FRONTIERS OF NMR IN MOLECULAR BIOLOGY

Organizers: David Live, Ian Armitage and Dinshaw Patel

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Plenary Sessions Page

Poster Sessions

DNA/RNA

CA 001 ASPECTS OF FOLDING NUCLEIC ACIDS, C.W. Hilbers, M.J.J. Bloemers, F.J.M. van de Ven, J.A.L.I. Walters, Laboratory of Biophysical Chemistry, Faculty of Science, University of Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands. G.A. van der Marel, J.H. van Boom, Gorlaeus Laboratories, State University Leiden, P.O. Box 9502, 2300 RA Leiden, The Netherlands.

The conformational space available to nucleic acids is not confined to the canonical double helix; polymorphism is a wide spread phenomena. Two types of nucleic acids, showing such behavior will be considered in this contribution: cyclic oligonucleotides and hairpin structures. Structures of cyclic oligonucleotides have been elucidated in our laboratory. Furthermore, these molecules have been subjected to multiconformer analysis to predict their structure. The same approach has been used to probe the conformational space available as constrained by NMR parameters derived to determine the structure of nucleic acids. Folding properties of nucleic acid molecules become clearly manifest in the loop formation in hairpin loop structures. This will be considered in a number of synthetic DNA hairpin loops. In particular, the influence of base composition of the loop on its structure will be examined.

CA 002 ON THE STRUCTURE OF HELIX V IN THE 5S RNA OF E. COLI, Peter B. Moore, Penghua Zhang, Daniel T. Gewirth, and Robert Rycyna, Departments of Chemistry and Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511

The most interesting part of 5S RNA from the point of view of RNA structure is its helix V region [bases 68-80, 96-106].

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      68        75        80
5'.....C C G A U G G U A G U .....3'
       | |                 | |
3'.....G G A U G A G A G C G .....5'
      105       100
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Despite the fact that it includes many mis-matches, it acts chemically as though it were normal double helix and virtually all its imino protons are NMR-visible at 300K, pH 7. We have completed the task of assigning these resonances, and will discuss the implications of the assignments for the structure of that region in light of the model for helix V recently proposed by Romby et al.[1] Our results on point mutants involving this part of 5S as well as our initial work on backbone assignments will also be mentioned.

[1]Romby, P. et al. (1988) Biochemistry 27, 4721-4730.

DNA/RNA and Ligand Interactions

CA 003 INTERACTION OF A SNAKE VENOM NEUROTOXIN AND SEQUENCES FROM THE ACETYLCHOLINE RECEPTOR α SUBUNIT, Aksel A. Bothner-by, P.K. Mishra, Carnegie Mellon University, Pittsburgh, PA 15213, and Barbara W. Low, College of Physicians and Surgeons of Columbia University, New York, NY 10032

From a study of the stereochemistry of the reactive site of protein α-toxins considered as a probe, one segment of the α-subunit of the acetylcholine receptor (residues 177-193) has been recognized by Low and Corfield[1] as the prime complementary binding domain. Intrinsic fluorescence studies showed very tight binding (K_D=5x10^{-8}) of the 179-191 region of calf peptide (KESRGWKHWVFYA). The dominant fluorophor, W187, is heavily quenched on binding suggesting a marked change in environment. We have applied 2-D proton spectroscopies (COSY, NOESY) to the study of this peptide, α-cobratoxin, and their complex. The results on the toxin agree well with those of Kondakov, et al.[2] and with the x-ray structure determination.[3] The peptide is small and appears rather flexible in solution, though anomalous shifts indicate formation of a hydrophobic region. The structure for the complex, suggested by fluorescence and binding measurements, is tested by the NMR data.

1 B.W. Low and P.W.R. Corfield, Asia Pacific Journal of Pharmacology, 2, 115 (1987).
2 V.I. Kondakov, Arsek'ev, K.A. Pluzhnikov, V.I. Tsetlin, V.F. Bystrov, and V.T. Iranov, Bioorgan. Khim, 10, 1606 (1984).
3 M.D. Walkinshaw, W. Saenger, and A. Mallicke, Proc. Nat. Acad. Sci. USA, 77, 2400 (1980)

CA 004 NMR STUDIES OF PROTEIN-DNA RECOGNITION, R. Kaptein, R. Boelens, R. Lamerichs, J.A.C. Rullmann and J. Breg, Department of Chemistry, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands.

