binds DNA in a sequence-specific manner. In addition to simplifying structural analysis of this new motif, this finding provides a new strategy for the design of DNA-binding peptides (Talanian et al., Science 249: 769-771 [1990]).

CG 016 NMR CHARACTERIZATION AND ACTIVITY OF CONSTRAINED PEPTIDES

David E. Wemmer, Joseph H.B.Pease, Richard W. Storrs, Dagmar Truckses and Brian Volkman; Department of Chemistry, University of California, Berkeley, CA 94720

Hybrid sequences peptides, created using a disulfide scaffold taken from natural peptides which occur in bee venom, provide an mechanism for examining the relationship between secondary structure and activity. Hybrid peptides have been synthesized which contain sequences from the proteins ribonuclease A, myosin light chain kinase and basic pancreatic trypsin inhibitor. NMR spectroscopy has been used to show that in all of these hybrid sequence peptides a structure homologous to the parent peptide apamin has indeed been formed. This is comprised of a β-turn, loop and two+ turns of α-helix. NMR and CD have been used to determine how the helix propagates beyond the anchored second turn as a function of sequence and solution conditions. The activity of these hybrid sequences has also been determined, showing that the introduction of structural constraints using disulfides can be done without significantly altering activity. The systems studied, their structural characterization and their activity will be described.

Large Proteins/Isotope Labeling

CG 017 STABILITY AND DYNAMICS OF THE FOLDED STATE OF T4 LYSOZYME, Lawrence P. McIntosh, D. Eric Anderson, Amy Roth, Jirong Lu, and F.W. Dahlquist, Institute of Molecular Biology, University of Oregon, Eugene, OR 974093

The folding equilibrium of T4 lysozyme is highly pH dependent. In 200 mM KCl and 20°C, the folded state is maximally stable near pH .5. At pH 1.0 this stability is lowered by about 10 kcal/mole. This pH dependent stability is due to specific interactions of several acidic residues in the folded state. We have used NMR techniques to measure the pKa values of all the carboxyl groups of T4 lysozyme. The changes in pKa value between the folded and unfolded states reflect the energetics of the specific interactions of the individual carboxyl groups in the protonated versus the unprotonated state. We have used this approach to dissect the contributions made by each acidic residue to the stability of the folded state. The salt bridge between Asp70 and His31 formed in the folded state is responsible for about 4 kcal/mole of the stabilization at pKa so this is reflected in substantial shifts in the pKa values of Asp70 and His31 from their noninteracting values. Three other acid residues have specific interactions in the folded state which contribute about 4 kcal/mole to the stability of the folded state. These include Asp47 (H-bonded to the NH of residue 54) Asp62 (salt bridge with Arg95), and Asp92 (capping a helix and in a salt bridge with Arg95). These four interactions account for most of the pH dependent stability in the acid range.

We have completely identified the backbone ^{1}H and ^{15}N resonances of isotopically enriched T4 lysozyme by two- and three-dimensional heteronuclear NMR methods. Using amide hydrogen exchange to probe the static and dynamic solvent accessibility of the wild type protein, we found that the amide protons in three α-helices (residues 5-11, 95-104, and 149-154) are highly protected from exchange. These helices contact each other, forming a stable hydrophobic core in T4 lysozyme. Currently we are assigning the ^{1}H-^{15}N HSMQC spectra of several T4 lysozyme variants including the temperature sensitive proteins D70N, A98V, and A146T, and the double mutant T21C/T142C. The latter protein is stabilized in the oxidized state due to a disulphide bridge across its active site cleft. We are mapping the structural and dynamic effects of these mutations on the backbone of T4 lysozyme by chemical shift , NOE, and hydrogen exchange measurements. For example, the substitution of Ala146 by Thr (A146T) reduces the thermal stability of T4 lysozyme ($\Delta\Delta G$ = 2 kcal/mol at pH 2). The insertion of the bulkier sidechain disrupts the static packing in the hydrophobic interior of the protein, as shown by crystallographic analysis (B.W. Matthews *et al.*). This sidechain replacement causes residues 137-150, which form two α-helices, to interconvert between NMR-distinct conformations on the millisecond timescale. Also, several amide protons near site 146 have accelerated amide exchange kinetics. The dynamically perturbed sites are broadly localized to position 146; however, the effects are more extensive than suggested by the crystallographic analysis of the lysozyme mutant. These results support a model in which destabilizing mutations enhance local fluctuations (entropy) and disrupt favorable contacts of the native protein.

CG 018 APPLICATION OF C13-LABELED AMINO ACIDS TO ESTABLISH THE C13,C13-CONNECTIVITIES FOR LARGER PROTEINS

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A key step in the NMR analysis of a protein is the extension of backbone sequential assignments to side chains. With protein of mederate size (up to about 30kDa by recent estimates), uniform N15 and C13 labeling coupled with multidimensional NMR spectroscopy appears to be the method of choice. It is clear, however, that selective labeling methods will be needed for the NMR analysis of much larger proteins, although such methods require the laborious preparation of numerous isotopiomeric proteins. In principle, assignments can be made step-by-step along the carbon skeleton of each amino acid from each carbonyl carbon to the side chain of each residue by providing suitable pairwise C13 labeled amino acids. As a test of this approach, we are applying it to *Streptomyces* subtilisin inhibitor (SSI, 23 kDa) and its proteinase complexs (78 kDa) whose carbonyl carbons have been assigned by the C13,N15 double labeling requential assignment method. We are evaluating strategies that involve the incorporation of a variety of multiple C13 labeled amino acids prepared by microbial fermentation or chemical synthesis. The goal is to establish an efficient combination of isotope labeling and multidimentional NMR experiments that will provide the assignments of side chain resonances from larger proteins.

CG 019 STABLE ISOTOPE ASSISTED STRATEGIES FOR NMR ANALYSIS OF LARGER PROTEINS, John L. Markley, Arthur S. Edison, Andrew P. Hinck, Andrej M. Krezel, Stewart N. Loh, Ed S. Mooberry, Byung-Ha Oh, Brian J. Stockman, Jinfeng Wang, William M. Westler, Biochemistry Department, University of Wisconsin-Madison, 420 Henry Mall, Madison, WI 53706-1569

Recent work in several laboratories has led to rapid progress in the NMR analysis of proteins in the 10 - 25 kDa range. Although the experimental approach is still evolving, some principles appear to be emerging. The most general strategy involves multinuclear multidimensional NMR studies of proteins enriched uniformly with ^{15}N and/or ^{13}C. Double (^{13}C + ^{15}N) labeling is needed for (^{13}N-^{15}N) correlations which are efficiently detected by triple resonance $^{1}H\{^{13}C,^{15}N\}$ experiments. Uniform enrichment permits the exploitation of heteronuclear coupling connectivities along with the greater chemical shift dispersion of ^{13}C and ^{15}N compared to ^{1}H. Higher dimensionality (3D and 4D) NMR experiments provide the uniqueness of frequency determination needed for straightforward spectral assignments. With isotopic labeling, sequential assignments can be based on ^{13}C- or ^{15}N-resolved NOEs or alternatively on through-bond coupling interactions that traverse the peptide bond. Uniform ^{13}C enrichment and the resulting ^{13}C-^{13}C coupling pathways facilitate side-chain assignments. Secondary structural elements can be readily recognized in certain types of heteronuclear 2D and 3D spectra. Measurement of the additional three-bond coupling constants that can be determined with labeled proteins provides additional constraints that can be used in structural determinations. I will discuss our experiences in applying the uniform labeling approach to staphylococcal nuclease (17 kDa), flavodoxin (19.3 kDa), ferredoxin (10 kDa) and cytochrome c_{553} (10 kDa). In several instances selective labeling had to be used in order to complete assignments or determine particular interactions.

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CG 020 STRUCTURAL STUDIES OF MEMBRANE PROTEINS, S. J. Opella, Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104

Although NMR spectroscopy is capable of determining the structures of proteins and membrane proteins can be prepared in two effective model membrane environments, detergent micelles and phospholipid bilayers, for biophysical studies including NMR spectroscopy, neither of these environments are well suited for the multidimensional NMR experiments that work so well with globular proteins in solution.

The difficulties associated with micelles arise from their relatively slow overall reorientation rate and can be overcome by judicious use of isotopic labels, in particular uniform ^{2}H labeling of carbon sites and ^{15}N labeling of all nitrogen sites. The analysis of the resulting three-dimensional spectra enable the residues in the hydrophobic and amphipathic helices of peptides and proteins to be identified. Proteins in phospholipid bilayers are immobile on NMR timescales and can be oriented between glass plates. The resonance frequencies and splitting observed in solid-state NMR spectra give the orientation of the helices relative to the plane of the bilayer, establishing the overall arrangement of the secondary structure of membrane proteins.

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Protein Dynamics

CG 021 MOLECULAR DYNAMICS OF STAPHYLOCOCCAL NUCLEASE IN SOLUTION AND IN THE CRYSTALLINE STATE. Dennis A. Torchia, Donna M. Baldisseri, Holly B.R. Cole, Lewis E. Kay and Ad Bax. National Institutes of Health, Bethesda, MD 20892

High resolution solution and solid state nmr techniques are well suited for studying internal dynamics of proteins through measurements of relaxation parameters, linewidths and hydrogen exchange rates. I will report measurements of ^{15}N relaxation times of the valine residues in Staphylococcal nuclease, SNase, complexed with thymidine 3',5'-bisphosphate and Ca^{2+}, in the crystalline state, and compare these measurements with relaxation times obtained in solution. The correlation of the solid state nmr measurements with X-ray temperature factors and with the results of a calculated dynamics trajectory (100 ps) will also be discussed. Sidechain dynamics in solution have been investigated by measuring relaxation parameters for the methyl carbons of the eleven leucine residues in SNase. Order parameters derived from these measurements using the method of Lipari and Szabo will be compared with X-ray temperature factors. The order parameters, together with various models of sidechain motion, were used to estimate the amplitudes of internal motions of the leucine sidechains. The uncertainties in the order parameters arising from experimental errors and from the neglect of dipolar cross-correlations will be discussed. Measurements of ^1H-^2H exchange rates of liganded and non-liganded SNase were made to determine the extent that ligand binding retards hydrogen exchange of assigned amide protons in SNase. I will discuss results obtained at 37 C and two pH values.

CG 022 NMR STUDIES OF PROTEIN STRUCTURE AND DYNAMICS, Gerhard Wagner, Jeff W. Peng, Sven G. Hyberts, Matthew Goldberg, Robert Clubb, Marc Adler, David Detlefsen and Thanabal Venkataraman, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115.

Conformation and mobility of a several small proteins were studied by NMR. Structures were determined for kistrin, an inhibitor of $GPII_bIII_a$, eglin c, an elastase inhibitor, and cytochrome c_{551} from *Pseudomonas aeruginosa*. Particular emphasis was on characterizing the conformations of surface regions that are important for the function of these proteins. For kistrin, this is a surface loop that contains an RGD sequence that is recognized by $GPII_bIII_a$, a receptor on platelet surfaces responsible for final events of blood clotting. For eglin c it is a loop that interacts with proteases. For proteins where we had isotope labeled samples we have measured heteronuclear ^1H-^{15}N and and homonuclear ^1H-^1H vicinal coupling constants in 2D and 3D experiments to characterize dihedral angles, in particular on protein surfaces. Based on these data a few differences of side chain orientations between solution and crystal structures were found. Furthermore, we have made extensive measurements of relaxation parameters for ^{15}N, such as T_1, T_2, $T_{1\rho}$, heteronuclear NOE and heteronuclear cross relaxation. An expression for ^{15}N $T_{1\rho}$ where the protons are not spin locked was derived, and the usefulness for characterizing the spectral density function will be discussed. Finally, measurements of these relaxation parameters will be shown that were used to characterize effects of mutations on eglin c.

CG 023 CORRELATION OF STRUCTURE, DYANAMICS AND STABILITY OF BPTI AND ITS 'MUTANT' FORMS Y35G, F22A, Y23A, AND F45A.

Clare Woodward, Dept.of Biochemistry, Univ. of Minnesota, St. Paul, MN 55108.

Bovine pancreatic trypsin inhibitor (BPTI) submolecular domains may be distinguished by their relative hydrogen isotope exchange rates. The hydrophobic core is identified by the 3 amide protons whose exchange with solvent is slower than any others in the molecule. We consider the cluster of atoms packing these protons, the backbone NH's of residues 21, 22, and 23, to be the hydrophobic core of the molecule. The core corresponds to the 'knot' described by Gregory & Lumry. In contrast, the two overlapping loops at the trypsin-binding end of the inhibitor, residues 9-17 and 36-42, constitute a quite separate dynamical domain. Exchange of buried NH's in this region is fast, and their rates approach those of some surface amide protons. The loops enclose 4 buried waters H-bonded to buried donor and acceptor protein atoms in crystals. We have produced BPTI with single amino acid substitutions in both regions, using the system of David Goldenberg and starting with WT and Y35G genes generously provided by him. In Y35G, tyrosine 35 is missing from the flexible loop region. In F22A, Y23A, and F45A, aromatic side chains in the hydrophobic core are replaced with alanine. Characterization of Y35G is completed; studies of F22A, Y23A, and F45A are more preliminary. The crystal structure of Y35G (D. Housset & A. Wlodawer et al., unpublished results), and Y35G NMR solution structure (Key Sun Kim & C. Woodward, unpublished results) are very similar. In Y35G, the core is unperturbed but the loops undergo major reordering, with some atom positions in the crystal structure differing fromWT by as much as 6 Å. Differential scanning calorimetry at pH 2 gives a Tm = 70° for Y35G, compared to 87° for WT; $\Delta G°$ is reduced to 4 kcal/mol compared to 8 kcal/mol in WT, and the change in $\Delta G°$ is entirely enthalpic. The change in ΔCp for Y35G versus WT is in the direction, but not the magnitude, expected if apolar surface equivalent to a tyrosine side chain is removed from the denatured state. Hydrogen exchange kinetics of Y35G indicate that in solution the loop region is very highly flexible, and freely accessible to solvent.

Late Abstract

RECENT DEVELOPMENTS IN MULTI-DIMENSIONAL NMR SPECTROSCOPY

Ad Bax, Mitsuhiko Ikura, Lewis E. Kay, Gaetano Barbato, Silvia Spera, Guang Zhu, Marius Clore, Angela Gronenborn and Dennis Torchia[a], Laboratory of Chemical Physics, NIDDK, and [a]Bone Research Branch, NIDR, National Institutes of Health, Bethesda, Maryland 20892.

Development of triple and quadruple resonance 3D and 4D NMR pulse sequences now permits assignment of proteins of up to 200 residues in a straightforward manner without relying on NOE information. Modifications and improvements to previously published pulse sequences are described that permit a small increase in the molecular weight limit of proteins that can be studied, and that may reduce sample preparation requirements.

The availability of isotopically enriched protein makes it relatively straightforward to measure the exchange rate of labile amide protons. Not only the slowly exchanging amides can be characterized, but also the HN protons that exchange on a time scale of seconds. The techniques will be demonstrated for calmodulin and for its complex with the target site of myosin light chain kinase.

The availability of $^{13}C/^{15}N$ labeled protein makes it possible to study for the first time the $^{1}H/^{2}H$ isotope shift of the backbone ^{15}N nuclei in a protein. Although the experimental setup is demanding and requires three external frequency sources in addition to a regular spectrometer operating in the inverse mode, this isotope effect could be measured accurately for nearly half the backbone ^{15}N nuclei in staphylococcal nuclease.

Structural Analysis of Proteins and Folding Intermediates

CG 100 STRUCTURE AND FUNCTION OF ACYL-COA-BINDING PROTEIN FROM BOVINE LIVER USING 2D ^{1}H NMR, Kim V. Andersen, Svend Ludvigsen, Jens Knudsen*, and Flemming M. Poulsen, Department of Chemistry, Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Copenhagen, Denmark. *Institute of Biochemistry, University of Odense, DK-5230 Odense M, Denmark.

A 86 residue acyl-coenzyme A binding protein from bovine liver (ACBP) has been studied by 2D ^{1}H NMR spectroscopy, in order to determine the three-dimensional structure in solution.

All of the amino acid residue spin systems have been identified, and full sequential assignment has been obtained.

The main feature in the secondary structure is a set of four α-helices. The three-dimensional structure has been calculated using a combination of distance geometry and simulated annealing. This structure will be presented, and the structural features will be discussed.

Spectra of the complex between Acyl-CoA and ACBP have been recorded for acetyl-CoA/ACBP and palmityl-CoA/ACBP, and the binding-site has been identifyed. The structure of the complex will be presented.

CG 101 SOLUTION STRUCTURE OF PARSLEY PLASTOCYANIN S. Bagby, P. C. Driscoll and H. A. O. Hill, The Oxford Centre for Molecular Sciences, University of Oxford, South Parks Road, Oxford, U. K.

Plastocyanin is a 'blue' copper protein which transfers electrons from cytochrome *f* to pigment $P700^+$ of photosystem I in the photosynthetic electron transport chain of organisms ranging from blue-green algae to higher plants. Higher plant plastocyanins show a high degree of primary structure conservation and possess a functionally important surface patch of negatively charged residues. In the exceptional case of parsley plastocyanin, this acidic recognition site may be disrupted by the deletion of residues Met-57 and Ser-58, and the change of residue 59 from a Glu to a Gln. Parsley plastocyanin also shows a significantly lower sequence homology (65%) to poplar plastocyanin (for which a number of crystal structures have been obtained) than do other higher plant plastocyanins.

In order to determine the structural and functional effects of these sequence differences, NMR data are being used to calculate the solution structure of parsley plastocyanin. The calculations employ simulated annealing protocols within X-PLOR, currently using approximately 1100 interproton and 50 hydrogen bonding distance restraints, supplemented by 35 φ backbone and 19 χ_1 side chain torsion angle restraints. Stereospecific assignments for β methylene and valyl methyl protons are now being obtained using line-fitting procedures. The parsley plastocyanin structure will be compared with crystal structures of poplar and *Enteromorpha prolifera* (green alga) plastocyanins, and with solution structures of French bean and *Scenedesmus obliquus* (green alga) plastocyanins. Further, we aim to rationalise cation binding data we have previously obtained for spinach and parsley plastocyanins.

CG 102 ESTIMATING α-β COUPLING CONSTANTS FROM NH-CαH CROSS PEAK FINE STRUCTURE. Kristin Bartik[a] and Christina Redfield[b]

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Stereospecific assignments of the β methylene protons in a protein lead to a significant improvement of the definition of the calculated solution structure of the protein both with respect to the backbone and the arrangement of the side chains. Methods used for stereospecific assignment require an estimate of the α-β scalar coupling constants and the relative NH to CβH NOE intensities. In this poster we will show that it is possible to obtain an estimate of the α-β scalar coupling constant from the analysis of the fine structure of the fingerprint cross peaks in high resolution phase sensitive COSY spectra. The fingerprint cross peaks from hen and turkey lysozyme spectra were analyzed and the estimate of the α-β coupling constants were compared to the values measured using E.COSY experiments.

CG 103 BINDING OF A SEGMENT OF FIBRINOGEN (α27-50) TO THROMBIN

C. G. Binnie, G. J. Pielak* and S. T. Lord. Department of Pathology, *Department of Chemistry, University of North Carolina, Chapel Hill, NC 27599.

The serine proteinase thrombin cleaves both Aα and Bβ chains of fibrinogen releasing fibrinopeptides A (residues 1-16) and B (residues 1-14), thereby converting the soluble fibrinogen to insoluble fibrin, the main protein component of a blood clot. We have previously shown that a 24-residue segment of fibrinogen, α27-50, (but with the 4 cysteines replaced by alanines) inhibits fibrinogen cleavage by thrombin but does not inhibit the catalytic site of the enzyme (C. G. Binnie & S. T. Lord, Thrombosis and Haemostasis, in press). These results indicate that this peptide binds to thrombin at a site distinct from the catalytic site. This interaction is being further investigated by NMR techniques to determine which of the residues are bound by thrombin and, ultimately, to establish the conformation of the proteinase-bound peptide.

COSY, relayed-COSY, and NOESY data have been collected for the peptide in solution for the purpose of residue assignment and to determine if the free peptide has structure. NOESY data have also been collected for the peptide in the presence of thrombin. Preliminary results indicate that a number of peaks are broadened in the presence of thrombin, suggesting direct interaction between the peptide and the proteinase. Peak assignments are continuing to identify which residues are involved. There were no time-dependent spectral changes for the mixture, indicating that thrombin does not cleave the peptide and simply inhibit by acting as an alternate substrate.

CG 104 **SOLUTION STRUCTURE AND LIGAND BINDING PROPERTIES OF PDC-109 DOMAIN B, A HOMOLOG OF FIBRONECTIN TYPE II MODULES,** Keith Constantine, Lazslo Patthy*, and Miguel Llinás, Department of Chemistry, Carnegie Mellon University, Pittsburgh, PA 15213 and *Institute of Enzymology, Biological Research Center, 1502 Budapest, Hungary.

Sequential ^{1}H-NMR resonance assignments for the second type II domain of bovine seminal fluid protein PDC-109 (PDC-109/b) have been obtained from analysis of 2D spectra recorded at 500 MHz. The data reveal two small antiparallel β-sheets, a *cis* Pro residue, and a cluster of aromatic residues. Initial low resolution molecular models demonstrate that the global fold of PDC-109/b is somewhat different from that predicted by the homology between type II and kringle domains. Models refined via simulated annealing and relaxation matrix protocols, as well as ligand binding studies using leucine analogs, indicate that the putative collagen binding site is structured largely by the above mentioned aromatic cluster, which consists of Tyr^7, Trp^{26}, Tyr^{33} and Trp^{39}.

CG 105 NMR STUDY OF THE SOLUTION STRUCTURE OF THE N-TERMINAL DOMAIN OF RAT CD2.

Paul C. Driscoll, Jason Cyster, Alan F. Williams & Iain D. Campbell.
Department of Biochemistry, Oxford University, Oxford OX1 3QU, UK and MRC Cellular Immunology Research Unit, The Sir William Dunn School of Pathology, Oxford University, Oxford OX1 3RE, UK.

The T-cell erythrocyte receptor (or CD2 antigen) is expressed on the surface of thymocytes and mature T-cells. CD2 is involved in the processes of adhesion of T-cells to antigen-presenting cells (by binding to a homologous protein known as LFA-3) and activation of the T-cell response (thought to be synergistic with the MHC-TCR-CD4/8 interaction). The domain structures of CD2 and LFA-3 have been recognised as belonging to the immunoglobulin superfamily. However the absence of disulphides in the N-terminal C2-type domain has resulted in much speculation as to the actual three-dimensional structure[1,2]. A 101-residue construct of the N-terminal domain of rat CD2 has been expressed in E. coli as a fusion protein with glutathione-S-transferase. The fusion product was purified by affinity chromatography on glutathione-agarose and subsequently cleaved with thrombin at a six residue linking sequence. ^{15}N incorporation has been achieved by producing the protein in mimimal media and three-dimensional ^{15}N-^{1}H NOESY-HMQC and HOHAHA-HMQC spectra have been recorded on a 5mM sample of the labeled protein. We will present the assignment of the spectra and the solution structure determination of this molecule. A further aim of this work is to investigate the binding characteristics of the N-terminal domains of CD2 and LFA-3, with a view to a NMR study of the complex.

1. Williams et al., Cold Spring Harbor Symp. Qaunt. Biology (1989) Vol. LIV, 637
2. Recny et al., J. Biol. Chem. (1990) Vol. 265, 8542.

CG 106 LOCALIZATION OF PROTON-TRANSFER EFFECTS OF MECHANISTIC IMPORTANCE IN THE ACTIVE-SITE REGION OF ESCHERICHIA COLI THIOREDOXIN USING TWO-DIMENSIONAL ^{1}H NMR, H. J. Dyson, L. L. Tennant and A. Holmgren, Department of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla, CA 92037, Department of Physiological Chemistry, Karolinska Institutet, Stockholm, Sweden

A series of two-dimensional (2D) correlated ^{1}H NMR spectra of reduced and oxidized Escherichia coli thioredoxin have been used to probe effects of pH in the vicinity of the active site -Cys_{32}-Gly-Pro-Cys_{35}-, using the complete proton resonance assignments available for thioredoxin. In either oxidation state, the majority of the thioredoxin molecule remains unchanged between pH 5.7 and pH 10, as indicated by the identical chemical shifts of the $C^{\alpha}H$, $C^{\beta}H$ and other protons. In reduced thioredoxin, a fairly widespread region around the active site dithiol is affected by the titration of a group or groups with pK_a approximately 7.1-7.4 in ^2H_2O. Another titration, with pK_a approximately 8.4, affects a smaller region of the protein. Oxidized thioredoxin contains a disulfide and no free thiol groups; nevertheless, the proton resonances of many groups in the active site region were observed to titrate with a pK_a of 7.5, probably as a result of an abnormally high pK_a value for the carboxyl group of the buried Asp 26 residue. For reduced thioredoxin, the results indicate that Asp 26 is titrating in this pH range, as well as both thiol groups. The new results are strongly suggestive that the mechanism of thioredoxin-catalysed protein disulfide reduction may be critically dependent on proton transfer as well as electron transfer within the active site.