The complexes of lac repressor headpiece with 11 and 14 bp half operators and with a full symmetric lac operator of 22 bp have been analysed using 2D NOE spectroscopy [1,2]. From the similar patterns of intra-protein and intra-nucleotide NOE cross peaks in the spectra of the complexes and those of the free constituents it could be concluded that the conformation of protein and DNA does not change significantly upon complex formation. Ca. 25 protein-DNA NOE's could be identified, which are very similar for all three complexes. Based on these NOE's a structural model for the protein-DNA complexes could be derived by docking procedures using ellipsoid and restrained molecular dynamics methods. The orientation of the recognition helix of headpiece in the major groove of the DNA is opposite in these complexes, compared to that observed for other prokaryotic repressors such as λ, 434 and trp. Some of the amino acid-base contacts as predicted by us based on the NMR results have now been confirmed in genetic experiments (Müller-Hill, personal communication) implying that intact lac repressor also uses the opposite helix orientation in DNA recognition. Other DNA-binding proteins that are being studied in our laboratory include the lex A repressor that controls the SOS system of E.coli and the Arc repressor of bacteriophage P22. It could be shown that lex A repressor contains the helix-turn-helix motif, while the DNA binding site of Arc repressor is completely different.

References

1. R. Boelens, R.M. Scheek, J.H. van Boom and R. Kaptein, J. Mol. Biol. 193, 213 (1987).
2. R. Kaptein and R. Boelens, Nucl. Acids Mol. Biol., Vol. 2, F. Eckstein and D.M.J. Lilley (Eds.), Springer-Verlag, Berlin, Heidelberg, 1988, pp.167-187.

CA 005 STRUCTURES OF RNA FOLDING DOMAINS, Arthur Pardi, Hans A. Heus, and Olke C. Uhlenbeck, Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309-0215

NMR spectroscopy is being used to probe the structure of RNA oligomers in solution with the goal of understanding the principle folding units that are used to build three-dimensional structures of RNAs. The RNA oligomers are synthesized in milligram quantities from DNA templates with T7 RNA polymerase. The molecules presently being studied are:

1) A 14 base pair double stranded RNA containing the sequence corresponding to the Dickerson DNA dodecamer (1).

2) A series of "hammerhead" catalytic RNA oligomers that undergo self-cleavage reactions (2). These systems are being studied as RNA-DNA hybrids in order to prevent self-cleavage while still maintaining the biologically relavent three-dimensional structure.

Biochemical studies indicate various degrees of secondary and tertiary structure in these systems. ^{1}H, ^{31}P, and 2D NMR spectroscopy are being used to obtain more direct structural information on these RNA molecules and the results of these studies will be presented.

1. Drew et al. Proc. Natl. Acad. Sci. 78 (1981) 2179-2183.
2. Uhlenbeck Nature 328 (1988) 596-600.

CA 006 NMR STUDIES OF NUCLEIC ACID COMPLEXES, David E. Wemmer and Jeffrey G. Pelton, Department of Chemistry, University of California, Berkeley, California 94720

Drug-DNA complexes are now being characterized with many different methods, including footprinting, x-ray diffraction, calorimetry and NMR. Each of these methods has advantages in providing certain types of information, however each also has certain limitations. We will discuss NMR studies of distamycin-A with a number of different DNA sequences, and compare the conclusions reached as compared with the other methods which have also been applied. In particular we will discuss the identification of multiple binding sites within a single sequence, and the processes through which the drug exchanges among them. Features which seem to lead to tight binding and slow exchange will also be discussed. Although distamycin is well known to bind preferentially to A-T rich sequences, we have evidence for cooperative binding involving G-C containing sites as well at drug/A-T site ratios above 1/1. The evidence for the existence of these sites, their possible identities, and the kinetics of exchange with the "normal" tight binding sites will be discussed.

Peptides/Hormones

CA 007 NMR, GROWTH FACTORS AND DOMAINS, Iain D. Campbell, Robert M. Cooke, Martin Baron, Timothy S. Harvey and Michael J. Tappin, University of Oxford, Department of Biochemistry, South Parks Road, Oxford OX1 3QU, U.K.

Structural studies of human epidermal growth factor (hEGF), transforming growth factor alpha (TGF-α), insulin like growth factor (IGF1) and interleukin 2 (IL2) will be reported. These polypeptides range in size from 50 to 132 residues thus different strategies are appropriate for different proteins. For EGF and TGF-α, for example, complete sequential NMR resonance assignment followed by distance geometry and restrained molecular dynamics have been applied. For IL2, site specific mutations and isotopic labelling have been more valuable so far. One aim is to identify important residues involved in binding to the receptors.
Another project is the investigation of the structure of some of the domains in proteins of the blood clotting, fibrinolysis and immune response systems. These domains are of suitable size for structural investigation by NMR. We are producing a variety of single domains and domain pairs, mainly by expression of the genes in a yeast cell system. Progress on structural studies of one such domain, the type I fibronectin finger, will be reported.

CA 008 NMR MEASURES OF CONFORMATIONAL MOBILITY IN CYCLIC PEPTIDES, K.D. Kopple, C.A. D'Ambrosio, and Y.-S Wang, L-940, Smith Kline & French Laboratories, P.O. Box 1539, King of Prussia, PA 19406.

Cyclization is used as a conformational constraint in searching for biologically active conformations of flexible oligopeptides, but cyclic peptides have considerable conformational mobility which must be characterized for valid conclusions to be drawn from their biological activity.

Conformation exchanges with rates 10-100 times faster than those producing obvious NMR line broadening can be detected by their contributions to spin-lattice relaxation in the rotating frame. Although conformation exchanges of a peptide may be kinetically complex, these contributions are dominated by the processes producing the largest chemical shift fluctuations. It is reasonable to hypothesize that larger fluctuations are associated with larger conformational excursions.

We have examined backbone conformation changes in the ten microsecond range in some cyclic tetra- hexa- and octapeptides using T_{1rho} measurements, and have begun to investigate the temperature dependence of the internal mobility reflected by the $1/T_{1rho}$ contributions. Some of the processes being observed appear to have very low activation enthalpies.

The NMR data, X-ray results and molecular modelling calculations give indications of the type of internal motion being observed.

CA 009 CALCIUM REGULATION IN MUSCLE CONTRACTION, Brian D. Sykes, Brian J. Marsden, A. Patricia Campbell and Robert S. Hodges, Department of Biochemistry and MRC Group in Protein Structure and Function, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7

Calcium plays an important role in many biological systems such as muscle where it effects a structural change in regulatory proteins such as troponin and tropomyosin. We have focussed on two aspects of this regulation; the binding of metals to the calcium binding protein troponin-C (TnC) and the calcium mediated interaction of troponin-I (TnI) and troponin-C. In both of these areas our approach has been to use synthetic peptide analogs to simplify the NMR spectrum and allow us to focus on one aspect of the system. The calcium-binding sites of proteins such as TnC exist in a helix-loop-helix conformation. In this study we have used high resolution 500 MHz ^{1}H nuclear magnetic resonance (NMR) techniques to determine the structure of the peptide Ac-(Asp105)-TnC (103-115) amide-metal ion complex in solution in order to compare it to the structure of the loop in intact TnC. The structural constraints were obtained from relaxation studies of assigned nuclei in the presence of the paramagnetic calcium analogue gadolinium, from two dimensional ^{1}H NMR nuclear Overhauser enhancement studies, and from observed NH-α CH NMR coupling constants. The comparison of distance restraints with the equivalent distances calculated from the X-ray structure yielded a RMS difference of $\pm$1.1 Å suggesting the structures are very similar. We have also studied a synthetic analogue of the inhibitory region of Tn-I, N α-acetyl Tn-I (104-115) amide, which represents the minimum sequence necessary for inhibition of actomyosin ATPase activity. Conformational changes induced by the formation of the synthetic peptide Tn-C (Ca^{2+}-saturated) complex were followed by the study of the transferred proton-proton nuclear Overhauser effect. The overall solution structures of these two peptides has been calculated using the computational techniques of restrained energy minimization and molecular dynamics wherein the experimentally determined distance restraints are used as pseudo potential energy terms [van Gunsteren & Berendsen, Groningen]. We have also used gadolinium relaxation measurements to locate the position of the TnI structure when bound to TnC.

CA 010 NMR OF COMPLEX CARBOHYDRATES. Herman van Halbeek. Complex Carbohydrate Research Center, University of Georgia, PO Box 5677, Athens, GA 30613, USA.