CG 107 LOW RESOLUTION SOLUTION STRUCTURE OF *Bacillus subtilis* ENZYME III^{glc} DERIVED FROM ^{15}N-^1H HETERONUCLEAR THREE DIMENSIONAL NMR SPECTROSCOPY

Wayne J. Fairbrother[1], Garry P. Gippert[1], Milton H. Saier[2], Jonathan Reizer[2], and Peter E. Wright[1]. [1] Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037 and [2] Department of Biology, University of California, San Diego, CA 92093.

The structure of enzyme III^{glc} (162 residues, M_r = 17,381) of the *Bacillus subtilis* phosphotransferase system (PTS) is being investigated using ^{15}N-^1H heteronuclear three dimensional NMR techniques. The enzyme is thought to play a regulatory role. The non-PTS sugar uptake systems are subject to negative control by free III^{glc}, and adenylate cyclase is subject to positive control by phospho-III^{glc}. The aim of the present structural study is to provide an understanding of the mechanisms by which phosphorylation of a specific histidine residue can affect the proteins activity.

Here we present the determination of a low resolution structure of unphosphorylated III^{glc} in solution based on NOE derived approximate interproton distance constraints involving principally the NH, $C^{\alpha}H$ and $C^{\beta}H$ protons. With the exception of $C^{\alpha}H$-$C^{\alpha}H$ NOEs, all the NOEs were assigned from a three dimensional NOESY-HMQC experiment.

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CG 108 Application of Two and Three Dimensional Proton NMR to the Structure Determination of Apo-neocarzinostatin

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Automated Edman sequencing of the apo-protein components of antitumor proteins mitomalcin (MMC) and neocarzinostatin (NCS) revealed that apo-MMC and apo-NCS are identical in sequence (113 residues). This conclusion was later confirmed by NMR experiments. Sequencing experiments of apo-MMC and apo-NCS also detected several mispositioned ASN and ASP residues reported previously [B. W. Gilson, et al., J. Biol. Chem., 259, 10801-10806 (1984); K. Kuromizu, et al., Arch. Biochem. Biophys., 246, 199-205 (1986)].

Apo-NCS was used as a model system to develop the application of combined two and three dimensional (2D and 3D) proton NMR spectroscopy to the structural determinations of proteins of moderate size without isotope labelling. The initial step of data analyses focused mainly on spin system identification and the assignments of the main chain resonances. A list was then generated, which identifies all possible proton-proton interactions for each of the cross-peaks in NOESY spectra. Extensive overlaps in such 2D data sets were evident. The 3D proton NMR spectra (NOESY-TOCSY or TOCSY-NOESY) were found powerful in resolving ambiguous resonance and cross-peak assignments in 2D data sets and especially useful in identifying long range proton-proton interactions characteristic of protein secondary folding. Strategies of 3D data analysis aimed at the assignments of overlapped 2D cross-peaks were developed.

By combined application of 2D and 3D NMR experiments, we have completely assigned 99% of protons including those of the side chains. The comparisons of our results and recent reports on the 2D NMR studies of apo-NCS [É. Adjadj, et al., Eur. J. Biochem., 190, 263-271 (1990); M. L. Remerowski, et al., Biochem. 29, 8401-8409 (1990)] demonstrated the advantage of using 3D NMR spectroscopy in the studies of molecules of moderate size, such as apo-NCS. We are able to obtain more complete proton resonance and secondary structural assignments and find several misassignments in the earlier report. Such improvements are of critical importance for accurate determination of three dimensional solution structure. Strategies utilized in this work should also be informative for developing automation procedures assisting spectrum assignments and data management, which is seen necessary for the application of multidimensional NMR techniques to the high resolution structure determination of macrobiomolecules becoming a routine practice.

A set of distance and torsional angle restraints derived from NMR data have been used to elucidate the three dimensional structure of the apo-NCS by distance geometry as well as restrained molecular dynamics. The solution structure of apo-NCS provides the basis for the characterization of the holo-NCS by NMR and molecular modeling methods.

CG 109 DISTANCE MEASUREMENTS IN A MEMBRANE PROTEIN FROM RELAXATION OF ^{1}H NMR RESONANCES BY A COVALENTLY ATTACHED NITROXIDE: SUBUNIT c OF THE F_1F_0 ATPASE. M.E. Girvin and R.H. Fillingame, Physiological Chemistry, University of Wisconsin, Madison, WI 53706. Subunit c is one of three subunits in the membrane-traversing F_0 portion of the H^+-transporting ATP synthase. It functions in translocating protons across the membrane. Subunit c is a protein of 79 amino acids that is predicted to fold as a hairpin of two α-helices in the native complex. Most of the ^{1}H NMR resonances of purified subunit c have been assigned using conventional 2D NMR techniques (Girvin and Fillingame, Biophys. J. 57, 349a 1990). The long range NOEs necessary for determining the 3D conformation of the protein have been difficult to observe, both because of the scarcity of approaches under 4Å between residues in the two helices, and because many of the observed NOEs involve protons in extremely crowded regions of the spectrum that cannot be resolved. By specifically modifying Asp61 in Helix II of the protein with a DCCD (dicyclohexyl-carbodiimide) analog containing a nitroxide radical (NCCD), it was possible to measure distances between the two helices by measuring the increase in linewidth of resolved and previously assigned COSY cross peaks due to paramagnetic relaxation. The nitroxide bearing ring of NCCD was found to be predominantly located in the vicinity of Gly58. Distances of 14Å and 16Å were calculated between the unpaired electron of NCCD in Helix II and the H^α of Ala24 and Ala25, respectively, in Helix I, and longer distances were calculated for other residues in Helix I. These distances are in agreement with genetic and biochemical results from the intact enzyme which have shown that substitutions for Ala24 reduce the reaction rate of DCCD with Asp61.

CG 110 A PULSE METHOD FOR MEASUREMENT OF PROTON T_2'S IN HOMONUCLEAR COUPLED SYSTEMS; APPLICATION TO PROTEIN STRUCTURAL STUDIES.

M. Gochin, T.L. James and I.D. Kuntz, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143-0446

A method is described for obtaining proton transverse relaxation times in homonuclear coupled systems. The oscillatory effect of the coupling on the T_2 decay was removed by using the attached heteronucleus as a filter. A BIRD pulse (1) was applied in the center of the T_2 decay period, causing directly and remotely connected protons to the heteronucleus to be decoupled from each other. Protons directly bound to the heteronucleus were inverted, leaving remote protons unaffected. Thus the method works well in natural abundance ^{13}C and ^{15}N systems or for enriched ^{15}N biological materials, where no N-N connectivities exist. The importance of obtaining protons T_2's pertains to their usefulness and sensitivity in quantitating structure and mobility in molecules. Sequences for obtaining proton T_2's were described and demonstrated on formate, alcohol and gramicidin-S. The accuracy of the measured T_2 as a function of X-nucleus offset and heteronuclear coupling constant was assessed. The method was applied to Bovine Pancreatic Trypsin Inhibitor (BPTI) and to spin-labelled BPTI to obtain a long range distance set of protons whose relaxation was influenced by the spin-label. This method has the potential for enhancing the accuracy and resolution of structures determined by NMR.

(1) J.R. Garbow, D.P. Weitekamp and A. Pines, *Chem. Phys. Lett.* **93**, 504 (1982)

CG 111 DETERMINATION OF THE THREE DIMENSIONAL FOLD OF ASCARIS TRYPSIN INHIBITOR IN SOLUTION USING NUCLEAR MAGNETIC SPECTROSCOPY, Bruce Grasberger, Angela M. Gronenborn, and G. Marius Clore, Laboratory of Chemical Physics, Building 2, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Md, 20892

Ascaris trypsin inhibitor is a member of a family of proteinase inhibitors whose three dimensional structure has not been determined to date. Proton resonance assignments and coupling constant measurements made at 30°C, pH4.75 using PE.COSY, P.COSY, HOHAHA, and NOESY experiments were used to obtain interproton distance and torsion angle restraints. These restraints were then used to calculate a series of structures using a hybrid distance geometry-dynamical simulated annealing method. The structures confirm the predicted secondary structural elements. Specifically, two regions of ß-sheet are observed, including residues 11-14 (strand 1) and 37-39 (strand 2) in the first, and 46-49 (strand 4) and 53-56 (strand 5) in the second, with a possible contribution of residues 20-22 to form a triple stranded sheet.

CG 112 AMIDE EXCHANGE AT PH 6.5: A SIMPLE TECHNIQUE FOR VERIFICATION OF ^{1}H NMR ASSIGNMENTS AND 2°/3° STRUCTURE IN PROTEINS, Sarah L. Heald†, Hans-Dietrich Hörlein, Jürgen Beunink and Jürgen Ebbers, †Miles Research Center, Miles Inc., 400 Morgan Lane, West Haven, CT 06516 and PH-P VE Biochemie, Bayer AG, Postfach 101709, 5600 Wuppertal 1, Germany.

The Alzheimer's Precursor Protein (APP) contains a Kunitz-type protease inhibitor domain (APPI). This isolated domain has recently been analyzed by 2D NMR techniques at pH 6.5 and 25°C. In the final stage of NMR resonance assignments, secondary structural elements similar to BPTI were identified. As a quick test of both the NMR resonance assignments and the proposed BPTI-like tertiary structure, the temperature was raised in 5°C steps in order to 'white-out' the amide resonances proposed to be on the protein's surface through rapid exchange. Three temperature points (25°C, 45°C and 65°C) were examined in more detail by collecting COSY spectra and assigning the residual amide resonances. Amides occurring in the freely-rotating residues on the N- and C-termini, as well as amides found in the loop regions of the protein's tertiary structure, were seen at 25°C but not at 45°C. Above 45°C, the amides located in the helices were 'whited-out' which left only the central β-sheet at 65°C yielding a spectrum similar to that obtained in D_2O. This phenomema was then studied in more detail using a ^{15}N-enriched BPTI-mutant, HMQC, HMQC-TOCSY, HMQC-NOESY and smaller temperature increments.

CG 113 CHARACTERISATION OF A DOMAIN RESPONSIBLE FOR INTER-SUBUNIT BINDING IN THE *BACILLUS STEAROTHERMOPHILUS* PYRUVATE DEHYDROGENASE MULTI-ENZYME COMPLEX

Deborah S. Hipps and Richard N. Perham, Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, UK.

A sub-gene encoding the N-terminal 170 residues of the *Bacillus stearothermophilus* dihydrolipoamide acetyltransferase polypeptide chain has been over-expressed in *Escherichia coli*. The expressed protein consists of the lipoyl domain, interdomain linker and peripheral subunit-binding domain. This latter is responsible, in *B. stearothermophilus*, for binding the pyruvate decarboxylase and the dihydrolipoamide dehydrogenase subunits of the intact pyruvate dehydrogenase complex. The expressed protein can be purified by a combination of anion and cation exchange chromatography. It binds to both types of matrix at pH 7.8 owing to its flexible dumb-bell like nature: the lipoyl domain has a net negative charge whereas the binding domain is positively charged. The di-domain is folded in a native conformation as assessed by enzymic assays and by limited proteolysis. It binds purified dihydrolipoamide dehydrogenase to form a stable complex capable of withstanding gel-filtration, electrophoresis in a native gel system and chemical cross-linking. The 400 MHz ^{1}H NMR spectrum of the di-domain shows peaks corresponding to those seen in spectra of the lipoyl domain plus others characteristic of residues in the mobile linker segment, and other as yet unidentified peaks which are likely to arise from the binding domain.

CG 114 THE RNA BINDING DOMAIN OF AN snRNP PROTEIN IS STRUCTURALLY RELATED TO RIBOSOMAL PROTEINS, David W. Hoffman, Charles C. Query, Barbara L. Golden, Stephen W. White and Jack D. Keene, Department of Microbiology and Immunology, Duke University Medical Center, Durham, NC 27710 USA

An RNA recognition motif (RRM) of approximately 80 amino acids constitutes the core of RNA-binding domains found in a large group of proteins involved in RNA processing. The U1 RNA-binding domain of the human U1snRNP-A protein containing an RRM sequence was investigated using NMR spectroscopy. The RRM in solution is a highly stable, monomeric domain consisting of four antiparallel β-strands and two α-helices. The highly conserved RNP1/octamer sequence and the RNP2 sequence, containing amino acids previously suggested to be involved in nucleic acid binding, are juxtaposed in adjacent β-strands. Conserved aromatic side chains that are critical for RNA binding are clustered on the surface of the protein adjacent to a variable loop that influences recognition of specific RNA sequences. The secondary structure and topology of the RRM are similar to those of ribosomal proteins L12 and L30, suggesting a distant evolutionary relationship between these two families of RNA-associated proteins.

CG 115 STRUCTURAL COMPARISON OF HUMAN EPIDERMAL GROWTH FACTOR, HUMAN TRANSFORMING GROWTH FACTOR-α AND A LOW ACTIVITY MUTANT.

Hommel U., Harvey T.S., Driscoll P.C., Dudgeon T., Campbell I.D., Department of Biochemistry, University of Oxford, Oxford OX1 3QU, Great Britain.

Epidermal and Transforming Growth Factors (EGF, TGF) are potent activators of cell growth and replication. Over the past few years, considerable progress has been made in understanding providing the underlying mechanisms of how EGF and TGF interact with their receptor. The three dimensional structures of human and mouse EGF as well as human TGFα have been previously published at low resolution (Montelione *et al.* 1986, Cooke *et al.* 1987, Khoda *et al.* 1988). Recently we have completed our study on hEGF and hTGFα, which resulted in structures at high resolution (see also accompanying poster Harvey *et al.*). On this poster we present a detailed comparison of the two proteins, which exhibit the same biological function, at atomic level. In addition, we compare wild-type EGF with a mutant, which has a drastically reduced affinity for the EGF receptor. We will also propose a model, which rationalizes the observed differences.

References:
Cooke *et al.* (1987) *Nature* **327**, 339 - 341.
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CG 116 PEGASUS: PROTEIN ENGINEERING AND GRAPHICS IN ANGLE SPACE USING SPECTROSCOPY, Bruce A. Johnson, Department of Biophysical Chemistry,Merck Sharp and Dohme Research Laboratories, Rahway, NJ 07065

A computer program for the determination of the three-dimensional structure of peptides and proteins from NMR data is described. The program has a rapid and flexible graphics display(1) and several minimization algorithms. The variable-target/ rapid analytical derivative method (2) is used as a gradient minimizer. Simulated annealing can be performed using the Metropolis algorithm. This provides a method that can escape from local minima. The energy corresponding to non-bonded contacts can be approximated using a simple but rapid repulsive function. A more complete, but slower, energy term (4) can also be used. Use of the rigid geometry model and full energy term (at later stages of minimization) results in structures with good fits to experimental data, ideal covalent geometries and low energies. Ramachandran plots can be displayed during and after structure-determination to check unreasonable main chain dihedral angles. Multiple structures can be superimposed and displayed. PEGASUS can assist the user in identifying unassigned NOEs. Simulated NOESY intensities can be calculated from the protein conformation using a relaxation matrix approach.

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CG 117 HIGH PRESSURE NMR STUDIES OF MODEL MEMBRANES AND PROTEINS, J. Jonas, X. Peng, S. Samarasinghe and A. Jonas* and D. A. Driscoll*, Department of Chemistry, School of Chemical Sciences and *Department of Biochemistry, College of Medicine, University of Illinois, Urbana, Illinois, 61801

Selected results of our recent NMR studies of the pressure effects on the structure and dynamics of biochemical systems will be reviewed. First, the effects of pressure, up to 5 kbar, on multilamellar vesicles of dipalmitoyl-3-sn-phosphatidylcholine perdeuterated in the acyl chains (d_{62}-DPPC) were examined using high-pressure ^2H NMR techniques. The experiments were performed on pure lipid bilayers in the liquid-crystalline state, and on bilayers in the liquid-crystalline state containing the local anesthetic tetracaine. ^2H line-shape measurements on various gel phases yielded a phase diagram for DPPC-d_{62}. Additional information was obtained from the measurements of the pressure effects on both ^{31}P lineshapes and relaxation times of the DPPC headgroup.

Secondly, the pressure induced reversible denaturation of hen egg white lysozyme in the presence and absence of tri-N-acetylglucosamine was studied by high resolution proton NMR. The reaction volumes for His-15, Leu-17, Trp-28, Cys-64 and Trp-108 residues were determined.

CG 118 EFFECT OF SITE-SPECIFIC MUTATION AND SOLVENT CONDITIONS ON INSULIN MONOMER STRUCTURE

Niels C. Kaarsholm, Melinda Roy, Henning Thøgersen, and Steen G. Melberg
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Protein aggregation and conformational flexibility together with unusual αH-NH exchange properties complicate solution structural studies of insulin. We have employed NMR and CD spectroscopy to evaluate the effects of several site-specific mutations and the response to changes in solvent conditions. Mutations at residues B9-Ser, B25-Phe and B16-Tyr are compared with respect to efficiency in providing sufficient monomerization as well as retaining native-like structure and biological activity. Secondary structure analysis shows considerable variability of monomer conformation. The results identify the B16-Tyr → His as a relevant species for sequential assignment and structure determination in aqueous solution.

CG 119 IDENTIFICATION OF 2D ^{1}H NMR PEAKS USING NEURAL NETWORKS, Mogens Kjær and Flemming M. Poulsen, Carlsberg Laboratory, Department of Chemistry, Gamle Carlsberg Vej 10, DK-2500 Valby, Copenhagen, Denmark.

A three layer forward-feed network has been trained to recognize AX ^{1}H COSY-type peaks in a protein NMR spectrum. The training set consists of simulated 2D peaks with different intensities and coupling constants as well as several types of artifacts and random noise. The network was thus trained not only to recognize the symmetry of the 2D peaks, but also the artifacts normally occurring in 2D NMR spectra of proteins (improper water signal suppression, ringing from data set truncation etc.). The training of the network takes several hundred hours of CPU time, however, the use of the network is faster than conventional peak picking algorithms, because of better utilization of modern parallel vector processors. The generality of the patterns used for training the network immediately suggests the possibility of training a network to recognize more complicated peak patterns.

CG 120 THE STRUCTURE AND DYNAMICS IN SOLUTION OF CALCIUM-FREE AND CALCIUM-BOUND CALBINDIN D_{9k}. Johan Kördel, Nicholas J. Skelton, Mikael Akke, & Walter J. Chazin, Department of Molecular Biology (MB2), Research Institute of Scripps Clinic, 10666 North Torrey Pines Road, La Jolla, CA 92037.

The long-term objective of structural research on the calmodulin superfamily of regulatory calcium-binding proteins is to understand the role of molecular conformation and dynamics in determining protein function and metal ion specificity. Since it has not been possible to crystallize any member of this family of proteins with different levels of calcium occupancy in a specific domain, structure determination in solution using ^{1}H NMR spectroscopy is uniquely suited to solve this problem. We have applied the NMR method to examine native porcine and recombinant bovine calbindin D_{9k} in the calcium-free and calcium-bound states. Three-dimensional solution structures have been calculated using the distance *geometry* algorithm (DISGEO implementation) followed by restrained molecular dynamics refinement in the AMBER force-field. The systematic grid search program HABAS was used to determine stereospecific assignments of prochiral methylene groups and to generate ~150 dihedral angle constraints. These were combined with distance constraints for 15-20 hydrogen-bonds and a large number (>750) of proton-proton distance constraints from unambiguous NOEs to generate structures of high quality. From these structures, we are able to begin to formulate a view of the molecular events associated with the binding of calcium by calbindin D_{9k}.

CG 121 2D NMR ON THE ACTIVE SITE IN PARAMAGNETIC METALLOPROTEINS: HEME PEROXIDASES, Gerd N. La Mar[1], Jeffrey S. de Ropp[2] and Liping P. Yu,[1] Department of Chemistry[1] and UCD NMR Facility,[2] University of California, Davis, CA 95616

While the information content of the ^1H NMR hyperfine shift in paramagnetic metalloproteins is particularly high, the full exploration of its potential has been hampered by the lack of effective methods for assigning resonances and determining structure. The application of nuclear 2D methods for paramagnetic proteins lags considerably behind that of diamagnetic macromolecules, based largely on the belief that the 2D methodology will be ineffective. The effective paramagnetic-induced relaxation was considered likely to "quench" the build up of NOEs due to short T_1s, and the broad lines and short T_2 were assumed to lead to cancellation of the antiphase components for the cross peak as well as signal loss due to rapid decay of coherence. We have explored the scope and utility of 2D dipolar and scalar connectivity experiments to the cyanide inhibited complex of resting state horseradish peroxidase, a 42 kDa glyco- hemoproteins which exhibits sizeable chemical shift dispersion (~60 ppm) and substantial (to 150 Hz) line broadening for key residues in the active site. Complete spin systems can be identified by COSY-type experiments even for resonances with linewidths to 170 Hz, although the efficacy of the variants to this experiment differ substantially. The double quantum filter was found to strongly discriminate against broad lines, with optimal detection of coherence and most effective suppression of the diagonal provided by the phase-sensitive COSY or PCOSY experiments. NOESY maps collected rapidly and with very short mixing times yield cross peak build-up slopes that directly provide distances within the active site. Preliminary results from applications to lignin peroxidase will be described. The results indicate that paramagnetism should not necessarily interfere with the three dimensional solution structure determination of a paramagnetic metalloprotein based solely on 2D NMR experiments.

CG 122 N-EXTENSION OF BPTI. A MODEL FOR FOLDING AND LONG-RANGE INTERACTIONS. Conni Lauritzen, Ole Skovgaard, Erik Tüchsen and Poul Erik Hansen. Institute for Life Sciences and Chemistry, Roskilde University, DK-4000 Roskilde, Denmark

BPTI is expressed in a recombinant E.coli as a MBP"BPTI fusion protein. A Factor X_a recognition sequence is placed next to Arg-1 of BPTI. A number of N-terminal extended BPTI molecules with 4-13 extra residues have been constructed. The BPTI+6 was obtained by off-target cleavage by Factor X_a. The extended forms serve as models for estimation of long-range effects on nuclear shielding and on pK_a values as a function of localized changes of charge. The N-extension changes the conformation of the two original terminals of BPTI. Furthermore, the interaction between BPTI and the extra residues are monitered in terms of changes in NH exchange rates. The overall changes of the BPTI part is discussed based on NOE effects and a number of long-range ^1H chemical shift changes seen both on NH back-bone protons resonances and of side-chain protons of central residues.
The folding pattern of the extra N-terminal residues are discussed and compared with similar sequences. The conformation of the BPTI+6 form is discussed in relation to the inability of Factor X_a to cleave at the specific site.

CG 123 IMPROVED ANALYSIS OF NMR SPECTRA OF PROTEINS USING THE LINEAR PREDICTION AND LEAST SQUARES METHODS, Jens J. Led, Henrik Gesmar and Søren Kristensen, Department of Chemistry, University of Copenhagen, The H.C. Ørsted Institute, Universitetsparken 5, DK-2100 Copenhagen Ø, DENMARK.

The linear prediction (LP) and least squares (LSQ) methods can greatly enhance the quality of protein NMR spectra and increase the amount and the quality of the information the can be obtained. Aspects of these improvements will be demonstrated. Thus, using a 2D CH-correlated spectrum of insulin as an example, it is shown that the spectral resolution and sensivity of 2D NMR spectra of proteins can be enhanced significantly by LP extrapolation of the time-domain signal. Second, it is shown that most of the non-carbonylic resonances of insulin can be assigned on the basis of the enhanced CH-correlated spectrum by applying a least squares analysis to the frequency-domain signal. Third, it is shown that the variation with pH of the chemical shifts of the assigned carbon resonances can be determined from a series of 1D ^{13}C spectra using a combination of LP- and LSQ analyses. This, in turn, provides valuable information about the folding and the dynamics of the protein.

CG 124 SEQUENCE SPECIFIC ASSIGNMENT AND STRUCTURE DETERMINATION OF bPP BY 2-DIMENSIONAL NMR.

[1] Xiang Li, [2] M.J. Sutcliffe, [3] T. W. Schwartz and [1] Christopher M. Dobson.