Complete structural characterization of complex carbohydrates involves determining *(i)* the type, number and primary sequence of the constituting monosaccharides (including the occurrence of branch points, and the location of appended non-carbohydrate groups such as alkyl, acyl, phosphate, sulfate groups), and *(ii)* the three-dimensional conformation(s) and dynamics in solution. NMR spectroscopy is the single most powerful technique for the accomplishment of these tasks.

The intelligent combination of multiple-pulse, ^{1}H and ^{13}C NMR spectroscopic techniques affords, in principle, a way of *de-novo* sequencing of a carbohydrate as large as a decasaccharide (*c.q.*, a polymer with a decasaccharide repeat unit), provided that a few μmol of pure substance are available for the analysis. 1-D and 2-D $\{^1H,^1H\}$ Hartmann-Hahn (HOHAHA) spectroscopy, along with double- and triple-quantum filtered $\{^1H,^1H\}$ COSY, are well suited to yield the complete assignment of the ^{1}H-NMR spectrum of a carbohydrate; the HOHAHA technique permits subspectral editing for each constituting monosaccharide. Reversed $\{^1H,^{13}C\}$ correlation-spectroscopy is a highly sensitive method that provides $\{^1H,^{13}C\}$ chemical-shift correlation maps of an oligosaccharide through the ^{1}H-detection of heteronuclear multiple quantum coherences (HMQC and HMBC). Once the HOHAHA and HMQC deduced assignments of the ^{1}H and ^{13}C spectra of a carbohydrate are complete, the HMBC spectrum then permits the deduction of the sequence of the monosaccharides, and the location of most types of appended groups.

Solution conformational analysis of carbohydrates by NMR relies on the measurement of scalar and dipolar couplings. The pure-absorption HOHAHA-edited monosaccharide subspectra provide the scalar couplings necessary to define the ring conformations. The torsional angles around the interglycosidic bonds are defined in terms of interproton distances through the measurement of dipolar couplings. 2-D NOE spectroscopy is an invaluable tool in 3-D structural analysis. Depending on the size of the oligosaccharide under investigation, the temperature of the sample, and the magnetic field strength at hand, one may resort to NOESY or rotating frame NOESY (ROESY) experiments for the measurement of the dipolar couplings. In addition to the experimental NMR approach, conformational analysis of complex carbohydrates requires the theoretical evaluation of the NOE-generated internuclear distance constraints by a potential-energy minimization method such as GESA, MM2, or AMBER.

The integrated approach of applying HOHAHA, HMQC, and HMBC NMR experiments for the sequencing of oligosaccharides, and NOESY/ROESY and GESA for their solution conformational analysis, will be illustrated for several plant-derived oligosaccharides, some bacterial polysaccharides, as well as for a number of oligosaccharide side chains of mammalian glycoproteins.

CA 011 FOLDING OF PEPTIDE FRAGMENTS OF PROTEINS IN AQUEOUS SOLUTION, Peter E. Wright, H. Jane Dyson, Victoria Feher, Richard Houghten, Linda Tennant, John J. Osterhout, Jonathan P. Waltho and Richard A. Lerner, Department of Molecular Biology, Research Institute of Scripps Clinic, 10666 North Torrey Pines Road, La Jolla, California 92037.

The molecular mechanism by which proteins fold remains one of the important unanswered questions in structural biology. It is generally accepted that protein folding proceeds via local folded intermediates which function as initiation sites for cooperative growth. However, direct experimental identification of the transient early folding intermediates of native proteins is difficult. Recent developments in two-dimensional NMR spectroscopy have provided very sensitive methods for the detection of folded structures in peptide fragments of proteins in water solution. NMR experiments which provide unequivocal evidence for the formation of β-turns, nascent helix and α-helix in short linear peptides in water solution will be described. The folded conformations are mostly relatively unstable and are in rapid dynamic equilibrium with unfolded states. Folded peptide structures can be identified by the presence of medium or long range NOEs, unusual $^3J_{HN\alpha}$ coupling constants and amide proton exchange measurements. In some cases, the structures can also be detected by circular dichroism spectroscopy. By systematic variation of the amino acid sequence we are able to elucidate the factors which stabilize the folded conformations. The observations that peptide fragments of proteins adopt secondary structures in water solution has enormous implications for initiation of protein folding, for the mechanism of induction of protein-reactive anti-peptide antibodies and for T-cell recognition.