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The 36 amino acid bovine pancreatic polypeptide (bPP) has been sequence-specifically assigned by two-dimensional NMR, using a combination of TOCSY, NOESY and pre-TOCSY COSY following standard procedures. Around 360 inter/intra residue NOEs have been collected. Coupling constants from backbone ($J_{NH\alpha}$) and side-chain ($J_{\alpha\beta}$) are also determined from E-COSY in H_2O and D_2O respectively. Secondary structure is easily assigned by examining the NOE pattern and coupling constant data. The 3D structure of bPP in solution is determined following the protocol of hybrid distance geometry-dynamical simulated annealing and molecular dynamics calculations. Comparison of the bPP structure with that of aPP in the crystal state is made and the characteristics of the peptide conformation in solution is discussed.

CG 125 SOLUTION STRUCTURE OF A 125 RESIDUE CHITIN BINDING PROTEIN FROM BARLEY, Svend Ludvigsen and Flemming M. Poulsen, Department of Chemistry, Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Copenhagen, Denmark.

A 125 residue chitin binding basic protein from barley seeds (CBBP) has been studied by 2D ^1H NMR spectroscopy with the aim to investigate the structure which is not known from other sources and to study the interaction between tetra-GlcNAc and the protein. Tetra-GlcNAc binds firmly to the protein and by analyzing NMR spectra of the complex and the protein alone, information of the binding site has been extracted. The structure of CBBP has been obtained on the basis of an almost complete assignment of the NMR spectra. 900 distance constraints and 103 torsion angle constraint have been determined. Structures have been calculated using a hybrid method combining either DISGEO or DIANA and Simulated Annealing. All 20 structures obtained in this way fulfill the distance and torsion constraints imposed. CBBP have three disulfide bridges. These were identified from an analysis of NOESY spectra and subsequently confirmed by chemical analysis combined with mass spectrometry. The protein has six non-glycine residues which have positive phi angles, these have been determined using precise measurements of coupling constants.

CG 126 ^1H-NMR STUDIES OF THE INTERACTION OF UREA WITH LYSOZYME

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The properties of the urea denatured state of hen lysozyme have been previously investigated by ^1H NMR (1). Here we consider the changes induced in hen lysozyme by urea prior to denaturation. Urea is found to induce two types of change in hen lysozyme prior to denaturation. One type of change mirrors that observed when GlcNAc binds to hen lysozyme. This suggests that the major conformational changes induced by GlcNAc and urea are remarkably similar. Urea is a structural analogue of the N-acetyl group, and both urea and the N-acetyl group form the same two hydrogen bonds to Asn 59 and Ala 107 (2). The formation of these two hydrogen bonds from a molecular fragment of the correct geometry may trigger the conformational changes within the enzyme which optimise the binding interactions between an N-acetylated saccharide and the enzyme. This interaction may form the basis of recognition for the correct substrate by hen lysozyme. In addition, changes are observed which are unrelated to those induced by GlcNAc. The majority of these changes are small and occur for surface residues and probably reflect interactions of the enzyme with the solvent. These slight changes in the NMR spectrum prior to denaturation suggest that the structure of the enzyme is only slightly perturbed prior to denaturation.

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(2) K. W. Snape. D. Phil Thesis, University of Oxford.

CG 127 THE EFFECT OF INTERNAL MOTION ON THE PROTEIN STRUCTURES DERIVED FROM CROSS-RELAXATION

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The effect of internal motion on the quality of a protein structure derived form nuclear magnetic resonance (NMR) cross relaxation has been investigated experimentally. Internal rotation of the tyrosine-31 ring of turkey ovomucoid third domain was found to mediate magnetization transfer; the effect led to underestimation of proton-proton distances in its immediate neighborhood. Experimental methods that distinguish pure cross relaxation from chemical-exchange-mediated cross relaxation were used to separate true distances from distorted ones. A normal set of distances and a set which took internal motion into account were used as input to a distance geometry program for structural modeling. The two resulting families of converged structures showed larger (2 Å) global differences. Their comparison points to the necessity of analyzing the effects of internal motions in order to obtain accurate NMR solution structures.

CG 128 MIDGE: MODEL-INDEPENDENT REFINEMENT OF INTERPROTON DISTANCES,

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We have tested a recursive method to refine interproton distances compatible with 2-dimensional nuclear Overhauser effect (NOESY) experiments. The method is equivalent to a recursive non-linear least squares procedure, and thus convergence is independent of the initial estimate of the parameters. The relaxation matrix (**R**) is calculated from the NOESY matrix, and its diagonal elements (ρ_i) are adjusted at each iteration until the difference between theoretical and experimental cross-peaks is a minimum. The improvement comes from using interproton distances calculated from the off-diagonal (σ_{ij}) elements to generate ρ_i values. The interproton correlation times (τ_{ij}) are also estimated from the **R**-matrix. The method was applied to alumichrome, a rigid cyclohexapeptide of virtually identical solution and crystallographic structures. Convergence was tested by using different initial conditions, one of them being a NOESY matrix in which the experimentally unobserved off diagonal elements were set equal to zero and the diagonal elements to 0.5. The iterations rapidly converge, in all cases, to a single set of distances whose root-mean-squares deviations (rmsd) from the crystallographic distances is < 0.05 Å.

CG 129 SEQUENTIAL RESONANCE ASSIGNMENT AND GLOBAL FOLD OF AN FK-506 BINDING PROTEIN (FKBP) FROM BOVINE THYMUS

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FK-506, rapamycin, and cyclosporin A are potent immunosuppressive compounds which have been shown to inhibit T-cell activation. These compounds bind with high affinity to a functional class of proteins we have termed "immunophilins". FK-506 binding protein (FKBP) and cyclophilin, although unrelated at the primary sequence level, are both enzymes, possessing a peptidyl-prolyl cis-trans isomerase activity which is strongly inhibited by FK-506 and rapamycin for FKBP, and by cyclosporin A for cyclophilin. The significance of this enzyme activity and the detailed role of the immunophilins in the mechanism of immunosuppression by these compounds is unknown. To better understand the function of the immunophilin proteins and their interaction with inhibitors, we are pursuing the solution structure of FKBP by multidimensional NMR methods.

Sequential resonance assignment of the ^{1}H NMR spectrum of an FKBP purified from bovine thymus has been carried out using a combination of the sequential assignment and main-chain directed methods. Spin system identification for the 107 residue protein was achieved for backbone and a majority of side chain protons using a variety of scalar and dipolar correlated two dimensional NMR experiments. Elements of secondary structure have been identified based on the observation of characteristic patterns of short and medium range NOE connectivities and amide proton exchange data, and the folding topology of the protein deduced on the basis of long range NOE connectivities between neighboring structural elements. Derivation of a complete set of NOE distance and dihedral angle constraints from the NMR data for use in generating high resolution solution structures is in progress. Determination of the solution structure for FKBP, as well as enzyme-bound complexes of FK-506 and other immunosuppressive compounds, should provide insight into the precise mechanisms of immunophilin involvement in T-cell activation.

CG 130 SEQUENTIAL ASSIGNMENT OF PROTON RESONANCES AND SECONDARY STRUCTURE OF VACCINIA VIRUS GROWTH FACTOR

Michael J. Overduin, David Cowburn, Yao-Zhong Lin, James P. Tam
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Vaccinia virus growth factor (VGF), a 77-residue polypeptide encoded by vaccinia poxvirus, reportedly plays an important role in stimulating the growth of uninfected cells to enable virus infection. The VGF analogue studied here consists of 51 residues that constitute the EGF homologous region (positions 19-69) of VGF. This protein has been shown to bind strongly to the EGF receptor although with different mitogenic effects than other EGF-family proteins. In particular, VGF is the only known EGF antagonist. The sequential assignment of the proton resonances of the 51-residue VGF analogue were determined by analysis of 2D NMR COSY, HOHAHA and NOESY spectra. The secondary structure and elements of tertiary structure of this protein are characterized from the nuclear Overhauser enhancements and coupling constants. By comparing this structure to previously reported structures of other members of the EGF family, similarities and differences in the function of this growth factor are discussed.

CG 131 MULTIDIMENSIONAL NMR DATA PROCESSING, USING SELECTIVE DISCRETE FOURIER TRANSFORMATION (SDFT), I. Pelczer, P. N. Borer, G. C. Levy, and S. Szalma, Chemistry Department, Syracuse University, Syracuse, NY 13244-4100, USA

Selective Discrete Fourier Transformation (SDFT) can replace the widely used FFT processing in many cases, as we recently proposed (Szalma *et al., J. Magn. Reson.*, in press). Advantages of SDFT occur when subregions of large data matrices are the subject of interest, as is often the case with increasing the number of spectral dimensions. For example, usually much of a 3D spectrum is empty in the frequency domain. SDFT also: (i) allows simple alteration of the digital resolution, apodization, etc. in local regions, (ii) provides the potential for savings in disk storage efficiency and processing time, especially in multidimensional applications, and (iii) allows arbitrary reconstruction of lower dimensional slices/projections from a multidimensional spectral space. These properties make it possible to use SDFT to optimize processing parameters, including the phase corrections appropriate for each dimension, by monitoring two- or one-dimensional slices. This process can be accomplished interactively since these slices can be computed very quickly using reduced digital resolution.
Examples to illustrate these features will be presented on 2D and 3D spectra of nucleic acid samples, including a 24-mer RNA hairpin loop and a 10-mer DNA duplex.

CG 132 THE STRUCTURE OF PORCINE PHOSPHOLIPASE A_2 IN SOLUTION , AS DEDUCED FROM NMR.

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Phospholipase A_2 is a lipolytic enzyme, which is actived on aggregated substrates. The PLA from porcine pancreas consists of 124 amino acids and has been cloned and overexpressed in *E.Coli* . Crystal structures of the enzyme, and an enzyme / substrate analog complex are known. In order to get insight into the interfacial activation process, we study the enzyme by NMR. With this method detailed structural information can be obtained for the protein bound to micelles and a substrate analog. As a first step, the PLA was studied free in solution. Using conventional 2D NMR, and heteronuclear 3D NMR on ^{15}N labelled PLA, assignments were made for the majority of the protons. Quantitive distance constraints were obtained from 2D NOE spectra. In cases of overlap, qualitative constraints were taken from the better resolved 3D spectrum. Distance geometry was used to generate structures, which were further refined with restrained molecular dynamics. The differences between the NMR and crystal structure are discussed. Most notably, the N-terminal part, which is known to bind to the lipid layer, does not adopt the α-helical conformation found in the crystal structures.

CG 133 APPLICATIONS OF GRADIENT PULSES IN THREE DIMENSIONAL NMR

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Three dimensional NMR experiments are routinely used to edit complex spectra but are often very time consuming. Field gradient pulses provide the ability to select a unique coherence pathway in a single scan per evolution increment which can substantially reduce the time required to collect the data. A 3D COSYCOSY2Q experiment has been developed which has the added advantage of eliminating the water signal without using selective excitation or presaturation techniques. The result is that correlations which are normally obscured by the water signal may now be observed. Gradient pulses have proven to be very efficient at suppressing the signal from the carbon–12 bound protons as well. A 3D HMQC–COSY experiment using gradient pulses has been developed which allows one to identify direct and long–range proton–carbon correlations. These novel techniques are illustrated with some common macromolecules.

CG 134 DETERMINATION OF LEFT HANDED TURNS IN PROTEINS BY NMR SPECTROSCOPY, Flemming M. Poulsen, Svend Ludvigsen and Mark Bycroft[1]. Department of Chemistry, Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Copenhagen, Denmark.
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The occurrence of left-handed turns in proteins is relatively low, however, it is still large enough that a typical small protein will contain one or even several of these. In the present work we have examined the left-handed turns and their manifestations in the NMR spectrum of four proteins: serine proteinase inhibitor 2 of barley seeds, bacterial ribonuclease, chitin binding basic barley seed protein, and hen egg white lysozyme. For the first two of these proteins the structure in the crystal and in the solution phase has been determined, and for the third the structure in solution has been determined, and for the fourth the assignment of the NMR spectrum is known as well as the three-dimensional structure in crystals. A method for the determination of the left-handed turns will be described and its application to the NMR spectrum of the four proteins presented.

CG 135 PARTIAL RESONANCE ASSIGNMENTS AND SECONDARY STRUCTURE DETERMINATION OF THE RNASE H DOMAIN OF HIV-1 REVERSE TRANSCRIPTASE BY TWO- AND THREE DIMENSIONAL HETERONUCLEAR NMR SPECTROSCOPY, Robert Powers, G. Marius Clore, Ad Bax, Paul T. Wingfield, and Angela M. Gronenborn, Laboratory of Chemical Physics, Building 2, National Institute of Diabetes and Digestive and Kidney Diseases, and Protein Expression Laboratory, Building 6B, National Institutes of Health, Bethesda, Maryland 20892

Approximately 80% of the ^{15}N, ^{13}C, and ^{1}H resonances of the RNAse H domain of the HIV-1 Reverse Transcriptase (138 residues, M_r = 15200) have been assigned by employing a number of novel three dimensional (3D) heteronuclear NMR experiments. The sequential assignments were obtained by using heteronuclear triple-resonance 3D experiments allow for a step-wise assignment of the backbone ^{1}H, ^{13}C, and ^{15}N nuclei through large one-bond J couplings. These experiments provide three independent sources of sequential connectivities which allow for an unambiquous method to link i and i+1 residues. Additional sequential information was obtained from $C\alpha H_i$-NH_{i+1} and NH_i-NH_{i+1} connectivities observed in the 3D NOESY-HMQC experiment. The side-chain resonances were identified by 3D ^{1}H-^{13}C-^{13}C-^{1}H correlated (HCCH-COSY) and total correlated (HCCH-TOCSY) spectroscopy based on the $^{13}C\alpha$ and $C\alpha H$ sequential assignments. The 3D ^{15}N-NOESY-HMQC and ^{13}C-NOESY-HMQC spectra's NOE pattern predict a secondary structure for RNAse H consisting of 3 β–strands forming an antiparallel β-sheet and 3 α-helical regions. NH solvent exchange rates have been measured and correlate well with the predicted secondary structure. A comparison of the HIV-1 RNAse secondary structure as determined by NMR with the *E. coli* RNAse H crystal structure indicates some distinct differences between the structures of both proteins.

CG 136 NMR STUDIES OF PHOSPHO-HISTIDINE *BACILLUS SUBTILUS* HPr, Ponni Rajagopal and Rachel E. Klevit, Department of Biochemistry, University of Washington, Seattle, Wa-98195.

HPr is a protein present in the bacterial PTS (phophoenolpyruvate: sugar phosphotransferase) system. The N-1 position of the His^{15} residue in HPr is phosphorylated by the PEP-Enzyme I complex. The phosphoryl group from HPr is then transferred to a number of sugar-specific proteins for transport across the membrane.

The NMR spectrum of *Bacillus subtilus* HPr has been assigned (M. Wittekind, J. Reizer and R. E. Klevit, (1990) *Biochemistry, 29*, 7191) and its structure determined by distance geometry methods (unpublished results). Phospho-histidine HPr is labile towards hydrolysis and so an *in situ* enzymatic regeneration system was used in the NMR studies. 2D NMR studies of *B. subtilus* HPr show that a large number of residues undergo changes in chemical shifts on phosphorylation at His^{15}. Notably, the chemical shifts of Ala^{16} and Arg^{17} amide protons and ^{15}N resonances change by as much as 1 ppm and 5 ppm respectively. In addition, a number of new NOEs are observed in the 2D NOESY spectra of phospho-histidine *B. subtilus* HPr. The exchange rates of certain amide protons in the vicinity of the active site are different in the phosphorylated and in the unphosphorylated forms. A detailed analysis of the structural changes undergone by phospho- histidine HPr will provide useful information regarding certain aspects of phosphorylation.

CG 137 HYDROGEN EXCHANGE IN NATIVE AND THERMALLY DENATURED RIBONUCLEASE A, Andrew D. Robertson and Robert L. Baldwin, Department of Biochemistry, Stanford University Medical Center, Stanford, CA 94305

Hydrogen exchange and proton NMR spectroscopy have been used to measure the relative stabilities of secondary structure elements in native ribonuclease A (RNase A) and to test for the presence of structure in the thermally denatured (pH < 4, 65° C) protein. The pattern of exchange protection in native RNase A is heterogeneous, with the most stable region being that which includes the two C-terminal strands of β-sheet. The helices containing residues 3 to 13 and 25 to 35 are the least stable elements of hydrogen-bonded secondary structure in native RNase A. The third helix, containing residues 50 to 60, shows exchange protection comparable to that of the C-terminal β strands, against which it packs in the X-ray crystal structure. The region showing the greatest protection from exchange also is involved in early events in the refolding of RNase A from guanidine-HCl [Udgaonkar & Baldwin (1988) *Nature 335*, 694-699]. Hydrogen exchange in thermally unfolded RNase A is approximately that predicted for a disordered polypeptide [Molday, Englander, & Kallen (1972) *Biochemistry 11*, 150-158]. We thus conclude that there is no stable hydrogen-bonded structure in thermally unfolded RNase A at low pH. In the course of our studies, we discovered that at least one of the approximations made by Molday *et al.*, that exchange from valine is similar to that from alanine, had to be modified; exchange from a small valine-containing peptide is approximately 5-fold slower than that measured for alanine peptides. In addition, the observed pH minima for hydrogen exchange from thermally denatured RNase A tend to occur below those predicted from the parameters of Molday *et al.*. Our results in this regard are similar to those of Roder, Wagner, & Wüthrich [(1985) *Biochemistry 24*, 7407-7411, see Table I]. The origin of this deviation is unknown, but may result from the high positive charge on unfolded RNase A at acid pH.

CG 138 **^{1}H Resonance Assignments of the E3 binding domain of *E. coli* 2-Oxoglutarate dehydrogenase by 2D NMR spectroscopy**

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2-Oxoglutarate dehydrogenase (2-OGDH) is a large multi-enzyme complex consisting of multiple copies of three units: 2-oxoglutarate decarboxylase [E1o], dihydrolipoamide succinyltransferase [E2o], and dihydrolipoamide dehydrogenase [E3]. Multimeric E2o is known to form an octahedral core; additionally E2o binds E1o and E3. Previous work with the *E. coli* 2-*OGDH has identified* that the E3 binding domain is located between residues 103-152 in E2o.

A 51 residue peptide based on residues 103-152 of E2o has been prepared by solid-phase synthesis techniques and 2D ^{1}H NMR studies have been started in an attempt to determine the three dimensional structure of this binding domain. The sequential assignment of this peptide has been completed using NOESY, HOHAHA, and PE-COSY experiments. Initial analysis of the resulting short range NOE data indicates a loop from residues 133-140 and a helix from 141-150. Further studies additionally utilizing long-range NOE data and $^3J_{HH}$ coupling data are in progress in pursuit of solving the tertiary structure.

CG 139 DATABASE FOR PROTEIN NMR RESULTS, Beverly R. Seavey, Elizabeth A. Farr, and John L. Markley, Biochemistry Department, University of Wisconsin-Madison, 420 Henry Mall, Madison, WI 53706-1569.

We have developed a database, *BioMagResBank*, for the storage and retrieval or NMR-derived protein data. Because NMR data are sensitive to experimental conditions, we have modeled experimental conditions extensively. NMR data such as shift assignments, coupling constants, NOEs, and pKas are stored and can be retrieved as indexed by pH, temperature, protein type, experiment type, and so on. To achieve this flexibility, we are using a relational database management system (RDBMS). We find that an RDBMS provides added benefits in enforcing data correctness and consistency during data entry, along with flexibility in generating input to analysis programs that expect a fixed format and atom nomenclature. Further development of the protein NMR field will likely bring changes in the sequence-dependent experimental parameters to be collected. The architecture of the database allows it to be redesigned incrementally to adapt to such changes without perturbing the existing data and organization. The database has been designed to be readily accessible to scientists at various levels of computer proficiency and to support automatic manipulation of and reasoning about the data by programs written in higher level languages.

[Supported by NIH grant LM 04958 and by the New Energy & Industrial Technology Development Organization (Tokyo, Japan).]

CG 140 RELAXATION STUDIES AND DYNAMICS IN MULTIDOMAIN FIBRINOLYTIC PROTEINS

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Recent work in this laboratory [1,2] has been directed towards understanding the structural and dynamical properties of a therapeutically important class of molecules for which little physical and crystalographic information is at present available. NMR studies on a number of fibrinolytic proteins including urokinase, plasminogen and tissue plasminogen activator have suggested the existence of segmental motion in these multidomain molecules resulting in the observation of considerable line-width narrowing of resonances from certain domains. Our high resolution one and two-dimensional spectra of these molecules have enabled us to examine quantitatively the nature of inter-domain motions and relaxation properties in particular by detailed analysis of well resolved methyl peaks. These studies should facilitate the understanding of domain-domain interactions. Recent evidence has suggested that the nature of these interactions may be dependent on solution conditions in a way that may be of functional significance. The observations in preliminary studies of changes in the spectra on varying ionic strength and following proteolytic cleavage associated with activation of zymogens suggest that NMR can be of considerable value in exploring the functional behaviour and pharmaceutical properties of these proteins.

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CG 141 STRUCTURAL CHARACTERIZATION OF sCD4-183 BY NMR SPECTROSCOPY, Eldon L. Ulrich, Kathleen A. Farley, and Paul E. Fagerness, Control Division, The Upjohn Company, Kalamazoo, MI 49001

CD4, a human T-cell surface protein, binds the HIV gp120 protein in the initial stage of an AIDS infection. Mutation mapping of CD4 interactions with HIV gp120 and the physiological functions of CD4 carrying cells have been carried out in other laboratories. These studies strongly implicate residues 41-55 as the gp120 binding site and domains I and II (residues 1-176) as the class II MHC binding region. Therefore, soluble N-terminal CD4 fragments are potential inhibitors of HIV binding and infection and may be useful in studies of CD4 physiological functions. We have investigated the conformation and stability of an N-terminal 183 amino acid fragment of CD4 that has an additional N-terminal methionine (sCD4-183). The one- and two-dimensional ^{1}H NMR spectra of the protein are consistent with a β-sheet structure having a large dispersion in the NH resonances and many low-field shifted α-proton peaks. Temperature, pH, buffer, ionic strength and guanidinium hydrochloride titrations have been carried out. The protein is stable from pH 4.5 to 12 in phosphate buffer and up to 47 °C. NaCl or phosphate concentrations of 1M at pH 7.6 do not significantly affect the protein spectrum. The C2 and C4 proton resonances of histidine residues 28 and 108 have been tentatively assigned and pKa values of 5.26 and 6.95 determined, respectively. Guanidine denaturation occurs over a broad concentration range (0.2 - 2.6 M) with a midpoint of about 1 M.

CG 142 STRUCTURE OF SOYBEAN TRYPSIN/CHYMOTRYPSIN BOWMAN-BIRK INHIBITOR AND STUDIES OF ITS INTERACTION WITH SERINE PROTEASE BY 2D-NMR,
Milton H. Werner and David E. Wemmer, Department of Chemistry, University of California, Berkeley, Berkeley, CA, 94720 and the Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory, Berkeley, CA 94720

Bowman-Birk inhibitor is a small serine protease inhibitor (M_r=8kD) that can simultaneously inhibit trypsin and chymotrypsin at kinetically independent binding sites. We have purified the major isoform and assigned the ^{1}H-NMR spectrum by two dimensional methods. The protein structure is characterized by two β-hairpins, one each comprising the inhibitory domains against trypsin and chymotrypsin. Each binding site is part of a *cis*-proline type turn at one end of its respective binding site. The solution structure of the protein and NMR studies of its interaction with serine protease will be presented.

CG 143 SOLUTION STRUCTURES OF AMYLOID (A4) β-PEPTIDE AND ITS CONSTITUENT FRAGMENTS: RELATIONSHIP TO AMYLOID DEPOSITION IN ALZHEIMER'S DISEASE,
Michael G. Zagorski and Colin J. Barrow, Suntory Institute for Bioorganic Research, Wakayamadai, Shimamoto-cho, Mishima-gun, Osaka 618 Japan.
Alzheimer's disease (AD) is the major cause of senile dementia in the U.S., affecting over 10% of the people over the age of 65 years; 47% over 85. It is characterized by neuronal dysfunction and extracellular deposits in the form of amyloid plaques and cerebrovascular amyloid, and intracellular deposits in the form of neurofibrillary tangles. The major constituent of amyloid plaques is the A4 or β-peptide, which is a small (42 or 43 amino acids) polypeptide derived from a larger amyloid precursor protein (APP). We have determined the secondary structures of the parent β-peptide, β-(1-42), and related fragments, β-(1-28), β-(29-42), and β-(1-39), using CD and 2D NMR. The β-(1-28) and β-(29-42) peptides occupy different domains of APP, while the β-(1-39) peptide is the predominate form in blood and is associated with cerebrovascular amyloid deposits. In aqueous trifluoroethanol solution, the β-(1-28), β-(1-39) and β-(1-42) peptides adopt *monomeric* α-helical structures at low pH (1-4), *tetrameric* α-helical structures at high pH (7-10), whereas at intermediate pH (4-7) an *oligomeric* β-structure (the probable structure in plaques) predominates. Thus, β-peptide is *not* an intrinsically insoluble protein (as originally thought) and that localized or normal age related alterations of pH are perhaps necessary for the self-assembly and deposition of β-peptide to produce the β-structure which is found in amyloid plaques. The hydrophobic carboxy segment, β-(29-42), exists exclusively as an *oligomeric* β-structure in solution regardless of differences in solvent, pH, or temperature, thus suggesting that this segment directs the folding of the complete β-(1-42) peptide to produce the β-structure found in amyloid plaques.

CG 144 MULTIDIMENSIONAL NMR METHODS FOR THE STUDY OF LARGER PROTEINS IN SOLUTION.
E. R.P. Zuiderweg, A.M. Petros, E.T. Olejniczak, R.T. Gampe, Jr., H. L. Eaton, G. Gemmecker, P. Neri and S.W. Fesik, Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL 60064.

The classical Wagner-Wuthrich methods for the determination of protein structure in solution cannot easily be applied to larger molecules because of overlap problems in the NMR spectra of these molecules. We present here an overview of the new methods which cope with these problems and discuss aspects of their practical implementation. The overview will comprise ^{15}N- and ^{13}C-resolved 3D NOESY spectroscopy, 4D NOESY spectroscopy and techniques to obtain scalar correlated data employing ^{13}C-^{13}C coherence transfers.

Nucleic Acid Structure

CG 200 2D NMR STUDIES ON INTERACTION OF DAUNOMYCIN WITH d-CpG

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Department of Biosciences and Biotechnology, University of Roorkee, ROORKEE 247 667 INDIA.

Proton NMR spectra of binding of daunomycin to deoxydinucleotide d-CpG have been taken at 500 MHz in temperature range 275K - 320K. Changes in chemical shift of ~ 0.1 ppm in GH8 and CH6 protons and 0.08 ppm in 3H proton of daunomycin at 277K shows intercalation of the drug chromophore between C and G bases. 2D NMR experiments show a change in glycosidic bond rotation of guanine from ANTI to SYN on binding to drug. Presence of strong NOE cross peaks GH8-GH1' and CH6-CH5' show existence of Z DNA in the bound complex. Other NOE's between drug protons and d-CpG protons yield specific geometry of complex in which D ring of drug is close to cytosine base.

CG 201 ROTATIONAL-ECHO DOUBLE-RESONANCE (REDOR) NMR SPECTROSCOPY OF THE ANTI-MICROBIAL PEPTIDE, MAGAININ 2, Jack Blazyk†, Andrew W. Hing*, Jacob Schaefer*, and Melody Ferguson†, †Chemistry Department, Molecular and Cellular Biology Program, and College of Osteopathic Medicine, Ohio University, Athens, Ohio 45701, and *Chemistry Department, Washington University, St. Louis, MO 63130.

Magainin 2, a naturally occurring 23-amino acid cationic peptide isolated from the skin of the African clawed frog, kills a wide variety of microorganisms by disrupting the the membrane systems of the target organism. Magainins have the potential to adopt a highly amphiphilic α-helical secondary structure, which is typical for many membrane-active peptides. Unlike other cationic peptides, such as melittin, which are cytotoxic, magainins are nonhemolytic at antimicrobial concentrations. Thus, magainins and related peptides may have therapeutic potential as antibiotics. The molecular nature of the interaction between magainin 2 and the cell surface has yet to be determined. One proposal suggests that the peptide forms channels across the membrane through which anions can flow. Another suggestion is that magainins bind primarily to the membrane surface and destroy the integrity of the lipid bilayer. We have applied a new solid-state NMR technique, REDOR, which measures dipolar coupling, to accurately determine distances between labeled nuclei. We have synthesized magainin 2 containing ^{15}N-Ala at position 9 and 2-^{13}C-Gly at position 13. When the peptide adopts an α-helical structure, the distance between the labeled atoms will be about 7 Å. Determination of this interatomic distance is useful in verifying α-helical content under different conditions. In addition, we have synthesized two phosphoglycerides, DPPC and DPPG, containing ^{13}C at the 12 position of the palmitoyl chains. Measurements of dipolar coupling between ^{15}N in the peptide and ^{13}C in the membrane lipids are used to reveal the penetration of the peptide into the lipid bilayer. These data will provide insight into the mechanism by which magainins interact with lipid bilayers and kill microorganisms.

CG 202 THREE-DIMENSIONAL FEATURES OF AN RNA HAIRPIN LOOP THAT CONTROLS EXPRESSION OF THE R17 VIRAL REPLICASE GENE,

P.N. Borer[1], I. Pelczer[1], M.W. Roggenbuck[1], K.-Y. Wang[1], G.W. Jeong[1], K.D. Bishop[1], J. Gott[2] & O.C. Uhlenbeck[2], [1]Department of Chemistry, Syracuse University, Syracuse, NY 13244-4100 and [2]Department of Chemistry & Biochemistry, University of Colorado, Boulder, CO 80309.

Unique conformations adopted by RNA sequences probably determine the specificity of their interactions with proteins. However, the important conformational features may not be easily predictable from sequence. The coat protein of R17 virus binds to a 24 nucleotide hairpin loop near the beginning of the replicase gene to shut down production of the replicase after the early stage of viral infection. An analog of the RNA binding site that preserves the important determinants for coat protein specificity was synthesized using T7 RNA polymerase and purified by gel electrophoresis. The 500 MHz ^{1}H-nmr spectra were assigned using a novel combination of deconvoluted 2D-NOE spectra, computer-assisted path analysis to distinguish the proper sequential NOE connectivities, and a uniquely phased 2 quantum correlation spectrum that allows easy editing of directly and remotely coupled spins. In the structure, a "bulged" A base is stacked inside the helical stem, two A's in the unpaired region stack on the stem, three of the unpaired residues have S sugar puckers, eight have mixed S/N puckers and only about half are purely in the N-forms usually associated with helical RNA. These results suggest that the rules for predicting RNA 3D-structure from sequence are complex.

CG 203 A NOVEL STRUCTURE OF A DNA SEQUENCE WHICH CAN FORM A HAIRPIN, Yves Boulard[a], Jacques Gabarro-Arpa[b], Jean Cobnet[b], Marc Le Bret[b], Andre Guy[c], Robert Teoulec[c], Wilhelm Guschlbauer[a] and G. Victor Fazakerley[a], Service de Biochimie et Genetique Moleculaire, Departement de Biologie Cellulaire et Moleculaire, CEN Saclay, 91191 Gif sur Yvette Cedex, France, Laboratoire de Physicochimie Macromoleculaire, Institut Gustave Roussy, 94800 Villejuif, France and Laboratoire de Chimie, Departement de Recherche Fondamentale, CEN Grenoble, 85x, 38041 Grenoble Cedex, France.

The sequence 5'(GGATCGTTTCGATCC) is observed, as a function of temperature and concentration, in an equilibrium between a duplex and a hairpin form. At 0°C the duplex form predominates, while at 33°C, 3mM strand concentration, the hairpin form is the major species. In the hairpin structure 6 Watson-Crick base pairs are formed giving rise to a B DNA type stem in which all sugars are C2' endo. The central 3 T residues form the loop. From NMR distance constraints and molecular mechanics calculations we show that the first two T residues in the direction 5'-3' fill the minor groove of the stem and are unstacked. The base plane of the second thymine lies parallel to the sugar phosphate backbone of the second strand. No distance measurements could be obtained for the third T in the loop. Calculations suggest that it should be orientated away from the main body of the double helix on top of the stem. This structure is quite different to that found in solution for a loop comprising 4 T residues (1) but, for the loop region, shows similarities with an X-ray structure of a 4 T residue loop on a Z form stem (2).

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CG 204 SELF-CLEAVING RNA: STRUCTURAL STUDIES OF CLEAVED AND UNCLEAVED HAMMERHEAD DOMAINS BY ^{1}H NMR, Ann Caviani Pease, Mark G. Kubinec, and David E. Wemmer, Department of Chemistry, University of California, Berkeley, CA 94720

A site-specific self-cleavage reaction has been observed for several plant viral satellite RNA's which results in 5'-hydroxyl and 2',3'-cyclic phosphate termini[1,2,3]. Symons and coworkers have proposed a hammerhead secondary folding model for the self-cleavage domain[4,5]. This domain consists of 13 conserved nucleotides and three variable length double-stranded helices. Cleavage occurs only at a specific phosphodiester linkage and requires no cofactor other than magnesium ions. Although the nucleotides directly at and near the cleavage site may vary, the position of cleavage within the hammerhead domain is conserved. Therefore it is clear that a specific three-dimensional folding of hammerhead domain is required for cleavage. The nature of the interactions giving rise to this folding are as yet unknown.

We have chosen to study the three-dimensional structure of such an RNA by nuclear magnetic resonance, and have recently reported the complete imino proton NMR assignments of a cleaved hammerhead domain[6]. Although these studies confirmed the secondary folding model, additional data is necessary to understand the three dimensional folding of the domain. We present here spectra of two uncleaved hammerhead domains, and discuss the structural results.

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CG 205 SOLUTION STRUCTURE OF THE DNA BINDING DOMAIN OF THE TRANSCRIPTION FACTOR GAL4 AS DETERMINED BY 2D NMR METHODS, Joseph E. Coleman, Tao Pan and Kevin Gardner, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06510.

GAL4 protein is a transcription factor from *Sacchromyces cerevisiae* required for the transcriptional activation of the genes encoding the galactose-metabolizing enzymes. Although the intact GAL4 consists of 881 amino acid residues, its DNA binding domain has been located within the N-terminal 62 residues by limited proteolysis. The DNA binding domain of GAL4 contains a $Cys^{11}X_2Cys^{14}X_6Cys^{21}X_6Cys^{28}X_2Cys^{31}X_6Cys^{38}$ sequence which binds two Zn(II) or Cd(II) ions. This motif is conserved among a group of eleven fungal transcription factors. Both ^{113}Cd NMR and phase-sensitive ^{1}H COSY show that only the six cysteine residues act as ligands to Zn(II) or Cd(II). Metal ion binding is essential for recognition of the specific DNA sequence, UAS_G, to which GAL4 binds. ^{1}H-^{113}Cd coupling patterns have identified two cysteine residues in which the $-S^-$ is shared between the two bound metal ions. We have completed the sequential assignments of the ^{1}H NMR resonances of $Zn(II)_2$GAL4(62*) and $^{113}Cd(II)_2$GAL4(62*). A 3D structure calculated from the long and short range NOE data show GAL4 to consist of a series of turns and loops from residues 8 to 42 accommodating the binuclear metal cluster and a small C-terminal subdomain (residues 43-62) folded back on the cluster. GAL4 is the first DNA binding protein which does not contain α-helix or β-strands involved in specific DNA recognition. It appears that the highly positively charged metal cluster (8 Lys, 2 Arg) wraps the DNA in a relatively nonspecific fashion, while the C-terminal subdomain contributes residues involved in specific DNA recognition. Supported by NIH Grants GM09070 and DK09070.

CG 206 SOLUTION STRUCTURE OF A DNA DUPLEX RECOGNIZED BY YEAST TRANSCRIPTION FACTOR REB1: 2D NMR STUDIES USING DISTANCE GEOMETRY AND NOESY BACK-CALCULATION. Darrell R. Davis[†], David J. Stillman[‡], and Dennis R. Hare[§]. [†]Department of Medicinal Chemistry and [‡]Department of Cellular, Viral, and Molecular Biology, University of Utah, Salt Lake City, UT 84112. [§]Hare Research Inc., 14810 216th Ave NE, Woodinville, WA 98072.

The DNA binding protein REB1 from yeast has been recently identified and shown to be essential for cell growth. This protein is quite abundant and appears to bind to a variety of sites in the yeast genome. The REB1 protein has been shown to modulate transcription and is essential, but a specific function has yet to be determined. Although REB1 binds specific DNA sequences with high affinity, it contains none of the classic structural motifs used for DNA binding such as zinc finger, leucine zipper, or helix-turn-helix. REB1 has some homology with the DNA binding region of the protein from the *myb* oncogene. This oncogene protein presumably binds to DNA via a structural motif that contains tryptophan repeats. We have synthesized a 13 base-pair DNA molecule that contains the 8 nucleotide consensus binding sequence for REB1. Inter-proton distance constraints obtained from time dependent NOESY spectra were used as input into the distance geometry program DSPACE. Initial DSPACE determined structures were further refined using an iterative back-calculation approach. Qualitative inspection of 2D NOESY spectra during the assignment phase of this project allowed the identification of several unique structural interactions that may form the basis for specific protein-DNA recognition. These observations and an analysis of the high-resolution structure will be discussed in light of some unusual results from methylation interference experiments on the protein-DNA system.

CG 207 NMR STUDIES OF THE 1:1 COMPLEX FORMATION BETWEEN THE DRUG HOECHST 33258 AND THE DODECANUCLEOTIDE DUPLEX d(GTGGAATTCCAC)$_2$

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The complex formation between the drug Hoechst 33258 and the dodecanucleotide d(GTGGAATTCCAC)$_2$ was monitored by observation of changes in the DNA imino proton resonances when the DNA was titrated with the drug. Using 2QF-COSY, clean-TOCSY, NOESY and 2Q-spectroscopy, most of the ^{1}H-NMR lines of the labile and non-labile protons of the drug and of each DNA strand in the complex were assigned. On this basis, 23 intermolecular NOEs were identified. These NOEs positioned the drug to the AATTC-site of the DNA duplex and provided the input for a molecular modelling treatment of the two species interacting in solution. Additional exchange cross peaks observed by ROESY proved that protons which are symmetrically related in the free DNA duplex give rise to exchange cross peaks in the ligand/DNA complex, thus indicating that the complex undergoes a dynamic process. This process could be envisaged to be the exchange of the drug molecule between two equivalent binding sites in the self-complementary dodecanucleotide. The rate of exchange for this process was measured to be $2.1 \pm 0.3\ s^{-1}$.

CG 208 *Abstract Withdrawn*

CG 209 STRUCTURAL STUDIES OF THE HIV-1 GAG PROTEIN, P6

Lora M. Green±, Paul R. Blake±, Michael F. Summers±, Raymond C. Sowder III*, Larry O. Arthur*, and Louis E. Henderson*, ±Department of Chemistry and Biochemistry, The University of Maryland Baltimore County, Baltimore, Maryland 21228 and *Retroviral Protein Biochemistry Laboratory, AIDS Vaccine Program, Program Resources Inc., NCI-Frederick Cancer Research and Development Center, Frederick, Maryland 21701

The retroviral genome consists of three major genes: gag, pol, and env. The env gene encodes for the viral coat proteins, the pol gene encodes for reverse transcriptase and integrase, and the gag gene encodes for the major structural proteins of the virion. The gag gene is expressed as a polyprotein precursor, which is cut enzymatically by an HIV-1 encoded protease to form the mature viral proteins, including p6. Little is known about the function of this protein, but initial studies by Haseltine and Goettlinger have shown that mutations in this region prevent the HIV-1 virus from budding from the infected cell (News and Comment Section, Science, 248, 1596, (1990)). Structural studies of p6 purified from HIV-1 virions were undertaken using standard two-dimensional nuclear magnetic resonance techniques. The pattern of amide to amide NOE connectivities, as well as the presence of long range NOE amide to alpha connectivities, suggest that regions of p6 are structured, and that this structure will be able to be determined using NMR-based methods.

CG 210 A ZINC-FINGER DOMAIN FROM YEAST ADR1: COMPARISON OF WILDTYPE AND MUTANT SOLUTION STRUCTURES GENERATED BY NMR AND DISTANCE GEOMETRY, Ross C. Hoffman, Grace E. Parraga, Suzanna J. Horvath, and Rachel E. Klevit, Department of Biochemistry, University of Washington, Seattle, WA 98195

ADR1, a transcription factor found in S. cerevisiae, contains two zinc-fingers of the TFIIIA class which are essential for binding to an upstream activator sequence of the Alcohol Dehydrogenase II (ADH2) gene. The two zinc fingers are designated ADR1b (residues 102-130) and ADR1a (residues 130-159). Using 2D-NMR, synthetic peptides of both sequences have been shown to fold in a zinc-dependent manner into small heat-stable domains. A quantitative analysis of ADR1b has been performed, resulting in a family of distance geometry structures. ADR1b consists of a loop-like structure (residues 2-13), a "fingertip" (residues 14-15), and an α-helix (residues 16-26). The zinc is tetrahedrally coordinated by cysteines 106 & 109 and by histidines 122 & 126. Adjacent to the metal cluster is a small hydrophobic core composed of conserved Phe and Leu residues. Point mutations of ADR1 that adversely affect DNA binding have been identified. In the context of the whole molecule, a His118→Tyr mutation completely abrogates DNA binding and a His118→Ala mutation results in a 20-30 fold decrease in binding. Qualitative analysis of ADR1b peptides containing these mutations indicates they have the same zinc-dependent global fold as wildtype ADR1b. Thus, the structural changes that affect DNA binding are more subtle. Distance geometry structures of the mutant peptides have been generated and show some interesting differences when compared to wildtype.
(This work was supported by NIH PO1 GM32681.)

CG 211 MAPPING PROTEIN SURFACE METAL-BINDING SITES BY 2D NMR PARAMAGNETIC DIFFERENCE SPECTROSCOPY, Robert Johnson and Frances H. Arnold. Division of Chemistry and Chemical Engineering 210-41, California Institute of Technology, Pasadena, CA 91125.

2D NMR difference spectroscopy was used to monitor the interactions between copper(II) iminodiacetate (Cu-IDA) and surface residues of *C. krusei* ferricytochrome c. 2D proton correlation (COSY) spectra were recorded in the presence of equal concentrations of Cu-IDA and copper(II)ethylenediaminetetraacetate (Cu-EDTA). The use of Cu-EDTA corrects for nonspecific paramagnetic effects of copper(II) in the bulk solution and allows us to identify specific binding sites for Cu-IDA. Diagonal and cross peaks of those protons near Cu-IDA binding sites are selectively broadened for the samples containing Cu-IDA compared to those containing Cu-EDTA. These selectively relaxed peaks appear in the 2D difference spectrum (COSY (EDTA) - COSY (IDA)). These experiments demonstrate that Cu-IDA binds preferentially to surface exposed histidine residues and does not appear to interact with other surface amino acids at low concentrations (<0.2 mM Cu(II)). This technique is generally useful for identifying the sites on protein surfaces which interact with chelated metals and can also be used to evaluate the relative strengths of metal-chelate binding. This information is critical for understanding the selectivity of protein separations based on interactions with chelated metals.

CG 212 *3D Solution Structure of Human Anaphylatoxin C3a: Comparison between the Solution and Crystalline State*

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The solution structure of Human Anaphylatoxin C3a (~8 kD, 77 amino acids), a component of the complement activation pathway involved in the inflammatory response, has been determined. Structures were generated using a metric matrix embed, refined by direct minimization of the residual distance violations (DISGEO), by a restrained molecular dynamics potential (AMBER), or by a potential based solely on geometric and NMR-derived information (DSPACE). A measure of the accuracy of these structures was gained by the agreement between the observed experimental NOE intensities and theoretical NOE intensities calculated with the relaxation matrix analysis portion of IRMA.

A comparison of the different refinement methods employed will be presented, as well as a direct comparison to the 3.2 Å resolution x-ray structure. Significant differences exist in the solution and solid-state structures especially at the TYR-15 to LEU-19 loop and the C-terminal portion of the molecule. The biological implications of these findings will also be discussed.

CG 213 SOLUTION STRUCTURES OF T_nA_n DNA TRACTS BY 2-D NMR

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In order to study the effects of T_pA steps in contiguous T tracts followed by A tracts we have investigated the solution structures of d[GCCGTTAACGGC]$_2$ and d[GCGTTTAAACGC]$_2$ duplexes by 2-D NMR and distance geometry/backcalculation methods. The six-bond backbone was constrained by various combinations of experimental NOE and coupling observations: γ was constrained via lower bound NOE distances between H5′/H5″ and H2′/base protons of the same residue as well as H4′ sums-of-couplings; δ was constrained by H1′-H4′ distances (as well as H3′-H4′ and H2″-H3′ couplings from E. COSY); ϵ was constrained via the maximum H3′-P coupling allowed by H3′ sums-of-couplings (or H3′ linewidth); β was constrained by the maximum H5′-P and H5″-P couplings consistent with observed H5′/H5″ linewidths; $\alpha + \zeta$ were indirectly constrained by lower bounds on the observed (n)H1′ to (n+1)H5′/H5″ NOEs combined with the prior partial constraints on β, γ, δ and ϵ. Initial DNA structures (random-embedded DG structures, canonical B- and A-form DNAs) were refined until their simulated spectra matched the experimental NOESY spectra. Coordinate RMSDs per atom between the refined structures are less than 1.0Å for the central 10 base pairs indicating good convergence. The ability to determine various helical parameters and the reliability of NMR-derived structures will be presented. Furthermore, the significance of backbone angle constraints in determining structures will be discussed.

CG 214 NMR OF *HIV-1* TAR RNA SPECIES AND THEIR INTERACTIONS WITH SIMPLE BIOCONJUGATES

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The TAR RNA sequence present at the 5' terminus of *HIV-1* RNA transcripts plays an important role in activation of transcription and is a potential target for AIDS therapy. The imino proton spectrum of an approximately full-length wild-type 59mer TAR sequence is consistent with the presence of three double-helical stem sections, three bulges and a loop. Homonuclear 2D and 3D methods have been used to assign signals and determine a first-stage structure for a 29mer comprising the most functionally significant region of the molecule.

The solution structure provides a starting point for the design of model oligopeptides, oligonucleotides and nucleopeptide conjugates with selective TAR binding properties. Intermolecular NOEs are used to position the two species relative to one another. Results obtained for the binding of a molecule comprised of a peptidyl nuclear localization signal conjugated with a deoxynucleotide trimer will be presented.

CG 215 STABLE ISOTOPE AIDED NMR STUDIES OF STRUCTURE AND INTERACTION OF λ-CRO, Yoshimasa Kyogoku, Masahiro Shirakawa, Hiroshi Matsuo and Yasuhara Serikawa, Institute for Protein Research,Osaka University,Suita,Osaka 565,Japan

λ-Cro repressor and its interaction with DNA have been studied by ^{15}N and ^{13}C aided NMR spectroscopy. Assignments of the most of the ^{15}N and ^{1}H resonances in the main chain of the protein were obtained by measuring heteronuclear multiquantum coherence (HMQC), SQC, relayed HMQC-COSY and HMQC-NOESY spectroscopy of the proteins uniformly labeled with ^{15}N, specifically labeled with ^{15}N-enriched amino acids and doubly-labeled with ^{13}C-carbonyl- and ^{15}N. Furthermore, 3D HMQC-NOESY and series of X-half filtered 2D spectra have been obtained for identifying sequential NOE connectivities. Some of the already reported assignments of the amide protons were revised. In order to study the interaction of Cro with DNAs, we prepared two different DNA fragments, one has identical sequence to the Cro binding site and the other not, corresponding to specific DNA and nonspecific DNA, respectively. Some $^{15}N/^{1}H$ cross peaks of ^{15}N-labeled Cro showed marked shifts differently depending on the addition of the specific DNA fragment and the nonspecific one to the ^{15}N-labeled cro protein. Plots of the amino acid residues which showed the shifts on the three dimensional structure of Cro demonstrate the binding surface of Cro with specific DNA and non-specific DNA. The results indicate that in the specific interaction, compared to the nonspecific interaction, a much wider region of Cro, including the α_3 helix, makes tight contact with DNA, inducing conformational changes on both Cro and the operator DNA.

CG 216 **PARALLEL MOLECULAR MODELING: A GENETIC APPROACH,** George C. Levy and Sophia Wang, NMR and Data Processing Laboratory, Syracuse University, Syracuse, New York, 13244-4100

A genetic algorithm approach is proposed for the tasks of determining from multidimensional NMR, crude structures of complex molecules including small proteins and nucleic acids, and for refinement to elucidate accurate and reliable 3-dimensional structures. The genetic algorithm approach offers natural advantages for molecules exhibiting conformational heterogeneity, and, further, genetic algorithms are inherently parallelizable. Thus, this approach utilizes one of the most important trends in high-performance computing, parallel processing. The implementation used in this project includes several extensions to standard genetic algorithms: (i)the blended inheritance genetic operation supplements crossover and mutation, leading to rapid convergence; (ii) a shared fitness function is added to try to stabilize multiple conformation sub-populations, where conformational heterogeneity is present. Current implementations include a version for the massively parallel Connection Machine from Thinking Machines Inc.(or similar SIMD machines) and a highly efficient network-distributed version suitable for Unix workstation computers.