Proteins

CA 012 APPLICATIONS OF IMINO PROTON EXCHANGE TO NUCLEIC ACID KINETICS AND STRUCTURES, M. Gueron, E. Charretier, M. Kochoyan and J.L. Leroy, Groupe de Biophysique, Ecole Polytechnique, 91128 Palaiseau, France

Imino proton exchange in duplexes is commonly limited by the chemical exchange step. Hence it may be changed by the addition of proton acceptors acting as exchange catalysts. In the limit of infinite catalyst concentration, the exchange time is equal to the **lifetime of the closed state**, since the evidence is that exchange occurs only when the base pair is disrupted. The efficiency of the catalyst at moderate concentrations provides a value for the **base-pair dissociation constant.** Using spectral broadening, longitudinal relaxation and magnetization transfer in H_2O solutions, exchange times between 1ms and 1s can be measured. Those longer than 2 minutes are accessible to direct measurement in D_2O.

Using such methods, we have shown that base pairs open one at a time. In B DNA, the lifetime is in the range of milliseconds at room temperature (1), and the influence of neighbors is not large. The lifetime in the Z form is 100 times longer.

In oligodeoxyduplexes containing tracts of A.T base pairs, the lifetimes are anomalously long **if the tract contains four consecutive A.T base pairs, possibly including a 5'-AT step but not a 5'-TA step.** This strongly suggests that, in such tracts, a conformation distinct from standard B-DNA is formed cooperatively.

All A.T tracts known to generate curved DNA exhibit anomalously long base-pair lifetimes. This is the first local and physical property shown to correlate with DNA curvature. Our observations suggest that the structure responsible for the long lifetimes is involved in the curvature of DNA.

Imino proton exchange in the absence of added catalyst is explained by intrinsic catalysis (2). This interpretation leads to predictions for the structure of the open state and for the exchange mechanism.

1. J.L. Leroy, M. Kochoyan, Tam Huyn-Dinh and M. Guéron, **J. Mol. Biol.**, **200**, 223-238 (1988).
2. M. Guéron, M. Kochoyan and J.L. Leroy, **Nature**, **328**, 89-92 (1987).

CA 013 STATIC AND DYNAMIC ASPECTS OF PROTEIN STRUCTURES, Gerhard Wagner, Biophysics Research Division, Institute of Science and Technology, University of Michigan, Ann Arbor, MI 48109.
Proteins are dynamic molecules. When conformations of proteins are determined by NMR on the basis of NOE distance constraints usually bundles of structures are obtained which all are consistent with the experimental constraints. This variability may be due to the limited number of constraints, the errors in measurements of NOE's and coupling constants, and to internal mobility. The experimental parameters used for structure determination are sensitive to molecular geometry and mobility. We have developed methods which better characterize both these aspects of protein structure. On the one hand, we have improved methods for quantitative measurements of NOE's and coupling constants which form the basis for structure calculations by distance geometry algorithms. On the other hand, we have concentrated on techniques for characterizing protein dynamics. We have performed T_1 and T_2 measurements of ^{13}C and ^{15}N by 2D heteronuclear correlation techniques, and we have used heteronuclear experiments to identify multiple conformations and to characterize the respective equilibrium constants and interconvertion rates. We will show that, for the proteins we have studied, multiple conformations are common features, and the interconvertion rates are slow on the NMR time scale, at least at low temperature. To detect these multiple conformations it was crucial to have powerful heteronuclear correlation techniques available.

Metalloproteins/Carbohydrates

CA 014 NMR SPECTROSCOPY IN PARAMAGNETIC PROTEINS, Gerd N. La Mar, L. B. Dugad, S. Donald Emerson and V. Thanabal, Department of Chemistry, University of California, Davis, California 95616, Lucia Banci, Department of Chemistry, University of Florence, 50121 Florence, Italy, S. G. Sligar and Mark Chiu, Department of Biochemistry, University of Illinois, Urbana, Illinois 61801
While paramagnetic linkage does render steady-state NOEs smaller and make more difficult the measurement of cross relaxation rates by 1D or 2D time-dependent measurements, we have found the scope of such studies surprisingly large. NOESY maps of several paramagnetic proteins (cytochrome b_5, met-cyano myoglobin), have been successfully determined and sufficient resonances assigned that allow the experimental mapping of the paramagnetic dipolar field. The dipolar field, in turn, is very useful in delineating the molecular and electronic structural changes exhibited by single point mutants in the heme cavity of sperm whale myoglobin. The NOEs from very rapidly relaxing protons are generally not detectable in the NOESY map because of signal-to-noise limitations due to the combination of the small NOE and the very large linewidth. However, conventional 1D methods allows the detection of the needed NOEs using very rapid pulsing methods. It is also found that the usefulness of NOEs in paramagnetic proteins increases with the molecular size of an electronically iso-structural chromophore, primarily because the paramagnetic relaxation is unaffected by changes in molecular reorientation time, while the cross relaxation increases linearly with the reorientation time. Thus the use of viscous solvents for small (15 kDa) proteins significantly increases the NOEs without adverse line broadening affects. While most successful applications to date have been for heme proteins, we have recently extended these studies to iron sulfur proteins where it has been possible to assign all hyperfine-shifted signals to individual cysteines, and thereby identify the discrete oxidation states of the two irons in 2-iron ferredoxins. Similarly promising NOE studies have been initialed on cobalt(II)-substituted carbonic anhydrase.

Isotope Directed Methods

CA 015 APPLICATIONS AND LIMITATIONS OF ISOTOPIC LABELING FOR THE NMR STUDY OF MEDIUM SIZE PROTEINS, Ad Bax, Lewis Kay, Dominique Marion, Mitsuhiko Ikura, Dennis A. Torchia, Steven W. Sparks, Paul Driscoll, G. Marius Clore and Angela M. Gronenborn, Laboratory of Chemical Physics, NIDDK, and Bone Research Branch, NIDR, National Institutes of Health, Bethesda, MD 20892.

Recent trends to incorporate stable isotopes in proteins permit the use of a large variety of heteronuclear NMR experiments for simplifying spectral appearance and for gaining additional assignment and structural information. Isotope-edited 2D spectra can be much simpler than their regular counterparts, without any significant loss in sensitivity. A variation on this isotope editing theme utilizes three-dimensional NMR for unraveling the complicated spectra, permitting non-selective labeling with ^{15}N or ^{13}C. Incorporation of ^{15}N in the protein also permits the use of a multiple quantum line narrowing procedure which yields enhanced resolution in the F_1 dimension of regular COSY or NOESY spectra. This type of line narrowing can also be exploited for measuring NH-Hα J couplings in medium size proteins where these couplings are normally unresolvable.

CA 016 NMR STUDIES OF NORMAL AND TRANSFORMING RAS GENE PRODUCTS.
Sharon Campbell-Burk, E. I. du Pont de Nemours & Co., Wilmington, DE 19880-0328.

The most prevalent oncogene identified in human tumors belongs to the ras gene family. Ras oncogenes differ from normal ras genes by one or two base changes, and encode 21kD proteins (p21) with amino acid substitutions in the guanine nucleotide binding domain. The decreased guanosine triphosphatase (GTPase) activity associated with ras oncogene-encoded p21 proteins is believed to stabilize the p21.GTP conformation and confer transforming capabilities.

Normal and transforming ras protein products have been studied using isotope-edited NMR and ^{31}P NMR spectroscopy. Stable isotopes (^{15}N, ^{13}C) have been biosynthetically incorporated into these proteins, and resonances corresponding to amino acids in important domains were identified. These resonances were used as site-specific probes to study differences between normal and transforming mutant p21.GDP complexes. Normal and mutant p21 proteins complexed to GTP and non-hydrolyzable guanosine triphosphate analogs [GMPP(NH)P, GTPγS] were also studied. Spectral differences between the putative biologically (transformation) active p21.GTP and inactive p21.GDP forms are observed. Results from these studies will be presented.

CA 017 APPLICATIONS OF ISOTOPE DIRECTED 2D NMR METHODS, David H. Live, Department of Chemistry, Emory University, Atlanta, GA 30322.

Recent developments in experimental techniques and instrumentation make it possible to carry out a variety of proton detected heteronucler 2D NMR spectra on both natural abundance and enriched samples. Applications of these experiments to studies of oligonucleotides and polypeptides will be discussed, including studies their use in H-1 - P-31 2D correlation experiments for structures of oligonucleotides and proteins.

CA 018 CONCERTED ASSIGNMENTS OF H-1, C-13, AND N-15 RESONANCES IN PROTEINS,[a] John L. Markley, Prashanth Darba, Ed S. Mooberry, Byung Ha Oh, Michael A. Reily,[b] Brian J. Stockman, Eldon L. Ulrich,[c] and William M. Westler, National Magnetic Resonance Laboratory at Madison, Biochemistry Department, University of Wisconsin, 420 Henry Mall, Madison, WI, 53706.