CG 217 2D HETERONUCLEAR NMR STUDIES OF OLIGONUCLEOTIDES, David Live, Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125.

With the increased interest in conformational analysis of oligonucleotides that deviate from conventional double stranded structures, or participate in binding to other molecules, exclusive use of ^{1}H data may be inadequate. ^{1}H detected heteronuclear experiments provide an important source of supplementary information. With the absence of facile isotopic labeling, investigations involving ^{13}C and ^{15}N require the ^{1}H detected approach. Dispersal in the ^{13}C dimension can be very helpful in identifying signals in regions where there may be high overlap in the ^{1}H spectrum. ^{13}C shifts can be useful in characterizing sugar conformation as well and provide immediate identification of the C3' and H3' resonances at the 3' end of a strand. ^{1}H - ^{15}N information can help in assigning assigning imino protons, particularly in distorted structures when conventional NOEs are not present, and can provide insight into interstrand and ligand hydrogen bonds. Even though information can be obtained from ^{31}P direct detection because of its high natural abundance, the ^{1}H experiments can be advantageous, particularly in making sequential assignments. Studies of on a number of oligonucleotides in duplexes and higher order aggregates, as well as of complexes with drugs will be described.

CG 218 DESIGN OF A MINIMAL PEPTIDE-OLIGONUCLEOTIDE COMPLEX FOR 2-D NMR STRUCTURAL STUDIES, C. James McKnight, Robert V. Talanian and Peter S. Kim, Howard Hughes Medical Instiiute, Whitehead Institute for Biomedical Research, M. I. T., 9 Cambridge Center, Cambridge, MA 02142.

The yeast transcriptional activator GCN4 controls the expression of several genes involved in amino acid biosynthesis. GCN4 is a member of the "bZIP" class of transcriptional activators that includes the oncogene products Fos and Jun. The bZIP activators contain a dimerization motif of approximately 30 amino acids containing a heptad repeat of leucine residues (the leucine zipper) and, immediately N-terminal to the leucine zipper, a basic region of approximately 30 residues. This basic region is thought to make the direct contacts with the target DNA sequence. No 3-dimensional structural information is currently available for the bZIP DNA binding motif. We have recently shown that the leucine zipper is not required for specific DNA binding by replacing the leucine zipper with a disulfide bond that serves to dimerize a 34 residue peptide (GCN4-br1) containing only the basic region and a gly-gly-cys linker (Talanian, R. V., McKnight, C. J., and Kim, P. S. (1990) **Science 249,** 769-771). The relatively small size of this disulfide linked basic region and the compactness of the cognate DNA binding sequence make this system a good candidate for structural studies by NMR. This poster describes experiments designed to determine the smallest basic region dimer capable of specific DNA binding and the shortest oligonucleotide that can be recognized and bound. Preliminary NMR experiments indicate that the complex of a disulfide-linked peptide dimer (60 residues total) with a 14 base pair oligonucleotide is in slow exchange on the NMR time-scale, and that the complex is highly symmetric.

CG 219 NMR STUDIES AND CONFORMATIONAL ANALYSIS OF TRIPLE HELICES FORMED FROM DNA-HAIRPINS

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Homopurine/homopyrimidine tracts have attracted considerable attention because of their unexpected sensitivity to S1-nuclease when present in supercoiled plasmids. S1-nuclease is regarded to be highly specific for single stranded nucleic acids, but the enzyme also appears to recognize distortions in the phosphodiester backbone. Homopurine/homopyrimidine DNA oligonucleotides readily form triple stranded structures.

We studied the 15-mer d(TCTCTC-TTT-GAGAGA), which adopts a hairpin conformation at high pH, but a triple helical structure at low pH by combination of two hairpins (1). For the triplex structure, a nearly complete assignment of the resonances in the 2D NMR spectra has been obtained. In the Hoogsteen paired strand, as well as in the Watson-Crick paired strands short H3' to H6/H8 distances are observed. This indicates that A-type conformations are mixed in. In addition some unusual backbone torsion angles are observed. Structural refinements of the triplex will be presented and comparison made with the hairpin form at high pH.

Binding of the oligomer d5'(TCTCTCT)3' to the hairpin also leads to formation of a triple helix although small structural differences may exist.

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CG 220 ^{1}H NMR STUDIES OF DNA DUPLEXES CONTAINING BASE PAIRS BETWEEN PYRIMIDINES WITH ALTERNATIVE HYDROGEN BONDING MODES AND THE NORMAL PURINES. Angus N. R. Nedderman, Martin J. Stone, Paul Kong Thoo Lin*, Daniel M. Brown*. University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U K, * MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK.

Two modified pyrimidine bases, N^4-methoxycytosine (M) and 6H,8H-3,4-dihydro-pyrimido[4,5-c][1,2]oxazin-7-one (P), were incorporated as their deoxyribosides into self-complementary octamers in order to investigate base pairing to the purine bases A and G. Base resonances were assigned by DQF-COSY and phase-sensitive NOESY experiments and the helix-coil transitions studied by variable temperature ^{1}H NMR spectroscopy. P formed base pairs of approximately equal stability with both A and G. M bound strongly to A but less so to G, due in part to the preferred *syn* conformation of the methoxyl group. Studies of the imino protons, using binominal water suppression techniques, indicated that A.P, G.P and A.M base pairs were of the Watson-Crick form whilst G.M base pairs oscillated between Watson-Crick and wobble mismatched conformations. These results demonstrate that both tautomeric forms of M and P can be utilised in base pair formation.

CG 221 SOLUTION STRUCTURES OF UNUSUALLY STABLE RNA HAIRPINS CONTAINING THE GNRA LOOP SEQUENCE, Arthur Pardi and Hans A. Heus, Department of Chemistry and Biochemistry, Campus Box 215, University of Colorado at Boulder, Boulder, CO 80309.
The most frequently occurring RNA hairpins in 16S and 23S ribosomal RNA contain a tetranucleotide loop with a GNRA consensus loop sequence. The solution structures of the GCAA and GAAA hairpins have been determined by ^{1}H and ^{31}P NMR spectroscopy, distance geometry and molecular dynamics calculations. The structures of both loops contain: a novel G•A base pair between the first and last residue in the loop, a G imino proton - phosphate hydrogen bond, extensive base stacking and possibly a sugar 2' OH - base hydrogen bond. The structures explain the unusually high stability of these hairpins, as well as sequence requirements for the variant and invariant nucleotides within the GNRA tetranucleotide loop family. Methods have also been developed for incorporating stable (^{15}N and/or ^{13}C) isotopes into RNA oligomers and examples of multidimensional heteronuclear NMR experiments on these RNA hairpins will be discussed.

CG 222 SOLUTION STRUCTURE OF THE HOMEODOMAIN OF YEAST α2 REPRESSOR AND NMR SPECTRAL CHANGES ON BINDING TO DNA, Cynthia L. Phillips*, Andrew K. Vershon, Alexander D. Johnson and F. W. Dahlquist*, *Institute of Molecular Biology, University of Oregon, Eugene, OR 97401 and Department of Microbiology, University of California, San Francisco, CA 94743.
The protein α2 is a transcriptional repressor of mating-type genes in yeast. The region of α2 which binds DNA contains a homeodomain of low sequence homology with respect to homeodomains in higher eukaryotes. We used solution ^{1}H and ^{15}N NMR spectroscopy to determine the secondary structure of the homeodomain of α2 contained by a C-terminal 83 residue fragment (α2-83). We have obtained sequence-specific assignments for the backbone protons of the entire 60 residue homeodomain, and most of the remainder of the α2-83. The secondary structure was determined using NOE connectivities between backbone protons, $^{3}J_{HN\text{-}H\alpha}$ coupling constants, and dynamical information from hydrogen exchange rates of the backbone amide protons. There are three helical segments in α2-83, consisting of residues 11-25, 32-42 and 46-59 (where residue 1 is the first residue of α2-83). This secondary structure of the α2 homeodomain is nearly identical to those of the Antp and engrailed homeodomains found in *Drosophila*. Titration of α2-83 with DNA derived from a consensus a-specific gene operator shows the greatest chemical shift changes in helices 1 and 3 of α2-83, with little change in helix 2. This is consistent with the structures of the Antp and engrailed homeodomain DNA complexes in which helices 1 and 3 contact the DNA, whereas helix 2 is not appreciably affected by DNA binding. Work is in progress to investigate structural and dynamical changes in α2-83 upon binding to DNA.

CG 223 CONFORMATION AND STACKING OF MULTIPLE-BASE BULGES IN DNA DETERMINED VIA TWO-DIMENSIONAL NMR AND COMPUTATIONAL METHODS.
Mark A. Rosen, Lawrence Shapiro, and Dinshaw J. Patel, Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY 10032.

Two-dimensional homonuclear NMR has been used to examine the solution conformation of an oligodeoxynucleotide duplex containing a centrally-placed bulge loop consisting of one base (A), two bases (AA) or three bases (AAA, AIA or ATA). NOE-based assignments of non-exchangeable protons in D2O indicate that base stacking is maintained throughout the bulge-containing strand but is interrupted on the opposite strand when the bulge contains two or more bases. NOESY experiments in H2O demonstrate that Watson-Crick pairing is intact for the basepairs adjacent to the bulge site, and confirm the intrahelical position of all bases within the bulge. Conformational modeling via NOE-restrained molecular dynamics and relaxation matrix analysis suggests that upon insertion of two or more bulged bases within the helix, the bases across from the bulge site are wedged apart, producing a kink in the helical axis at the bulge site. These results are interpreted in light of published studies that have shown decreased gel mobility of bulge-containing oligodeoxynucleotides.

CG 224 SOLUTION STRUCTURE OF THE MITHRAMYCIN-OLIGONUCLEOTIDE COMPLEX USING TWO-DIMENSIONAL NMR SPECTROSCOPY. Mallika Sastry, Xiaqlian Gao and Dinshaw J. Patel, Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY 10032.

The antitumor antibiotic mithramycin has been shown to act against a variety of experimental and human tumors. This activity is achieved by binding to double-stranded DNA and inhibiting DNA dependent RNA synthesis. The presence of divalent metal ions (e.g. Mg^{2+}) is essential for drug binding. Enzymatic and chemical footprinting studies on mithramycin-DNA complexes show that the drug binds to sites containing two contigous GC base pairs with the dinucleotide step GpG as the preferred binding site. We have studied the mithramycin-dTGGCCA complex in the presence of Mg^{2+} ions using one and two dimensional NMR spectroscopy. Our studies show that the drug binds as a dimer to the oligonucleotide sequence. The symmetry of the duplex is retained upon drug binding. Intermolecular NOE's show that this drug binds in the minor groove of the duplex. Based on NOE-restrained molecular dynamics we propose a model for the three-dimensional structure of the mithramycin-DNA complex. A comparison with the structure of the chromomycin-DNA complex[1] will be presented.

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CG 225 NMR Relaxation Matrix Refinement of DNA Oligomer Solution Structures

Lawrence Shapiro, Magdalena Eriksson, Michael Nilges, Axel T. Brunger, and Dinshaw J. Patel. From the Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY 10032, and the Howard Hughes Medical Institute and Department of Molecular Biophysics, Yale University, New Haven, CT.

We have refined the solution structure of the DNA duplex d(GGTATACC) using a molecular dynamics refinement scheme which accounts fully for spin diffusion by iteratively minimizing the difference between observed and calculated NOESY spectra. To achieve this, a term proportional to this difference was added to the potential function of the molecular dynamics program X-PLOR. The refined structures converge to an RMSD around 1 A from both A- and B-form starting structures. The conformational parameters of the relaxation-matrix refined structures become more regular and move closer to B-DNA values relative to structures refined using two-spin approximation distances.

CG 226 DISTANCE ERRORS IN DNA STRUCTURE DETERMINATION PRODUCED BY SEQUENCE-SPECIFIC BINDING OF PARAMAGNETIC IMPURITIES - EFFECTS ON THE EcoRI DODECAMER DUPLEX d[CGCGAATTCGCG]$_2$.

Einar Sletten*, Willy Nerdal* and Brian R.Reid
Department of Chemistry, University of Washington, Seattle, WA 98195

In general, the magnitude of NOESY cross-peaks is related to the internuclear H ··· H distances. However, in the presence of minute amounts of paramagnetic impurities the NOE-effects may be partially quenched. In molecules where site specific metal binding occurs this could lead to serious local errors in distance measurements. In NMR studies on the EcoRI dodecamer duplex in the absence of chelating buffers [Nerdal *et al.*, *Biochemistry*, (1989), **28**, 10008] the observation of anomalously weak G4-H8 NOEs to other protons led to the generation of an apparent kink or discontinuity between the third and fourth base pair from either end. We now show that titrating the 10 mM DNA sample with EDTA to 0.6 mM restores these broadened G4-H8 NOEs to full strength. The exposed G12 residue is also affected while G2 and G10 show minimal effects. In several other guanine-containing oligonucleotides studied in this laboratory similar sequence-specific metal binding to guanine is observed when guanine occurs in the context -pyrimidine-guanine-purine-. This observation may prove to be valuable in assigning and/or measuring longer distances between proton signals in polynucleotides utilizing paramagnetic shift and/or broadening probes.

*On leave from Department of Chemistry, University of Bergen, Norway.

CG 227 Structural studies of the gene-5 proteins of the filamenteous phages M13 and IKe as studied by NMR.

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In the lifecycles of the filamenteous phages M13 and IKe [1] gene-5 plays a fundamental role. In both systems it encodes for a ssDNA binding protein of which the amino acid sequences are consisting of 87 and 88 residues respectively. In solution the proteins are present as 20 kD dimers. Further comparison shows that they are 60% homologous in their amino acid sequence and share the same ssDNA binding characteristics.
2D-NMR studies performed on the M13 and IKe gene-5 proteins allowed sequential assignment of the backbone resonances and a major part of the side-chain resonances. It will be shown that the dimeric molecules consist mainly of antiparallel β-sheet elements that are remarkably well conserved [2,3]. Spinlabeled oligonucleotides were employed [4] to overcome overlap problems in the analysis of the ^{1}H-spectra and to map the DNA binding domains. The conservation of the amino acid residues as well as the secondary structure elements involved in ssDNA binding are demonstrated. The observed similarity in the observed structure-function relationships suggest a common ssDNA gene-5 protein interaction model for this class of DNA binding proteins.

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CG 228 A 2D NMR STUDY ON THE STRUCTURE OF THE COMPLEX OF THE ANTITUMOR DRUG NOGALAMYCIN WITH DNA-FRAGMENTS, Leo P.A. van Houte, Carla J. van Garderen and Dinshaw J. Patel, Department of Biochemistry & Molecular Biophysics, College of Physicians & Surgeons, Columbia University, New York, NY 10032.

The anthracycline antibiotic nogalamycin isolated from *Streptomyces nogalator* binds to double-stranded DNA via intercalation. *In vivo* nogalamycin selectively inhibits RNA synthesis and exhibits an antitumor activity against a number of tumor cell lines.
Footprinting experiments established a preferential binding of nogalamycin at d(C-A).d(T-G) pyrimidine-purine steps in DNA. NMR studies on the structure of the complex of nogalamycin with the d(GCATGC) (ref.4) and d(AGCATGCT) (ref.5) duplexes, in which the two binding sites are separated by 2 base pairs, clearly showed that the aglycone rings of nogalamycin intercalate at the d(C-A).d(T-G) sequence. In these structures the nogalose and bicyclic amino sugars are positioned in the minor and major groove, respectively, and point in the direction of the ends of the duplex.
The complex of nogalamycin with the d(*CGTsA*CG) duplex (*C is either C or me^{5}C; ref. 1,2 and 3) was investigated with crystallographic methods. In this case the aglycone rings of nogalamycin intercalate at the two d(*C-G).d(*C-G) steps, which are separated by 4 base pairs and located at the end of the oligomers. The nogalose and bicyclic amino sugars are also found in the minor and major groove, respectively. In contrast to the nogalamycin-duplex structures containing the d(C-A).d(T-G) steps, the nogalose and bicyclic amino sugars are directed to the central basepairs of the complex.
In order to investigate the sequence specificity and the position of the sugars the 1:1 complexes of nogalamycin with d(GCAT).(ATGC) and d(GCGT).d(ACGC) were studied with 2D NMR techniques. The results will be presented on the poster.

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CG 229 ADR1a, A ZINC FINGER PEPTIDE, EXISTS IN TWO FOLDED CONFORMATIONS.
Robert X. Xu, Suzanna J. Horvath, and Rachel E. Klevit. Department of Biochemistry, University of Washington, Seattle, WA 98195
Two-dimensional NMR (2DNMR) studies of several different zinc finger peptides have yielded a picture of the three-dimensional structure of this small DNA-binding motif. The global fold is quite similar to that predicted by Berg on the basis of known metal-binding proteins. The details of the differences among fingers with different sequences may provide some insight into how these domains interact with DNA. Towards this end, we have reanalysed the 2DNMR spectra of the C-terminal zinc finger sequence from the yeast transcriptional factor, ADR1. Although this was the sequence on which our original report describing the overall fold of zinc fingers was based, complete spectral assignments (reported here) were needed to compare this sequence in detail with that of ADR1b, for which we have reported an atomic level structure. In the process of analyzing the spectra of ADR1a and a point mutant of ADR1a, it was noted that the peptides give two sets of NMR lines, indicating that this sequence, unlike the other ADR1 zinc finger sequence, exists in two different folded conformations in solution. This conformational flexibility may have functional significance.
(This work was supported by NIH PO1 GM32681)

Peptides and Oligosaccharides

CG 300 THE STRUCTURE OF THE ANTICOAGULANT PROTEIN KISTRIN DETERMINED BY ^{1}H NMR SPECTROSCOPY, Marc Adler and Gerhard Wagner, Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02154, Mark S. Dennis and Robert A. Lazarus, Department of Biomolecular Chemistry, Genetech Inc., South San Fransico, CA 94080

A solution structure has been obtained for kistrin, a small protein of 68 residues and 6 disulfide bonds, using ^{1}H NMR spectroscopy. Kistrin is a naturally occurring inhibitor of blood clotting which is isolated from the venom of the Malayan pit viper *Agkistrodon rhodostoma*. It competes with an RGD sequence of fibrin for the binding to GP II_b-III_a, a cell surface receptor that mediates the attachment of platelets to a fibrin clot. GPII_b-III_a is a member of the integin family, a large class of cell surface glycoproteins that is deeply involved with many types of cell-cell interactions. There are no available structures for any protein that is homologous to Kistrin in either sequence or function. To date, structure calculation have been performed based on 554 assigned NOE's. Constraints have also been included for 25 ϕ's and 15 ψ_1's angles. The protein contains almost no regular secondary structure. This is surprising since a third of the amides are protected from exchange with the solvent. The active site, an RGD sequence, is located at a bend in a long arm like structure and is exposed to the solvent. The rest of the protein consists of a series of tightly packed loops.

CG 301 STRUCTURE OF AN FSH ANTAGONIST AND STIMULATOR OF ESTRADIOL SYNTHESIS, Paul F. Agris, Laura Easter, Charles Hardin, Wanda Smith, Hanna Sierzputowska-Gracz, Department of Biochemistry North Carolina State University, Raleigh, NC 27695, and Thomas A. Santa Coloma, Leo Reichert, Department of Biochemistry, Albany Medical College, Albany, NY 12209.

A peptide of 21 amino acids in length and corresponding to the follicle stimulating hormone (FSH) β-chain sequence of amino acids 33 to 53 had been found to be an effective antagonist of FSH binding to its testicular receptor.(Coloma, etal Biochemistry 29, 1194, 1990) In addition the peptide stimulates testicular estradiol synthesis. Such a peptide or a derivative has potential as a male contraceptive; thus an understanding of its structure and chemistry has importance in reproductive physiology. The peptide was first studied by circular dichroism (CD) under various solvent conditions (pH, and polarity). The more polar the solvent such as trifluroethanol, the more α-helical structure taken by the peptide. Under essentially physiological conditions of pH 6.5, and saline solution, the peptide exhibited 31% pleated sheet, 6% α-helix, 30% β-turn and 33% random coil, as calculated by VARSELEC (a program by W. Curtis Johnson, Jr.). Single dimension and two dimensional NMR (COSY, NOESY) techniques were used to establish signal assignments, and secondary structure for the peptide. All signals have been assigned, and the β-pleated sheet has been confirmed as the major secondary structural element. A model of structure will be proposed. (Supported by the N.C.A.R.S.).

CG 302 QUANTITATIVE NOESY-BASED CONFORMATIONAL ANALYSIS OF ENDOTHELIN-I IN ACIDIC AQUEOUS MEDIA, Niels H. Andersen, Chinpan Chen, Thomas M. Marschner, Stanley R. Krystek, Jr., Donna A. Bassolino, and Jiri Novotny, Dept. of Chemistry, University of Washington, Seattle, WA 98195; and Dept. of Macromolecular Modeling, Bristol-Meyers Squibb Research Institute, Princeton, NJ 08543.

Multiple NOESY data sets for endothelin-1, an endogenous 21-residue bisdisulfide vasoconstrictor, have been recorded in 60% aq. glycol at pH 3.2-5.0. A set of 168 conformationally-dependent distance constraints (ave. precision ±0.37 Å) were derived using program DISCON. These were used for distance constrained dynamics using programs CONGEN, DISCOVER, and XPLOR. The resulting set of structures implies that endothelin is a mixture of conformers in rapid equilibrium. All significantly populated species share a helical conformation from K9 through H16. This helical region is not a regular α-helix – several psi values (ψ_9, ψ_{11}, ψ_{14} in particular) are shifted to much smaller values than normal and ϕ_{15} approaches -120°. These features are reproduced by all three dynamic protocols. Another consensus structural feature is a reverse turn at residues 5→8. The C-terminus is structured but further studies (prochirality assignments) are required to define this feature. The regions that serve as"hinges" for the conformational reorganization appear to be: the C1-C15 disulfide, the C3-S5 segment, the 8(9) amide unit (ψ_8,ϕ_9), and L17. Structure details for at least two defined conformers will be presented, together with data implicating a pH dependent change in the conformational equilibrium.

CG 303 NMR SOLUTION STRUCTURE OF ω-CONOTOXIN GVIA.

Jonathan H. Davis[a], George Miljanich[b], D. Yamashiro[b], J. Ramachandran[b], and Vladimir J. Basus[c],

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The ω-Conotoxin GVIa from *Conus Geographus*, a fish-hunting sea snail, has been shown to bind essentially irreversibly to neuronal voltage-sensitive calcium channels (1). We have synthesized this peptide in order to obtain a large enough amount for NMR structural studies. The ^{1}H NMR spectrum of this peptide was completely assigned. We have obtained a single family of closely related structures consistent with the NOE and coupling constant data, using distance geometry for generating starting structures, followed by restrained molecular dynamics calculations. Due to the restrictive nature of the three disulfide bridges present in this 27-residue peptide, initial molecular dynamics were carried out with the disulfide bonds replaced by soft constraints. This allowed sufficient flexibility to obtain energetically reasonable structures from the restrained molecular dynamics calculations, consistent with the NMR data. The final structures had an average rms deviation of 1.5Å for the backbone atoms.

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CG 304 SOLID-STATE NMR OF MAGAININ AND PGLa PEPTIDE ANTIBIOTICS IN BILAYERS

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Magainins (23 residues) and PGLa (21 residues) are peptides found in the skin and other organs of frogs that act as broad spectrum antibiotics by increasing ion permeability of prokaryotic but not eukaryotic membranes (1). Multidimensional solution NMR experiments of magainin show that these peptides exhibit a high α-helix content in trifluoroethanol/water solution (2) and in micelles (3).

We synthesized several specifically ^{15}N labeled magainins and PGLa and incorporated them into multilamellar bilayers of varying lipid composition. The resulting membranes can be oriented between glass plates. In order to perform solid-state NMR experiments the aligned samples were placed in a flat coil with high sensitivity due to the nearly ideal filling factor. The ^{15}N resonance frequencies indicate that these amphipathic helices are aligned with their axes approximately parallel to the plane of the bilayer.

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CG 305 CONFORMATION OF MORPHICEPTINS AND ITS DERIVATIVES BY TWO-DIMENSIONAL NMR SPECTROSCOPY AND ENERGY MINIMIZATION CALCULATIONS, Bernardo Celda[1], Roberto Tejero[1], María J. Arnáu[1], Gregorio Valencia[2], José L. Torres[2] and Eduardo Bardaji[2], [1]Departamento de Química Física, F. Químicas, Universidad de Valencia, 46100-Burjassot(Valencia), Spain, [2]Unidad de Química de Péptidos y Bioquímica, C.I.D.-C.S.I.C., 08034-Barcelona, Spain.

The biological activity of three new N-terminal morphiceptin derivatives was recently evaluated in the GPI bioassay. To elucidate the structure-activity relationship of this opiate-active peptides the conformational properties of the N,N-diallyl-tyrosine (DT) have been investigated and compared with morphiceptin by use 1D and 2D-NMR, molecular modeling based on the NMR results, and molecular mechanics energy minimization. At least two of the four discernibles configurational isomers observed for both morphiceptin and DT analogue have been assigned by using ^{1}H and ^{13}C NMR experiments. The combination of NOE and coupling constants (via simulation of the cross-peaks using SPHINX and LINSHA programs) allow an almost complete definition of all the relevant torsion angles for the major configuration isomers of morphiceptin and DT. In principle, the largest isomer, more than 50%, for the DT, like for morphiceptin, exist with all the amide bonds in a trans conformation, whereas, in the second isomer the arrangement between ^{1}Tyr-^{2}Pro seems to be cis. The values of NH chemical shifts as function of temperature for both morphiceptins are all within 8 to 10 ppb/T, and are very typical for freely accesible protons. Utilizing NMR constrains and parameters in conjuntion with model building, extensive energy minimization led to the preliminar configuations for morphiceptin and DT. The variation of activity of morphiceptin with respect to DT is discussed as function of the conformations observed.

CG 306 PROTON NMR ASSIGNMENTS AND STRUCTURE OF SNAKE VENOM PEPTIDE ECHISTATIN. Yuan Chen[1] and Jean Baum[1]. [1]Chemistry Department, Rutgers University, Piscataway, NJ 08854. Steve Pitzenberger[2], Victor M. Garsky[2], Patricia K. Lumma[2], William C. Randall[2], 2Department of Medicinal Chemistry, Charles R. Middeugh[3], Gaupam Sanyal[3], [3]Department of Pharmeceutical Research, Adel M. Naylor[4], [4]Department of Molecular Systems, Merck, Sharp & Dohme, West Point, PA.

Echistatin is a forty-nine amino acid protein containing the tripeptide unit Arg-Gly-Asp, which has been shown to occur in a variety of proteins that interact with certain cell surface receptors. In particular, echistatin has been shown to be a potent inhibitor of fibrinogen-dependent platelet aggregation. The sequence-specific ^1H nuclear magnetic resonance assignments of echistatin have been obtained through two-dimensional experiments. The NMR results suggest that the conformation of the protein does not contain any regular α-helix or β-sheet, but mainly consists of turns and random coil, which is consistent with circular dichroism measurements. However, two short and distorted antiparrallel β-sheets which form the core of the protein have been suggested by the NMR data. The Arg-Gly-Asp sequence is located in a loop connecting the two strands of one of the distorted β-sheets. We are currently working on the three-dimensional structure of echistatin using Monte Carlo search algorithm and Distance Geometry to sample conformations which satisfy distance constraints derived from nuclear Overhauser effect measurements, torsional constraints from vicinal spin-spin coupling constants, and the known location of the disulfide bonds. Subsequent refinement of the sampled conformations is by Molecular Dynamics and energy minimization.

CG 307 **Structural Characterization of a 4-Helix Bundle by 2- and 3-Dimensional NMR.** Peter Domaille, 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 (Ac-GELEELLKKLKELLKG-$CONH_2$), that self-associates into a four-helix bundle. In particular, it was possible to to show that the peptide is helical between residues 2 and 15 by 2-D NOESY experiments (W. Degrado, T. Handel, J. Hoch, D. Live, J. Osterhout, D. Weaver). However, because of the symmetry of the bundle and the degeneracy of the amino acid sequence, it was not possible to establish the orientation of the helices with respect to each other based on cross peaks observed in NOESY spectra. Because of overlap, in this work we describe an analogue of the original peptide with Val and Phe at positions 3 and 13, respectively (V3F13). 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. In order to obtain additional ***interhelical*** NOE's, we have also synthesized V3F13 peptides in which 3 of the 4 remaining Leu's are deuterated. 2-Dimensional NOESY data for each of the 6 possible mixtures of deuterated peptides (i.e. V3F13 with H-Leu6 + V3F13 with H-Leu10) were not interpretable due to insufficient dispersion of the amino acid side chains. To alleviate the overlap problem we have carried out 3-dimensional NOESY-TOCSY experiments on the deuterated mixtures. The additional information gained in these 3-dimensional experiments allow us to distinguish between intra- vs inter-residue NOE's, even in cases of accidental degeneracy.

CG 308 SOLUTION STRUCTURE OF ENDOTHELIN-1 AND BIG ENDOTHELIN, M. E. Donlan, S.C. Brown, & P.W. Jeffs, Glaxo Research Institute, 5 Moore Drive, Research Triangle Park, NC 27709

Endothelin-1 (ET-1) is a 21 amino acid peptide which is one of the most potent vasoconstrictors known. From analysis of the cDNA of endothelin, it has been proposed that ET-1 is produced from a 38 amino acid peptide (big ET-1) by cleavage of a Trp-Val bond (1). Big ET has been found to be considerably less active than ET as a vasoconstrictor (2).

In order to gain a better understanding of this activity, we are investigating the three-dimensional structures of both ET-1 and Big ET-1. Proton resonances for both peptides were assigned at pH 3.4 in aqueous solution by standard two-dimensional NMR techniques. From the NOESY spectra, interproton distances were determined and used in distance geometry (DSPACE, Hare Research) and molecular dynamics (AMBER) calculations to generate families of structures These structures were further refined via back calculation of the NOESY spectra in an iterative procedure. The resulting structures were compared to one one another to understand the difference in their physiological activity.

(1) Yanagisawa, M *et. al.* (1988) Nature, **332**, 411-415
(2) Kimura, S. *et. al.* (1989) J. Cardiovascular Pharmacology, **13**, S5-7

CG 309 **CONTINUOUS ANGULAR DISTRIBUTION OF ROTAMER PROBABILITIES OBTAINED FROM VICINAL SPIN-SPIN COUPLING CONSTANTS,** Željko Džakula, Arthur S. Edison, William M. Westler, Andrzej Krezel, and John L. Markley, *Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin-Madison, 420 Henry Mall, Madison, WI 53706.*

A procedure is presented for determination of the continuous angular distribution of rotamer probability, $\rho(\phi)$, from measured, vicinal homonuclear and heteronuclear spin-spin coupling constants in conformationally labile molecules. Fourier coefficients of the distribution are obtained by a least-squares fit of the measured coupling constants to the integral of the product of a Fourier series expansion of $\rho(\phi)$ with the known angular dependence of $J(\phi)$ (i.e. the Karplus equation). In this approach, we do not make the physically unrealistic assumption that the distribution can be represented by a weighted average of a normalized set of three ethane-like staggered conformations. To determine the Fourier coefficients of the expansion of $\rho(\phi)$, four or more measured coupling constants are required. We have applied this method to find the distribution of rotamers about the χ_1 angle in L-leucine on the basis of 6 measured coupling constants[1] between various pairs of spins across the C^{α}-C^{β} bond. The resulting function, $\rho(\phi)$, agrees well with the distribution predicted with quantum statisical physics on the basis of a calculated rotational potential for L-leucine.

[1]Fischman, A. J., Wyssbrod, H. R., Agosta, W. C., and Cowburn, D., *J. Am. Chem. Soc.* **100**, 54-58 (1978).

CG 310 SOLUTION STRUCTURE OF THE C-TERMINAL DOMAIN OF PROTEIN TAU, Denise Fréchet, Fréderic Herman, Marc Vuilhorgne, Francois Fréderic Clerc, Jean Dominique Guitton, Evelyne Surcouf, Christine Pernelle and Laurent Bracco. Rhône Poulenc Rorer, CRVA, 13 quai Jules Guesde, 94403 Vitry-sur-Seine, FRANCE.

Tau is a microtubule associated protein involved in the formation of neurofibrillary tangles, a pathological lesion formed in the brain of Alzeihmer disease patients . A short hydrophobic α-helical domain , located at the carboxyl terminus of this protein, has been postulated to form a symmetric dimer, which might play a role in the formation of microtubule bundles. In order to gain insight into the molecular basis of this interaction, a peptide corresponding to this domain was synthesized (AcQLATLADEVSASLAKQGLY-OH) . Its conformation was studied by 2D and 3D ^{1}H NMR spectroscopy. Interproton distances obtained by quantitative treatment of NOESY data were used as constraints in molecular dynamics and energy minimization calculations, allowing to obtain a model of the conformation of the peptide in solution.

CG 311 CONFORMATION OF OXYTOCIN BOUND TO NEUROPHYSIN DETERMINED BY 2D-TRANSFER NOE AND ROE EXPERIMENTS AT 600 MHZ, K. Hallenga*+, G. Lippens*, C. Russell° and V.J. Hruby°, PGS+/UCMB+*, ULB, Ave. P. Héger, Bât. P2, 1050 Brussels; °Department of Chemistry, University of Arizona, Tucson, AZ 85721.

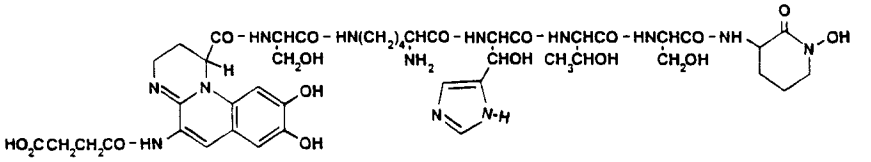
A comparison of NOE and ROE build-up curves at 600 MHz will be presented for the peptide hormone oxytocin bound to neurophysin. Differences in NOE and ROE cross-relaxation rates explain the earlier observation that 2D-transfer NOE experiments at 360 resp. 400 MHz give much weaker cross-peaks than experiments at 500 resp. 600 MHz for certain residues. This phenomenon can be explained by a detailed model of the oxytocin binding in which certain residues are firmly attached to the protein, while others maintain a great deal of flexibility.

The NOE/ROE comparison allows a determination of correlation times of many proton pair interactions across the oxytocin molecule in the bound form.

CG 312 CONFORMATIONAL ORIGIN OF DIFFERENTIAL IMMUNE RESPONSES IN TRANSMEMBRANE PROTEINS OF HIV-1, HTLV-1 AND MuLV,
Kyou-Hoon Han*, Per Johan Klasse+, Jonas Blomberg+ and James A. Ferretti*, NHLBI*,National Institutes of Health, Bethesda, MD 20892 and Department of Microbiology+, University of Lund, Lund, Sweden

Solution secondary structures of peptides corresponding to phylogenetically conserved regions in the transmembrane envelope glycoproteins of HIV-1, HTLV-1 and MuLV are determined by NMR and CD in combination with restrained molecular dynamics computations. All of the peptides are found to have appreciable α-helical content. For HIV-1, this region encompasses residues 576-592, and represents the most conserved region of gp41. A point mutation from Ala to Thr at position 582 in peptides and the intact gp41 proteins significantly decreases the ability of HIV-1 antibodies to recognize the peptide and to neutralize the virus. Analogous mutations to Gly or Ser do not result in diminished antibody recognition for either the peptide analog or the mutant protein. Antibody recognition of a protein must be occurring through a very precise mechanism which involves only a minute conformational change in the epitope.

CG 313 STRUCTURE DETERMINATION OF AN UNUSUAL PYOVERDINE TYPE SIDEROPHORE FROM PSEUDOMONAS FLUORESCENS 244,
Diane K. Hancock[1,2], Bruce Coxon[2], Shi-Yong Wang[2], Edward White V[2], Dennis J. Reeder[2],and Jon M. Bellama[1], [1]Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742, [2]Center for Analytical Chemistry, National Institute of Standards and Technology, Gaithersburg, MD 20899



This iron-chelating peptide was biosynthesized by growing Pseudomonas fluorescens 244 bacteria in a chemically defined, iron deficient medium. The primary structure was determined by amino acid analysis, FAB mass spectrometry, and NMR. The peptide contains an unusual amino acid, threo-ß-hydroxyhistidine, not previously observed in pyoverdines or other siderophores. The three-dimensional structures of this unique siderophore and its gallium chelate are being determined by ^{1}H, ^{13}C, and ^{15}N NMR.

CG 314 DETERMINATION OF SOLUTION STRUCTURE OF Y35G BPTI
Key-Sun Kim, James A. Fuchs and Clare K. Woodward, Department of Biochemistry, University of Minnesota, St. Paul, MN55108

The solution structure of Y35G BPTI (tyrosine 35 replaced by glycine) has been determined. Assignments of all crosspeaks in COSY spectra were made sequentially from COSY/NOESY spectra and verified by spin systems in HOHAHA and 3QF cosy spectra. NOE distance constraints were derived from NOESY spectra at mixing times of 80 ms, 100 ms, and 200 ms. Distance geometry was used to create the starting structure which was refined with restrained molecular dynamics. Alternatively, NOE distances were used with XPLOR starting with wild type BPTI X-ray crystal structure. In the solution structure the hydrophobic core region is similar to the wild type crystal structure, but the residues from 11 to 18 and from 35 to 42 have major reordering. Hydrogen exchange in Y35G is much faster than in wild type BPTI.

CG 315 NMR STUDIES OF GLYCOSPHINGOLIPIDS: EVIDENCE THAT INTERRESIDUE AMIDE-CARBOXYL AND AMIDE-AMIDE HYDROGEN BONDING CONTRIBUTE TO PREFERRED GLYCAN CONFORMATIONS, Steven B. Levery, The Biomembrane Institute, 201 Elliot Ave West, Seattle, WA, 98119.

In recent ^{1}H-NMR-supported studies of the three-dimensional structure of the ganglioside G_{M1} (≡Galβ1→3GalNAcβ1→4[NeuAcα2→3]Galβ1→4Glcβ1→1Cer), it has been proposed that the allowed geometries for the core GalNAcβ1→4(NeuAcα2→3)Galβ1→ trisaccharide are favorable for formation of a hydrogen bond between the GalNAc acetamido NH and the NeuAc carboxylate group [Acquotti et al (1990) JACS 112:7772-7778; Scarsdale et al (1990) Biochemistry 29:9843-9855]. New evidence, based primarily on a comparative analysis of ^{1}H-NMR data for the exchangeable amide protons of G_{M2} (≡GalNAcβ1→4[NeuAcα2→3]Galβ1→4Glcβ1→1Cer), and two constituent glycosphingolipids (G_{A2}≡Gg_3Cer≡GalNAcβ1→4Galβ1→4Glcβ1→1Cer and G_{M3}≡NeuAcα2→3Galβ1→4Glcβ1→1Cer), indicate that a such a hydrogen bonding interaction exists, and could contribute to the stabilization of a single conformer somewhat different from that proposed by Sabesan et al [(1984) Can J Chem 62:1034-1045]. Similar studies on the neutral lacto-ganglio hybrid structure GalNAcβ1→4(GlcNacβ1→3)Galβ1→4Glcβ1→1Cer (LcGg_4Cer), and its constituent glycosphingolipids (G_{A2} and Lc_3Cer≡GlcNacβ1→3Galβ1→4Glcβ1→1Cer), suggest the existence of a hydrogen bonding interaction between the amide groups of the two terminal 2-N-acetamido-2-deoxyhexose residues.

CG 316 FORMATION OF NATIVE HELICAL HAIRPIN CONFORMATION NEEDS ADDITIONAL INTERACTIONS: IMPLICATIONS FOR PROTEIN FOLDING

Gene Merutka, Hang-Cheol Shin, Jonathan P. Waltho, H. Jane Dyson, and Peter E. Wright, Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

The initiation of protein folding begins with the formation of transient secondary structures that are stabilized by subsequent events. In order to examine early folding events more closely, we have focused on the formation of a helical hairpin encompassing helices G and H of myoglobin. We have synthesized the individual G and H helices, disulfide-bridged combinations of G and H helices, and a peptide comprising the entire hairpin region. Helicity, degree of association between helices, and hairpin formation have been characterized by CD and NMR. The individual G and H helices are about 5 and 25% helical, respectively, and show no evidence of association when mixed together. Furthermore, the peptide comprising the entire hairpin region shows a similar amount of helicity and there is no evidence of interaction between helices. However, crosslinked peptides GssH and HssH give evidence of being highly helical dimers. The above data suggest that helical hairpin formation in globular proteins is not a simple interaction of secondary structures but may require additional stabilizing tertiary contacts.

CG 317 **SOLUTION STRUCTURE DETERMINATION OF A HINGE PEPTIDE DERIVATIVE**, Siggi Mronga, Horst Kessler, Gerhard Müller, Guido Kurz, Organisch Chemisches Institut, TU-München, D-8046 Garching, Germany, Louis Moroder, Erich Wünsch, MPI, D-8033 Martinsried, Germany

The hinge region links the antigen binding F_{ab} part to the constant F_c domain in immuonoglobulins. For the hinge peptide derivative [AcThr(OtBu)-Cys-Pro-Pro-Cys-Pro-Ala-ProNH_2]$_2$ fully ^{1}H and ^{13}C assignments were achieved by 2D NMR techniques: TOCSY, NOESY, ROESY, E. COSY, HMQC and HMQC with TOCSY transfer and a modified Overbodenhausen experiment with extreme high resolution in F_1, which was several times folded in F_1 but still phase correctable. Conformational relevant parameters (78 NOE distance restraints, $^3J_{HH}$ for prochiral assignments, temperature gradients) were determined by NMR and served as input data for MD structure refinement. A simulated model compond representing the (Cys-Pro-Pro-Cys)$_2$ core elongated by the peptide chains in the F_{ab} and F_c direction served as starting structure for the final MD run. The conformation in vacuo does not agree with the C_2 symmetry required from NMR data, but the structure obtained by a water simulation fulfills the requirement. Here the core of the hinge peptide derivative adopts a poly proline II double helix as in the X ray structure of IgG1. Hence, segments responsible for the internal flexibility are located outside the core, confirmed by the flexibility of the solvent exposed C terminus.

CG 318 CIDNP STUDIES OF IMMUNOGENIC DIVERSITY IN SYNTHETIC ANTIGENIC PEPTIDES, Karol A. Muszkat[a], Michael Sela[b], and Bilha Schechter[b], Departments of Structural Chemistry[a] and Chemical Immunology[b], The Weizmann Institute of Science, Rehovot 76100, Israel

We apply 500Mhz photoCIDNP measurements to elucidate side chain interactions responsible for the immunogenic diversity of the synthetic multichain copolymers Tyr1Tyr2Glu3Glu4[polyDL-Ala-poly-L-Lys], A, and Tyr1Glu2Tyr3Glu4[polyDL-Ala poly-L-Lys],B. Previous investigations (see e.g. Schwartz, Geiger, Hooghe, Bar Eli, Gallily, Mozes and Sela, Immunology 1979, 3, 389) show that A and B differ remarkably in their immunogenic attributes (T cell activation, macrophage binding and uptake, and MHC control of response), despite their far-reaching structural similarity and identity in the Ala-Lys chain. PhotoCIDNP Tyr signals of 3,5 ring protons indicate widely different correlation times for the Tyr rings in A and B. In A, Tyr1 and Tyr2 ring are free and rotate fast, while in B Tyr1 and Tyr3 give rise to one broad signal due to a strong Tyr1-Tyr3 hydrophobic interaction. Protein modelling suggests a preference for conformations deduced from photoCIDNP. CIDNP effects in free B tetrapeptide establish the presence of Tyr3-Glu4 (α-carboxylate) hydrogen bonding, replacing Tyr3-Ala hydrophobic interaction in the B-copolymer.

CG 319 CONFORMATIONS OF WILD TYPE AND MUTANT OmpA SIGNAL SEQUENCES IN SODIUM DODECYL SULFATE MICELLES BY NUCLEAR MAGNETIC RESONANCE. Josep Rizo, Francisco J. Blanco, Bostjan Kobe and Lila M. Gierasch; Departments of Pharmacology and Biochemistry, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235.

Isolated signal peptides show a high propensity to adopt α-helical conformations in membrane-like environments. This property seems to be necessary but not sufficient for biological function, and the signal sequence hydrophobicity also appears to influence its activity (McKnight et al. [1989], *J. Biol. Chem.* **264**, 17293-17297). We have studied synthetic peptides corresponding to wild type and mutant signal sequences of the OmpA protein of *E. coli* by one and two-dimensional NMR in perdeuterated sodium dodecyl sulfate micelles, to characterize in detail their conformational behavior in this membrane-mimetic environment and map their association with the micelles. An α-helix extended along most of the sequence is observed in the peptides. The helix is in general more stable in the functional peptides, especially in the hydrophobic core. Substantial broadening of the NMR signals of the functional peptides is observed, which indicates an intimate association with the micelles. The deuterium exchange rates of the amide protons of functional peptides are much smaller than the ones observed for peptides lying on the micelle surface. Some amides in the hydrophobic core remain protonated after 54 hours of exchange, suggesting a deep insertion of this part of the molecule into the micelles, with both termini more close to the surface. The implications of these observations for current models of protein secretion will be discussed.

CG 320 COMPARISON OF ANTIMICROBIAL PEPTIDE STRUCTURE TO AMIDE EXCHANGE KINETICS, Jack J. Skalicky, Michael. E. Selsted, Arthur Pardi, Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309-0215.

Defensins are a family of small, cationic peptides with antimicrobial activity against bacteria, fungi, and enveloped viruses. The peptides are found in macrophages and neutrophils. Antimicrobial activity appears to target the membrane and may involve ion channel formation. We present hydrogen exchange kinetics and temperature dependence of amide chemical shifts for three defensin peptides: NP-2, NP-5, and HNP-1. The structures of the peptides have been previously determined. Amide hydrogen exchange rate constants have been determined over a range of pH and temperature values for the slowly exchanging amides ($k_{ex}<1.0x10^{-2}$ min^{-1}) observed in these structurally related peptides. Nine slowly exchanging amides are at positions common to all three peptides. These and other slow exchanging amides are found in β-sheet structure, β-turns, and possibly in intermolecular contacts. Amide retardation factors (k_{ch}/k_{ex}) have been calculated and a clear relationship exists between the retardation of an amide exchange rate and its position in the secondary structure. The relationship between these additional NMR parameters and the 3-dimensional structures will be discussed.

CG 321 STRUCTURAL ANALYSIS OF THE GCN4 TRANSCRIPTIONAL ACTIVATION CORE REGION, Michael Van Hoy, Andrew Hansen, and Thomas Kodadek, Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, TX 78712.

Many genes in eucaryotes are regulated by transcriptional activation proteins. Several of these transcriptional activators contain discreet DNA-binding and activation domains required for proper activity. One type of activation domain is characterized by the great abundance of acidic residues it contains. Indirect experiments have suggested these acidic activation domains can adopt the conformation of amphipathic α-helices. GCN4, a yeast transcriptional activator protein, contains one such acidic activation domain. It has been shown that a 19 amino acid core region of the GCN4 domain can stimulate transcriptional activation when fused to a heterologous DNA-binding domain. We have used CD as well as ^{1}H-NMR spectroscopy to examine the structure of this polypeptide in solution.

CG 322 ^{1}H NMR STRUCTURE DETERMINATION OF GIANT RAGWEED ALLERGEN 5 (*Amb. t.* V) G.L. Warren*, K. Beshah#, L. Goodfriend+, G.A. Petsko%, L.J. Neuringer#.*Dept. of Chemistry & Francis Bitter Nat'l Magnet Lab, MIT, Cambridge, MA, 01239, +Royal Victoria Hospital, McGill University, Montreal, Canada,H3A 1A1, %Rosenstiel Lab, Brandeis University, Waltham, MA, 02254

Among the many foreign molecules recognized and dealt with by the immune system, there are a few substances, otherwise harmless, which prompt a deleterious hypersensitive response known as allergy. *Amb. t.*V (Ra5G), a small disulfide rich protein, elicits an allergic response in a small human population. A three dimensional structure has been determined for *Amb. t.*V at two temperatures, 10 and 35°C, using two-dimensional NMR spectroscopy. Three different upper-limit distance categories were used in the the structure calculations. These were derived from data obtained using at least six NOESY mix times. The structure calculations were done using distance geometry (DSPACE) and restrained molecular dynamics (XPLOR). From these structures we have been able to determine the disulfide bonding pattern, which heretofore has not been determined chemically, and have shown there is no well-defined secondary structure. The structure determined at 10° C seems to be more ordered than the one at 35°C. *Amb. t.*V may be an example of structural resolution being limited by motional disorder.

CG 323 NMR STUDIES OF LOOP E FROM 5S RIBOSOMAL RNA, B. Wimberly, G. Varani and I. Tinoco, Jr., Department of Chemistry and Laboratory of Chemical Biodynamics, University of California, Berkeley, CA 94720

Loop E from the Xenopus laevis 5S ribosomal RNA (5S rRNA) is part of the binding sites for the regulatory protein TFIIIA and ribosomal protein L25. As an asymmetric internal loop, it can adopt many different mismatched conformations. The structure of loop E in intact 5S rRNA has been probed using chemical modification and enzymatic digestion, but the base-pairing remains obscure. One- and two-dimensional NMR were used to investigate the solution structure of a 27-nucleotide duplex containing the X. laevis 5S rRNA loop E sequence. A combination of NOESY, correlated experiments, and selective deuteriation were used to assign most nonexchangeable proton resonances. Interproton distances and scalar couplings were used to determine sugar puckers and glycosidic torsion angles. Exchangeable and nonexchangeable proton spectra indicate the formation of two helical stems separated by a closed internal loop containing mismatched base pairs (including A•A and U•U pairs). Base stacking is conserved in the loop except for one G, which is bulged out. The preliminary structure is generally consistent with the patterns of chemical modification and enzymatic digestion observed for loop E in intact 5S rRNA. Comparison of this structure with that of a very similar internal loop (Varani et al., Biochemistry 28, 7760) shows that the structure of an internal loop can be dramatically changed by the addition of a single loop nucleotide.

Large Proteins/Isotopic Labeling/Dynamics

CG 400 ROLE OF IONIZABLE GROUPS IN THE STABILITY OF THE FOLDED STATE OF T4 LYSOZYME, D. Eric Anderson, Amy Roth, and F.W. Dahlquist, Institute of Molecular Biology, University of Oregon, Eugene, OR 974093

The equilibrium between the unfolded and folded forms of T4 lysozyme is highly pH dependent. In 200 mM KCl and 20°C, the folded state is maximally stable near pH .5. At pH 1.0 this stability is lowered by about 10 kcal/mole. This pH dependent stability is due to specific interactions of several acidic residues in the folded state. We have used nuclear magnetic resonance techniques to measure the pKa values of all the carboxyl groups of T4 lysozyme. The changes in pKa value between the folded and unfolded states reflect the energetics of the specific interactions of the individual carboxyl groups in the protonated versus the unprotonated state. We have used this approach to dissect the contributions made by each acidic residue to the stability of the folded state. The salt bridge between Asp70 and His31 formed in the folded state is responsible for about 4 kcal/mole of the stabilization at pKa so this is reflected in substantial shifts in the pKa values of Asp70 and His31 from their noninteracting values. Three other acid residues have specific interactions in the folded state which contribute about 4 kcal/mole to the stability of the folded state. These include Asp47 (H-bonded to the NH of residue 54) Asp62 (salt bridge with Arg95), and Asp92 (capping a helix and in a salt bridge with Arg95).

CG 401 BACKBONE DYNAMICS OF CALMODULIN AS STUDIED BY ^{15}N INVERSE DETECTED NMR, Gaetano Barbato, Lewis E. Kay, Mitsuhiko Ikura, Ad Bax, Laboratory of Chemical Physics, NIDDK, NIH, Bethesda, MD 20892

The measurement of the backbone ^{15}N relaxation properties of the calcium binding protein calmodulin provides an experimental approach for the study of the local mobility in this protein. Existing inverse detected pulse sequences for the measurement of heteronuclear Overhauser effect (NOE), spin lattice (T1), and spin spin (T2) ^{15}N relaxation times (1) were modified to permit recording of the spectra without water presaturation. This enables the measurement of ^{15}N relaxation parameters with high sensitivity even in the presence of moderate hydrogen exchange rates. T1, T2 and NOE values were obtained for 123 of the 148 total backbone amide nitrogens. Order parameters S^2 and effective correlation times (2) describing the motion of the backbone have been obtained as a function of backbone position, and provide a detailed picture of the dynamics of this protein, including the controversial "central helix".

1. L.E. Kay, D.A. Torchia, A. Bax, Biochem. 1989, 28, 8972

2. G. Lipari, A. Szabo, J. Am. Chem. Soc., 1982, 104, 4546

CG 402 FUNCTIONAL IMPLICATIONS RESULTING FROM DISRUPTION OF THE CALCIUM BINDING LOOP IN BOVINE α-LACTALBUMIN. L. J. Berliner, Yukihiko Hirai, Dore C. Meinholtz, Giovanni Musci and Marvin P. Thompson, Department of Chemistry, The Ohio State University, 120 W. 18th Avenue, Columbus, OH 43210, and the Eastern Regional Research Center, U. S. Department of Agriculture, Philadelphia, PA 19118.

The strong calcium binding site of α-lactalbumin is comprised of the carboxylate side chains of Asp 82, 87 and 88 and the carbonyl oxygens of residues 79 and 84. A single Met residue was selectively modified by controlled CNBr cleavage to yield homoserine at position 90.

CNBr cleaved (Met 90) α-lactalbumin lost the ability to bind calcium as monitored by intrinsic fluorescence, fluorescence labeling, electrophoretic mobility, atomic absorption and x-ray fluorescence measurements. Fluorescence, CD, and CIDNP-NMR evidence showed that the CNBr cleaved protein maintained a conformation which was similar to that of the molten globule apo-protein. Further, the V_{max} for lactose biosynthesis was severely reduced. This work was supported in part by grant from the USPHS (GM/HD 17270) and the National Science Foundation (DMB 8703794).

CG 403 ^{1}H-^{15}N, ^{1}H-^{13}C AND ^{1}H-^{15}N-^{13}C DOUBLE- AND TRIPLE-RESONANCE NMR STUDIES OF GRANULOCYTE COLONY-STIMULATING FACTOR. *A.L. Breeze, J. Boyd, N. Soffe, A.J. Wilkinson* and I.D. Campbell, Department of Biochemistry, University of Oxford, Oxford OX1 3QU, U.K., and *ICI Pharmaceuticals, Mereside, Alderley Park, Cheshire SK10 4TG, U.K.*

The proliferation and differentiation of bone marrow stem cells into mature neutrophilic granulocytes or macrophages is regulated by protein growth factors known collectively as the colony-stimulating factors. One such, granulocyte colony-stimulating factor (G-CSF), is currently attracting interest as a potential therapeutic agent in cases of immune suppression. However, there is to date a paucity of information concerning the location and architecture of receptor-binding regions for G-CSF, not least because of the absence of high-resolution structural information for any of the colony-stimulating factors. We are at present engaged in a study of G-CSF in solution by NMR. Our approach to achieving sequential resonance assignments for G-CSF (174 amino acid residues; 18.7 kDa) has been to adopt the strategy of uniform isotopic labelling with ^{15}N and $^{15}N/^{13}C$ in conjunction with the recording of a series of ^{1}H-^{15}N, ^{1}H-^{13}C and ^{1}H-^{15}N-^{13}C double- and triple-resonance 3-dimensional experiments; this approach should also facilitate extraction of the experimental constraints necessary for definition of the structure of G-CSF. The results of these studies will be presented.

CG 404 RESONANCE ASSIGNMENTS OF Ras-p21 PROTEINS USING HETERONUCLEAR TWO AND THREE-DIMENSIONAL NMR. Sharon Campbell-Burk and Peter Domaille, Central Research and Development Department, E. I. du Pont de Nemours and Company, Wilmington, DE 19880-0328, Luciano Mueller, Bristol Meyers Squibb Corporation, Princeton, NJ 08543.

Ras-p21 is the 21 kD protein product of the ras oncogene; the most prevalent oncogene family found in human cancer. Our efforts to assign the ^{1}H NMR spectrum of ras-p21 has proven difficult. This is probably due to the tendency of ras-p21 to dimerize at concentrations required for NMR analysis. We have recently applied 3 dimensional isotope-directed NMR techniques on uniformly enriched ^{15}N, [^{15}N, ^{13}C], and [^{13}C, ^{2}H] ras-p21 proteins. Intraresidue and sequential resonance assignments are in progress. Results will be presented.

CG 405 COMPARATIVE NMR STUDIES OF INTRACELLULAR LIPID BINDING PROTEINS David P. Cistola, James J. Toner, Jr, Katherine R. Miller, Jeffrey I. Gordon, and William C. Hutton, Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO 63110 and Physical Sciences Center, Monsanto Corporation, Chesterfield, MO 63198

Absorptive enterocytes in the small intestine abundantly express three homologous lipid binding proteins that appear to serve distinct functional roles in the absorption and transport of fatty acids, bile salts, and cholesterol. Comparative studies of these homologues provide an excellent opportunity to elucidate the structural basis for the apparent functional differentiation within this family of transport proteins. We have developed a three-faceted NMR strategy to determine and compare their binding, transport, and conformational properties in solution. First, site- and residue-specific labelling and 1D and 2D hetero-correlated NMR are used to monitor binding parameters such as stoichiometries, specificities, affinities, and mechanisms. Second, site-specific labelling and wide-line ^{2}H NMR are used to monitor the thermodynamic, kinetic, and structural mechanisms governing the protein-mediated transfer of lipids from membranes to organelles. Third, uniform ^{13}C and ^{15}N enrichment and isotope-edited 2D and 3D NMR methods are used to generate sequence-specific resonance assignments for conformational studies. To facilitate the NMR studies, the cDNAs for all three homologues have been cloned and introduced into plasmid vectors for expression in *E. coli*, yielding gram quantities of purified proteins from small volume fermentations. The comparative NMR results will be discussed in the context of regional physiology within the intestine.

CG 406 IMPROVED MULTIPLET EDITING OF ^{1}H - DETECTED, NATURAL ABUNDANCE ^{13}C NMR CORRELATION SPECTRA OF PROTEINS, Donald G. Davis, Laboratory of Molecular Biophysics, National Institute of Environmental Health Sciences, PO Box 12233, Research Triangle Park,NC 27709.

Two dimensional, proton- heteronuclear correlation spectra of proteins can be quite complicated and difficult to assign even for proteins of modest molecular weights. Some of the strategies that have been adopted to simplify such spectra involve site-specific isotopic labeling as well as 3- dimensional correlation methods. An alternative method for simplifying spectra is to label the cross peaks in terms of the multiplicity of the heteronuclear resonance. Although several editing schemes involving either single quantum (SQ) or multiquantum (MQ) coherences have been proposed, these methods all have drawbacks that limit their sensitivity. Here, new multiplet editing sequences that avoid these limitations are described.By using sequences in which the rare or low-gamma isotope (S-spin) chemical shift is encoded in antiphase single-quantum states of the form: $I_zS_{x,y}$, the transfer efficiency of multiplet labeled magnetization from the S-spins to protons (which limits the in-phase SQ methods), is made independent of the multiplicity of S, and inhomogeneous broadening of F_1 domain spectra by passive proton-proton scalar interactions (which limits the MQ schemes), is also eliminated. These properties are particularly advantageous when using editing methods to find and identify cross peaks from methylene groups in amino acid side chains of peptides and proteins. Examples of the simplification and partial assignment of multiplet edited ^{1}H-{^{13}C} natural abundance correlation spectra for some proteins are shown.

CG 407 NMR STUDIES OF PHOSPHOLIPASE A_2-INHIBITOR-MICELLE COMPLEXES.
N.Dekker[1], A.R. Peters[2], A.J. Slotboom[1], R. Boelens[2], R. Kaptein[2], R. Dijkman[1], and G.H. de Haas[1] [1]Center for Biomembranes and Lipid Enzymology, [2]Bijvoet Center for Biomolecular Research State University of Utrecht,P.O. Box 80.054,3584 CH Utrecht, The Netherlands

Recently a class of substrate-analogs have been synthesized which display good inhibitory properties towards porcine pancreatic phospholipase A_2 (PLA). These inhibitors have a 2-amide instead of a 2-ester linkage in the phospholipid structure. From kinetic studies it appeared that these phospholipid-analogs are effective inhibitors of the enzyme *only* after incorporation into an organized lipid-water interface. NMR was used to study the 38 kD enzyme-inhibitor complex bound to micelles of fully deuterated dodecylphosphocholine. It was found that (R)-dodecyl-2-amino-hexanol-1-phosphoglycol binds stoichiometrically with a high affinity to the active site of PLA in the enzyme-micelle complex, and that this binding is highly stereospecific.
Using selectively deuterated inhibitors a number of intermolecular NOEs have been assigned to interacting parts of the inhibitor and the enzyme. These experiments allowed us to localize at an atomic level the regions in the enzyme where the inhibitor is bound.
References:
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J. Fisher, W.U. Primrose, G.C.K. Roberts, N. Dekker, R. Boelens, R. Kaptein, and A.J. Slotboom (1989) *Biochem* 28, 5939-5946.
G.H. de Haas, R. Dijkman, M.G. van Oort, and R. Verger (1990) *Biochim. Biophys. Acta* 70, 538-553.

CG 408 PSEUDO 4-CHANNEL TRIPLE RESONANCE: MINIMIZING THE HARDWARDE DEMANDS, B. T. Farmer II, R&D Department, NMR Instruments, Varian Associates, 3120 Hansen Way, Palo Alto, CA 94304

The recent 3D triple resonance experiments of Bax have relied heavily on 4 independent RF channels. In most cases, it can be shown that 3 independent RF channels, one of which can be rapidly switched between 2 independent frequency sources, are sufficient. An experimental setup to accomplish this rapid switching will be described. Pulse sequences modified to suit this experimental setup and results from several pseudo 4-channel triple resonance experiments on a [^{13}C, ^{15}N] doubly labeled protein will be presented.

CG 409 NMR STUDIES ON CYCLOSPORIN A BOUND TO CYCLOPHILIN

Gerd Gemmecker, Robert T. Gampe, Jr., Edward T. Olejniczak, Hugh L. Eaton, Placido Neri, and Stephen W. Fesik. Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL 60064.

The immunosuppressive drug cyclosporin A has been shown to bind with high specifity to cyclophilin, an 18 kD protein with peptidyl-prolyl isomerase activity. We have applied a variety of multidimensional NMR techniques to investigate the 3D structure of the cyclosporin A / cyclophilin complex. Resolution problems in 2D NMR spectra of this system can be overcome by adding additional heteronuclear dimensions to these NMR experiments, and by the use of ^{13}C and ^{15}N isotope labeling of either the ligand or the protein. NMR techniques are presented that not only facilitate signal assignment in protein / ligand complexes, but also allow the extraction of conformationally relevant parameters such as NOE effects and scalar coupling constants.

CG 410 IN VIVO NMR STUDIES OF THE NEMATODE LONGIDORUS ELONGATUS, Bernard A. Goodman and Walter M. Robertson, Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, Scotland and John A. Chudek, Department of Chemistry, University of Dundee, Dundee DD1 4HN, Scotland.

Longidorus elongatus is a member of the family Dorylaimida and is probably one of the most important migratory plant parasitic nematodes in Great Britain. Apart from direct damage to plants it is also responsible for the transmission of raspberry ringspot and tomato black ring viruses. The first stages of a detailed in vivo NMR investigation of this nematode are described in the present paper. Conventional ^{13}C measurements reveal four principal mobile components; glucose, trehalose, a triglyceride and a C-18 monounsaturated fatty acid. Seasonal variations in composition are observed and these are reported along with experiments on the metabolism of labelled substrates.

CG 411 AMIDE PROTON EXCHANGE IN REDUCED AND OXIDIZED *RHODOBACTER CAPSULATUS* CYTOCHROME c_2 BY ^1H-^{15}N NMR SPECTROSCOPY, Paul R. Gooley*, Dezheng Zhao and Neil E. MacKenzie. Department of Pharmeuceutical Sciences, University of Arizona, Tucson, AZ 85721. *Present address: Merck, Sharpe and Dohme Research Laboratoties, Rahway, NJ 07065.

The hydrogen-deuterium exchange rates of the reduced and oxidized forms of *Rhodobacter capsulatus* cytochrome c_2 were studied by ^1H-^{15}N NMR spectroscopy. Minimal differences were observed for the N- and C-terminal helicies suggesting that these helicies are structurally important but do not control the redox potential differences observed amongst the cytochromes c. Significant differences were observed for other regions of the protein. As all slow exchanging protons of the helix spanning Phe-82 to Asp-87 are similarly effected, the unfolding equilibrium, but not the structure of this helix is altered between the two redox states. Other regions are not as simple to interpret, however, the difference in NH exchange rates between the redox states for a number of residues including His-17, Leu-37, Arg-43, Ala-45, Gly-46, Ile-57, Val-58, Leu-60, Gly-61 and Leu-100 suggest that interactions effecting the exchange rates of these residues may be important factors in determining redox potential.

CG 412 DETERMINATION OF THE TERTIARY STRUCTURE OF A LIPOYL DOMAIN FROM THE E.COLI PYRUVATE DEHYDROGENASE MULTIENZYME COMPLEX

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The dihydrolipoamide acetyltransferase (E2) subunit plays a central role in the operation of the pyruvate dehydrogenase multienzyme complex. As well as containing the acetyltransferase active site, the C-terminal half of the protein also aggregates with octahedral symmetry to form the inner core of the complex, around which the E1 and E3 subunits bind. The N-terminal part of E2 protrudes from the central core as a number of separately folded domains, interdigitating between the E1 and E3 subunits. The N-terminal domain of E2 carries the lipoic acid cofactor involved in acetyl group transfer. Movement between the different active sites is thought to be facilitated by a short, flexible, interdomain region of 20-30 residues (for review, Perham & Packman, 1989). Although the number of 'lipoyl' domains per E2 chain varies between 1 and 3 according to the species, they share similarities in primary structure which may explain some characteristic properties. SDS-PAGE, sedimentation equilibrium and gel filtration all predict different values for the *Mr* of the excised domain. A high net negative charge and an unusual elongated shape may be responsible. With the construction of subclones over-expressing a single lipoyl domain from the *B.stearothermophilus* (Dardel *et.al.*, 1990) and *E.coli* pyruvate dehydrogenase complexes (the latter kindly provided by Ali & Guest, 1990), a detailed study by NMR has now been possible. We report here the assignment and 3-D structure of the *E.coli* domain. A comparison with the equivalent domain from the *B.stearothermophilus* pyruvate dehydrogenase complex (Dardel *et.al.*, in press) suggests an unusual structure common to all lipoyl domains.

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CG 413 THE STRUCTURE AND DYNAMICS OF HUMAN EPIDERMAL GROWTH FACTOR

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The structure of human epidermal growth factor (hEGF), residues 1-48, has been previously reported (Cooke *et al.* 1987). We present a higher resolution structure of the whole molecule (1-53) which is the product of continued research into structure-function relationships in growth factors in this lab. This more accurate structure allows a detailed analysis of the spatial distribution of residues thought to be important in receptor recognition and binding (see the accompanying poster of Hommel *et al.*). The dynamics of hEGF have been studied experimentally through measurement of ^{15}N relaxation parameters. Stochastic dynamics simulations are being calculated using the improved 3-dimensional structure, and the dynamic properties derived from these trajectories will be compared with the experimental relaxation data.

Cooke et al (1987) *Nature* **327**, 339-341,

CG 414 *CIS:TRANS* PROLINE CONFORMERS OF STAPHYLOCOCCAL NUCLEASE AS STUDIED BY AN NMR DOUBLE ISOTOPE EDITING APPROACH, Andrew P. Hinck and John L. Markley,

Biochemistry Department, University of Wisconsin-Madison, 420 Henry Mall, Madison, WI 53706.

Two of the six proline residues of folded staphylococcal nuclease H124L (a recombinant protein produced in *E. coli* whose sequence is identical to nuclease A isolated from the V8 strain of *S. aureus*) have been shown to exist as equilibrium mixtures of *cis* and *trans* isomers (1,2). The configuration about the Lys^{116}-Pro^{117} peptide bond is 95% *cis*, whereas that about the His^{46}-Pro^{47} peptide bond is 85% *trans*. Previous NMR results have demonstrated that the *cis* and *trans* configurations can be recognized on the basis of unique ^1H-^1H NOEs. Because the region of the NOESY spectrum that contains this information is crowded, we are using isotope labeling to filter out the NOEs of interest. The only Lys-Pro sequence is at residues 116-117. We have synthesized and incorporated selectively ^{13}C labeled Lys and Pro into nuclease H124L and a mutant that has an altered *cis/trans* ratio. The *cis* configuration is being probed by incorporation of [$^{13}C^{\alpha}$]Lys and [$^{13}C^{\alpha}$]Pro, and the *trans* configuration is being probed by incorporation of [$^{13}C^{\alpha}$]Lys and [$^{13}C^{\beta,\gamma}$]Pro. A ^{13}C half-filter is used in a standard two-dimensional ^1H-^1H NOESY pulse sequence to suppress signals from protons not attached to ^{13}C.

1. J. Wang, A.P. Hinck, S.N. Loh, and J. L. Markley, *Biochemistry* **29**, 4242-4253 (1990).
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[Supported by NIH grant GM35976. A.P.H. is a trainee on Molecular Biophysics Training Grant GM08293.]

CG 415 MULTI-NUCLEAR MULTI-DIMENSIONAL NMR STUDY ON CALMODULIN AND ITS COMPLEX WITH A MYOSIN LIGHT-CHAIN KINASE FRAGMENT: Mitsuhiko Ikura, Silvia Spera, Gaetano Barbato, Lewis Kay, Guang Zhu, Marius Clore, Angela Gronenborn, and Ad Bax, Laboratory of Chemical Physics, NIDDK, NIH, Bethesda, MD 20892

Novel heteronuclear [^{1}H,^{13}C,^{15}N] triple-resonance 3D and 4D-NMR techniques, recently developed in our laboratory, have permitted us to make complete backbone and side-chain assignments for Ca^{2+}-loaded calmodulin (CaM) in the absence and presence of a 26-residue peptide derived from skeletal muscle myosin light-chain kinase (M13). The binding constant of CaM and M13 is an order of 10^{-9} M. In the absence of M13, the solution conformation is quite similar to the X-ray crystal structure for both N- and C-terminal globular domains. However, in contrast to the crystal structure, the "central helix" portion has a flexible kink at residues 77-81. The NMR structure includes a tightly bound water molecule near this region, that causes the helix to bend and stabilizes the "bent" form of the helix. In the complex with M13, substantial changes in ^{1}H, ^{13}C, and ^{15}N chemical shifts of the backbone atoms occur throughout the entire CaM molecule, although these changes are most distinct in three helices of E_I, F_{II}, and F_{IV} in the "EF-hand" Ca^{2+}-binding motifs. Preliminary NOE analysis indicates that all secondary structural elements, observed in the absence of M13, are preserved in the complex, except that a distortion of α-helix is observed for residues 74-77. Hydrogen exchange experiments indicate that amide protons of residues 110-114 are significantly protected from solvent upon the formation of complex with M13.

CG 416 MULTI-DIMENSIONAL NMR OF ISOTOPICALLY LABELED CARDIAC TROPONIN C, George A. Krudy, Rui M. M. Brito, John A. Putkey and Paul R. Rosevear, Dept. Biochemistry and Molecular Biology, Uni. of Texas Med. School at Houston, Houston, TX 77225.

Cardiac TnC has a molecular weight of 18.4 kDa and three functional helix-loop-helix Ca^{2+} binding sites. The critical role for cTnC in muscle contraction emphasizes the need to understand its solution structure, dynamics, and calcium induced conformational changes. Toward these goals, uniformly and selectively ^{15}N-enriched recombinant cTnC proteins have been prepared to aid in backbone resonance assignment by NMR. Two dimensional HMQC spectra of uniformly ^{15}N-enriched cTnC permitted detection of at least 159 of the 176 expected ^{15}N-^{1}H correlations. Analysis of the HMQC-TOCSY spectrum allowed identification of at least 140 of the expected 168 correlations in the fingerprint region. Selective ^{15}N labeling with [^{15}N]Gly was used to unequivocally identify the 12 Gly and 4 Ser ^{15}N-^{1}H connectivities. Both NOESY and HMQC-NOESY spectra were used to identify short NH(i)-NH(i+1) connectivities as well as inter-residue NOEs between downfield shifted CαH resonances and NH and aromatic resonances. The ability to identify unique patterns of NOE connectivities permitted sequence specific backbone assignments of calcium binding loops II and III. As a consequence of these assignments, β-sheet structures formed between helix-loop-helix Ca^{2+} binding domains could be studied both in the presence and the absence of calcium.

CG 417 DYNAMIC STUDIES OF THREE MUTANTS OF STAPHYLOCOCCAL NUCLEASE BY TWO-DIMENSIONAL ^{1}H DETECTED ^{15}N NMR SPECTROSCOPY, Stewart N. Loh and John L. Markley Department of Biochemistry, University of Wisconsin-Madison, Madison, WI 53706.

Staphylococcal nuclease H124L is a naturally occuring variant differing from the wild type enzyme by a Leu in place of His^{124}. In solution, it exhibits four native conformational substates that are linked through cis/trans isomerism at two proline residues, Pro^{117} and Pro^{47}. The isomerism about the Lys^{116}-Pro^{117} bond is manifested by the doubling of many NMR resonances, indicating that conformational effects of this isomerism are propagated throughout the molecule. In contrast, cis/trans heterogeneity at Pro^{47} has only been detected by splitting of the His^{46} spin system. Pro^{47} is contained in a large solvent-exposed loop (amino acids 42-50), which, owing to weak electron density and large temperature factors, was found to be flexible and/or disordered in the X-ray crystal structure of wild type nuclease. In solution, the unusual dynamics of the loop are borne out by broad NMR linewidths and the lack of resonance assignments within this region of the protein. Previous studies of ^{15}N relaxation times (Kay, L.E., Torchia, D.A. & Bax, A. *Biochemistry* **1989**, *28*, 8972) indicated that conformational averaging, with exchange on the millisecond timescale, is responsible for the observed line broadening. Here we report ^{15}N T_2 relaxation measurements of nuclease H124L as well as two mutants in which Pro^{42} and Pro^{47} have been replaced by Gly. Both proline mutants display sharp His^{46} $^{1}H^{\varepsilon 1}$ signals in contrast to the broad peak exhibited by nuclease H124L. We will discuss potential sources of flexibility of the loop, including the role of Pro^{47} and the possibility of a local multiple state conformational equilibrium involving Pro^{42} and Pro^{47}. Supported by NIH grant GM35976. S.N.L. is funded by a Training Grant in Cellular and Molecular Biology (NIH GM07215).

CG 418 ATTEMPTING THE BACKBONE ASSIGNMENTS OF ^{15}N-ENRICHED SUBTILISIN (27 kDa),

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Subtilisin consists of more than 260 amino acids. The conventional 2D-NMR-approach to assigning the backbone of a protein of this size is impossible. Hetero-3D-NMR-techniques such as NOESY-SQC, TOCSY-SQC and SQC-NOESY-SQC have been employed to obtain information on the backbone. LPSVD is used to increase the resolution in the ^{15}N-dimension.

CG 419 CONFORMATIONAL STUDIES OF BACTERIAL CHEMOSENSORY PROTEINS. A ^{19}F NMR APPROACH. Linda A. Luck and Joseph J. Falke, Department of Chemistry and Biochemistry, University of Colorado, Boulder, Colorado 80309-0215.

Conformational changes play an important role in sensory and signaling proteins. These changes regulate the interaction between an activated protein and its target protein or nucleic acid. ^{19}F NMR can be used to probe these conformational changes in proteins too large for NMR structural determination. Presented in this poster will be a study of two chemosensory proteins of the E.coli chemotaxis sytem: the periplasmic D-galactose and D-glucose receptor (GGR) and the cytoplasmic "switch" protein (CheY) which regulates the rotational sense of the flagellar motor. The crystal structure of the GGR is known and reveals a sugar binding site between two domains as well as a Ca(II) binding site closely related to the eukaryotic EF-hand sites. A conformational change takes place upon sugar binding which enables the receptor to dock to membrane transducer and transport proteins. Our ^{19}F NMR studies, which incorporate fluorine into the phe and trp sites in the receptor, indicate that a global structural change involving spatial shifts of alpha helices and beta strand elements takes place upon binding of sugar. Using an aqueous paramagnetic broadening agent, we have been able to better define this global conformational change. Our results suggest that the cleft is in an open conformation (cleft angle >15^{o}) when the sugar site is empty. In contrast, Ca(II) binding induces only a localized conformational change in the protein. Studies of the phosphorylated and dephosphorylated conformations of CheY will also be presented.

CG 420 NMR STUDIES OF THE EFFECTS OF AMINO ACID SUBSTITUTIONS ON THE STRUCTURE AND DYNAMICS OF BACTERIOPHAGE T4 LYSOZYME, Lawrence P. McIntosh, D. Eric Anderson, Jirong Lu and Frederick W. Dahlquist, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

The goal of our research is to characterize the relationships between the amino acid sequence of bacteriophage T4 lysozyme and the properties of its folded (and unfolded) conformations. We have completely identified the backbone ^{1}H and ^{15}N resonances of isotopically enriched T4 lysozyme by two- and three-dimensional heteronuclear NMR methods. Using amide hydrogen exchange to probe the static and dynamic solvent accessibility of the wild type protein, we found that the amide protons in three α-helices (residues 5-11, 95-104, and 149-154) are highly protected from exchange. These helices contact each other, forming a stable hydrophobic core in T4 lysozyme.

Currently we are assigning the ^{1}H-^{15}N HSMQC spectra of several T4 lysozyme variants including the temperature sensitive proteins D70N, A98V, and A146T, and the double mutant T21C/T142C. The latter protein is stabilized in the oxidized state due to a disulphide bridge across its active site cleft. We are mapping the structural and dynamic effects of these mutations on the backbone of T4 lysozyme by chemical shift , NOE, and hydrogen exchange measurements. For example, the substitution of Ala146 by Thr (A146T) reduces the thermal stability of T4 lysozyme ($\Delta\Delta G$ = 2 kcal/mol at pH 2). The introduction of the bulkier threonine sidechain disrupts the static packing in the hydrophobic interior of the protein, as shown by crystallographic analysis (B.W. Matthews *et al.*). This sidechain replacement causes residues 137-150, which form two α-helices, to interconvert between NMR-distinct conformations on the millisecond timescale. Also, several amide protons near site 146 have accelerated amide exchange kinetics. The dynamically perturbed sites are broadly localized to position 146; however, the effects are more extensive than suggested by the crystallographic analysis of the lysozyme mutant. These results support a model in which destabilizing mutations enhance local fluctuations (entropy) of the native protein.

CG 421 SOLUTION STRUCTURE OF RIBONUCLEASE H FROM *E. COLI* BY 3D NMR AND DISTANCE ANALYSIS

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Ribonuclease H from *E. coli* is an enzyme to cleave the RNA moiety of a RNA-DNA hybrid duplex. It consists of 155 amino acid residues with molecular weight of 17.6kDa. Our way to determine protein structure with NMR consists of 4 steps; 1) assignment of backbone nuclei (^{1}H, ^{13}C and ^{15}N) and secondary structure, 2) assignment of methyl and aromatic nuclei on sidechains, 3) distances between secondary structures and global fold, 4) refinement. To overcome the problem of overlapping of ^{1}H resonances in larger proteins, several kinds of heteronuclear 3D NMR experiments were applied to the protein;
1) ^{15}N NOESY-HMQC, ^{15}N TOCSY-HMQC, ^{13}C HMQC-NOESY, ^{13}C HMQC-TOCSY, ^{15}N (selective) HMQC
2) ^{13}C HSQC, ^{13}C TOCSY-HMQC, ^{13}C NOESY-HMQC and 3) ^{12}C-filtered ^{13}C (selective) HMQC-NOESY
Secondary structure of the protein was determined from the pattern of strength of cross peaks on the 3D TOCSY and NOESY spectra. There were five β-strands and five α-helices. In the spectra of ^{13}C and ^{15}N HMQC-NOESY about 400 NOE cross peaks were assigned and 32 NOE cross peaks between the secondary structures were picked up (those within the β-sheet were not counted). Through the calculation by DADAS90 which was extended from DISMAN to include second derivative minimization and Monte Carlo annealing, the global fold of the protein was determined.

CG 422 AUTOMATIC ASSIGNEMNT OF AMBIGUOUS CROSSPEAKS IN SYMMETRIC DIMERS, Michael Nilges and Axel T. Brünger, The Howard Hughes Medical Institute and Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511

In symmetric dimers, the structure determination by NMR is impeded by the ambiguity of inter-monomer and intra-monomer NOESY crosspeaks. In a molecular dynamics based simulated annealing approach this ambiguity can be treated in a natural and rigorous way. Results obtained with experimental data on a troponin C calcium binding domain (provided by Lewis Kay), and with model data obtained from the X-ray structure of the DNA binding domain of Met repressor will be presented. The calculations started from randomly generated initial structures. *All* NOESY peaks were treated as potentially intra- or inter-monomer, and *no* knowledge of secondary structure was assumed. Most ambiguities could be resolved in the calculations. The method gives a more reliable estimate of the size of the conformational space consistent with the data, and will thus be useful to pinpoint the regions in the molecule where labelling is necessary. It is readily extended to higher order aggregates.

CG 423 APPLICATION OF 3DNMR SPECTROSCOPY TO ^{15}N AND ^{13}C ENRICHED HUMAN DIHYDROFOLATE REDUCTASE, N. R. Nirmala, Brian J. Stockman, Michael De Yarman* , Tavner J. Delcamp*, James H. Freisheim* and Gerhard Wagner, Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 240 Longwood Avenue, Boston MA 02115 and (*)Department of Biochemistry, Medical College of Ohio, Toledo, OH 43699.

Dihydrofolate reductase is the target enzyme for several clinically important drugs, including methotrexate (antitumor) and trimethoprim (antibacterial). Human dihydrofolate reductase consists of a single polypeptide strand with 186 amino acid residues and a molecular weight of approximately 22000. Expression of the enzyme in E.coli has enabled the production of samples labelled with ^{15}N alone and with ^{15}N and ^{13}C. In addition, samples with selective ^{15}N enrichment of the leucine and the valine residues were produced. 3DNMR experiments were performed on the enzyme-methotrexate complex in order to exploit the advantage of spreading the resonances in the heteronuclear dimension to reduce overlap. Triple resonance experiments performed on the double labelled samples further facilitate sequence specific assignment. Over 40% of the backbone has been assigned from the spectra obtained with the ^{15}N-enriched sample alone. Pulse sequences used and the results obtained will be discussed.

CG 424 INTRAMOLECULAR MOTIONS OF A ZINC FINGER DNA-BINDING DOMAIN FROM XFIN CHARACTERIZED BY PROTON-DETECTED NATURAL ABUNDANCE ^{13}C NMR SPECTROSCOPY.
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The zinc finger DNA-binding domain Xfin-31 is a 25-residue peptide that binds a single zinc atom and forms a compact globular structure in solution. The ^{13}C spin-lattice and spin-spin relaxation rates and the $\{^{1}H\}$-^{13}C Nuclear Overhauser Effect (NOE) enhancements have been measured for the backbone and side chain methine carbon atoms by two-dimensional proton-detected ^{13}C NMR spectroscopy at natural ^{13}C abundance. The relaxation rate constants and the NOE enhancements have been analyzed using a model free formalism to characterize the intramolecular motions of Xfin-31. The results show that, with the exception of the terminal residues, the motions of the backbone α-carbon atoms are highly restricted. The two residues at the C-terminus have considerably greater conformational flexibility than the residues at the N-terminus. Residues 10-13, which form a turn between the β-sheet and the helix present in the three-dimensional structure of Xfin-31, have a slightly higher mobility than the remainder of the interior backbone. The side chains of the hydrophobic Val and Leu residues are more mobile than the backbone, but are still significantly restricted, which indicates that Xfin-31 is compact despite its small size. Systematically large spin-spin relaxation rates for residues in the zinc binding site imply that conformational exchange occurs in this region on a time scale longer than the overall rotational correlation time of the molecule.

CG 425 ISOTOPIC LABELING (^{13}C, ^{15}N) OF PROTEINS IN MAMMALIAN CELLS, Andrew M. Petros and Erik R. P. Zuiderweg, Abbott Laboratories, Dept. 47G, AP9, Abbott Park, IL 60064

The determination of solution structures of medium-sized proteins (15-25 kD) by NMR has become possible due to the expression of isotopically labeled proteins. Proteins overexpressed in bacteria can be labeled in a relatively straightforward manner (for a review see [1]). However, not all proteins can be expressed in bacterial systems and need to be obtained from mammaliam cell growth cultures. Labeling of these proteins is not that easy since, unlike bacteria, mammalian cells do not accept labeled salts and glucose as their sole nutrients. We have carried out experiments to better understand the exact growth requirements of CHO (Chinese Hamster Ovary) cells and thus develop a scheme for isotopic labeling of proteins in CHO cells. Furthermore, we have explored the use of algal biomass, which can be obtained both ^{13}C and ^{15}N enriched, at a reasonable cost, as a source of amino acids for preparation of mammalian cell media.

[1]Muchmore *et. al.* (1989) *Meth. Enzyml.* 177, 44-73.

CG 426 RECOGNITION OF SUBSTRATE AND MECHANISM OF ACTION OF PHOSPHOLIPASE A2: USE OF ISOTOPICALLY LABELLED SUBSTRATE ANALOGUES, William U. Primrose, Pritpal K. Slaich, Gordon C. K. Roberts, Colin Benyon[$], Stephen Connolly[$] and David H. Robinson[$], Department of Biochemistry, University of Leicester, Leicester LE1 7RH, U. K. and [$]Fisons plc, Pharmaceuticals Division, R & D Laboratories, Loughborough, LE11 0RH, U. K.

Phospholipases A_2 (PLA_2; EC 3.1.1.4) are a ubiquitous family of calcium-dependent enzymes that catalyse the hydrolysis of the 2-acyl ester bond of phospholipids. Both membrane-associated and secreted forms of PLA_2 are present in, and produced by, cells participating in the inflammatory reaction. PLA_2 catalyses the release of *arachidonic acid from membrane phospholipid*, which is likely to be the rate-limiting step in the production of the metabolites of the arachidonate cascade. These compounds have been implicated in inflammatory and acute allergic responses such as arthritis, psoriasis and asthma. There is a continuing effort worldwide to develop a *specific* inhibitor of PLA_2 as an anti-inflammatory drug.

Recently, the crystal structure of the pancreatic enzyme complexed with a non-hydrolysable substrate analogue has been published (**1**), which has suggested the mode of binding of true substrate. Nevertheless there remain many questions regarding the mechanism of action of the enzyme, its method for binding to its aggregated substrate and how much we can extrapolate findings from the extracellular enzymes to the physiologically important tissue enzyme. We have been using ^{13}C-labelled substrate analogues to investigate binding to this enzyme and the manner by which the enzyme stabilises the transition state intermediate of the reaction. Techniques including standard structural determination by multi-dimensional ^{1}H nmr, directly observed ^{13}C nmr, isotopically edited ^{1}H - ^{1}H NOESY spectra and FTIR are being employed to increase our understanding of the pancreatic enzyme and extend it to the tissue species.

Ref **1** Thunnissen et al., **Nature**, 1990, 347, 689

CG 427 STRUCTURE AND DYNAMICS OF MEMBRANE BOUND FILAMENTOUS BACTERIOPHAGE COAT PROTEINS, Ki-Joon Shon, Patricia McDonnell, Yongae Kim, and Stanley Opella, Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104

The structure and dynamics of the membrane bound forms of the coat proteins from class I (fd, M13) and class II (Pf1) filamentous bacteriophages, as described by NMR spectroscopy, will be presented. Comparisons between class I and class II bacteriophages are of interest because, although both types of viruses have very similar lifecycles and overall structures, there are no apparent sequence homologies in their coat proteins. These coat proteins are typical of membrane proteins in having long membrane spanning hydrophobic helices and smaller amphipathic helices parallel to the plane of the membrane. Both proteins have mobile N- and C- terminal regions, but differ significantly in their internal dynamics.

Three-dimensional NMR experiments on uniformly ^{2}H and ^{15}N labeled samples in micelles are used to determine the residues in the helices. Solid-state NMR experiments on specifically and selectively ^{15}N labeled samples in oriented bilayers are used to determine the orientation of the helices. The dynamics are described by motional averaging of powder pattern lineshapes and relaxation analysis.

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CG 428 **The Solution Structure of Neuronal Bungarotoxin**

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We are interested in understanding the structural features of agents which bind to the nicotinic acetylcholine receptor (nAChR). Of particular interest is the interaction of neuronal bungarotoxin (nBgt, a minor protein component of the venom of (*Bungarus multicinctus*) with the nAChR. Most of the sequence specific ^{1}H NMR assignments have been made for nBgt. On the basis of the observed NOEs, hydrogen exchange data and amide-alpha coupling constants, nBgt has been shown to consist of a three looped structure with a central triple stranded antiparallel β-sheet. The NOEs, in conjunction with the results of sedimentation equilibrium ultracentrifugation, show that nBgt forms a dimer in solution with the interface along one edge of the β-sheet, thereby forming a six stranded intermolecular β-sheet. The paucity of constraints which define the dimer interface has resulted in difficulties when producing the tertiary structure using either distance geometry or simulated annealing. An alternative approach has been adopted which produces model structures based to varying degrees on the homologous α-bungarotoxin (40% amino acid identity). These structures are subsequently used as the starting point for refinement using simulated annealing followed by restrained molecular dynamics.

CG 429 HETERONUCLEAR THREE-DIMENSIONAL NMR SPECTROSCOPY OF UNLIGATED STAPHYLOCOCCAL NUCLEASE H124L, Jinfeng Wang, Ed Mooberry, William F. Walkenhorst, and John L. Markley, Biochemistry Department, University of Wisconsin-Madison, 420 Henry Mall, Madison, WI 53706-1569.

Chemical shift and NOE changes occur in spectra of the staphylococcal nuclease upon binding calcium ion (required for catalytic activity) and 3',5' thymidine bisphosphate (pdTp, an inhibitor that binds at the active site). Extensive assignments have been published for the nuclease H124L·Ca^{2+}·pdTp ternary complex (1,2). To interpret the spectral changes induced by Ca^{2+} and pdTp binding, it was necessary to assign the spectrum of unligated nuclease H124L. Protein labeled uniformly with 95% ^{15}N was analyzed by 3D $^{1}H\{^{15}N,^{1}H\}$ nuclear Overhauser-multiple quantum coherence spectroscopy (NOESY-HMQC) on a Bruker AM600 spectrometer. Characteristic amide proton NOE connectivity patterns provided the sequential assignments. NOESY-HMQC data also provided connectivity patterns that were diagnostic for secondary structure. In α-helical domains, the $^{1}H^{N}$-$^{1}H^{N}$ sequential pathway shows a number of rectangles in series without any sequential overlap. In antiparallel β-sheet domains the $^{1}H^{N}$-$^{1}H^{N}$ NOE connectivities between adjacent strands form a number of discrete rectangles, with one rectangle covering another. Structural changes that accompany binding of Ca^{2+}, pdTp, and both ligands will be discussed.

1. J. Wang, D.M. LeMaster, and J.L. Markley, *Biochemistry* **29**, 88-101 (1990).
2. J. Wang, A.P. Hinck, S.N. Loh, and J.L. Markley, *Biochemistry* **29**, 102-113 (1990).

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CG 430 ϵ-[^{13}C]METHIONINE, 5-[^{19}F]TRYPTOPHAN AND DEUTERATED AROMATIC AMINO ACIDS INCORPORATED INTO RECOMBINANT HUMAN TRANSFERRIN N-TERMINAL HALF-MOLECULE SERVE AS NMR PROBES OF CONFORMATIONAL CHANGE, R.C. Woodworth*, A.B. Mason*, R.T.A. MacGillivray+ and W.D. Funk+, Departments of Biochemistry, *University of Vermont, Burlington, VT 05404, and +University of British Columbia, Vancouver, BC V6T 1W5.
Recombinant human transferrin N-terminal half-molecule (hTF/2N) and its D63S and K206Q site-directed mutants have been produced in transformed baby hamster kidney cells cultured in media containing ϵ-[^{13}C]M, 5-[^{19}F]W, 2,3,4,5,β_2[D_6]F, 2,4,α-[D_3]H, 2,4,5,6,7-[D5]W and 2,6-[D_2]Y. The five ^{13}C-M residues give rise to five well-resolved resonances, the one to highest field being appreciably broadened. Certain of these resonances shift on binding of synergistic anion (oxalate) and further shifts occur on binding of metal ion (Ga(III)). Fe(III) as the bound metal ion broadens selectively the three lowest field resonances. The three ^{19}F-W residues yield three well-resolved resonances which also shift on various treatments. The 3,5-[^{1}H] resonances of 11 of the 13 2,6-[D_2]Y residues are well-resolved. Two of these shift to higher field on formation of the ternary complex, consistent with ionization of the phenolic OH. Two or three resonances of ring-shifted methyl residues move down field on formation of the ternary complex. Supported by USPHS Grant DK21739.

CG 431 PARTIAL PROTON NMR ASSIGNMENT OF ADENYLATE KINASE. Honggao Yan and Ming-Daw Tsai, Department of Chemistry and Ohio State Biochemistry Program, The Ohio State University, Columbus, OH 43210.

As part of the structure-function studies of adenylate kinase (21.7 kDa) from chicken muscle (overproduced in *E. coli*), we have performed partial proton NMR assignment of adenylate kinase and its complex with a bisubstrate analogue $MgAP_5A$. The aromatic spin systems were first assigned with standard TOCSY and NOESY experiments. Then HMQC and HMQC-NOESY experiments were performed on uniform N-15-labeled enzyme and its complex with $MgAP_5A$. The spectral patterns are dramatically different between the free enzyme and its complex with $MgAP_5A$. Specifically labeled enzymes, including [N-15-Leu], [N-15-Leu/C-13-Val], and [C-13-Leu/N-15-Val], were then used to provide information for specific assignments. Partial assignments have been accomplished for a few segments of α-helices. The implication of such assignments in enzyme-substrate interactions will be discussed.

Late Abstracts

STRUCTURAL STUDIES OF THREE-WAY DNA JUNCTIONS CONTAINING UNPAIRED NUCLEOTIDES, Neocles B. Leontis, Department of Chemistry, Bowling Green State University, Bowling Green, Ohio 43403 USA

We recently showed that non-paired nucleotides stabilize the formation of three-way helical DNA junctions (1). Two or more unpaired nucleotides located in the junction region enable oligomers ten to fifteen nucleotides long to assemble, forming conformationally homogeneous junctions, as judged by native gel electrophoresis. The unpaired bases can be present on the same strand or on two different strands. UV melting studies of the order-disorder transition of representative three-way junctions allowed us to quantify thermodynamically the stabilization provided by unpaired bases in the junction region. We found that $\Delta\Delta G°(37°C) = +0.5$ kcal/mol for increasing the number of unpaired adenosines from two to three. Thus it appears that two unpaired nucleotides optimally stabilize DNA three-way junctions. We therefore have commenced solution NMR studies on a three-way junction having two unpaired adenosines on one strand and six base-pairs in each helical arm of the junction. TOCSY and NOESY proton spectra have been obtained at 500 and 600 MHz for this 38-nucleotide complex in collaboration with David Gorenstein, Purdue University. Our progress in assigning the spectrum of this novel nucleic acid structure and in determining its three-dimensional structure from NOESY distance constraints will be reported, along with computer modelling studies carried out in collaboration with Steve Harvey and Arun Malhotra of the University of Alabama.

1. Leontis, N.B., Kwok, W., and Newman, J. (1991) "Stability and Structure of Three-Way DNA Junctions Containing Unpaired Nucleotides." Nucleic Acids Research. *In Press.*

NMR STUDIES OF DNA LESIONS: PAIRING ALIGNMENTS INVOLVING THE 1,N^2-PROPANODEOXYGUANOSINE EXOCYCLIC ADDUCT AND THE 7-HYDRO-8-OXO-DEOXYGUANOSINE OXIDATIVE ADDUCT.

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The deoxyguanosine base can be modified at the N1/N2 positions and at the N7/C8 positions resulting in DNA helical lesions. Such modifications can result in either termination of chain elongation or base mis-incorporation during occasional read-through past the lesion site. The former modifications result from generation of exocyclic adducts such as 1,N2-propanodeoxyguanosine (pdG), while the latter modifications result from generation of oxidative adducts such as 7-hydro-8-oxo-deoxyguanosine (8-oxo-dG). We have incorporated these adducts at specific sites in DNA oligomer sequences and characterized structural features of the lesion site by two-dimensional NMR spectroscopy. The pdG adduct was studied opposite dA as a function of pH while the 8-oxo-dG adduct was studied opposite dA and dC. These studies establish the importance of *syn* glycosidic orientations at these modified dG analogs and demonstrate an interplay between hydrogen bonding and hydrophobic contributions modulated by pH in defining pairing alignments.