When used in concert, one-bond carbon-carbon correlations (obtained from $^{13}C\{^{13}C\}$ double-quantum correlation spectra), one-bond and multiple-bond proton-carbon correlations (obtained from $^{1}H\{^{13}C\}$ single-bond correlation and $^{1}H\{^{13}C\}$ multiple-bond correlation spectra, respectively), and multiple-bond proton-nitrogen correlations (obtained from $^{1}H\{^{15}N\}$ multiple-bond correlation spectra) provide a reliable method of assigning proton, carbon, and nitrogen resonances in proteins. In contrast to procedures that simply extend proton assignments to carbon or nitrogen resonances, this technique assigns proton, carbon, and nitrogen resonances coordinately based on their mutual coupling networks. Redundant spin coupling pathways provide ways of resolving overlaps frequently encountered in homonuclear ^{1}H 2D NMR spectra and facilitate the elucidation of complex proton spin systems. Main-chain carbon and nitrogen assignments obtained with this technique provide input for sequential assignment strategies based on inter-residue scalar coupling. These methods will be illustrated by assignments of ^{1}H, ^{13}C, and ^{15}N NMR signals from three electron transport proteins from the cyanobacterium Anabaena 7120: cytochrome c_{555} (M_r 10,000), ferredoxin (M_r 10,000), and flavodoxin (M_r 21,000). This approach led to the discovery that the cytochrome is a glycoprotein and has assisted in the structural determination of the sugar and its attachment site. Complete multinuclear assignments obtained for the heme of the cytochrome and the flavin of the flavodoxin provide information about the properties of these bound prosthetic groups. Isotope-directed nuclear Overhauser enhancements have been used to map out the flavin binding site in the flavodoxin. The ferredoxin results represent the first extensive multinuclear assignments of a paramagnetic protein.

a. Support from the NIH, NSF, and USDA is gratefully acknowledged.
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c. Present address: Upjohn Co., Kalamazoo, MI 49001.

CA 019 LOCAL ENVIRONMENTS AND DYNAMICS IN LARGER PROTEINS AND NUCLEIC ACIDS, Alfred G. Redfield, Brandeis University, Waltham, MA 02254.
Methods and prospects for structural study of larger protein, aided by stable isotope-labeling, will be discussed. The ability to selectively label molecules using modern genetic engineering opens the possibility of studying the large number of macromolecules in the 50 KD range. Methods used are generally restricted to simpler 2D proton-observe sequences as well as one-dimensional isotope-edited methods. I will present examples of these methods applied to tRNA's,[1,2] to T4 lysozyme,[3] and to ras P21 (by S. Campbell-Burk).

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2. R. Griffey, M. Jarema, and others, in Structures and Dynamics of RNA (ed. van Knippenberg and Hilbers), NATO ASI Series 110, pp. 99-112 and 176-189 (1986).
3. L. P. McIntosh et al., J. Biomolecular Structure and Dynamics 5, 21-34 (1987).

Larger Systems/Ordered Systems

CA 020 SOLUTION NMR STUDIES OF FAB'-PEPTIDE COMPLEXES, P. Tsang, T.M. Fieser, R.A. Houghten, R.A. Lerner and P.E. Wright, Dept. of Molecular Biology, MB-2, Research Institute of Scripps Clinic 10666 N. Torrey Pines Rd., La Jolla, CA 92037
Complexes formed between monoclonal anti-peptide Fab' molecules and their complementary nitrogen-15 labelled peptides have been studied by reverse-detection NMR techniques. The antibodies were raised against a synthetic peptide corresponding to residues 69-87 of the C-helix of the protein, myohemerythrin. Several complexes consisting of Fab' bound to synthetic peptides of identical sequence but labelled with nitrogen-15 at a variety of amide positions were made for these studies. We have been able to detect significant differences in the NMR spectra recorded from the series of labelled peptide complexes studied thus far. The assignment of these resonances, along with the distinctive linewidth and chemical shift differences observed among them, has proven to be interesting in light of what is already known about the epitope region of this peptide. These results, along with some other general properties regarding the binding of the peptide to the Fab' molecule, will be further discussed.

Methods for Elucidating Total Structures

CA 021 REFINEMENT OF MACROMOLECULAR STRUCTURES BY RESTRAINED MOLECULAR DYNAMICS, Axel T. Brunger, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511

The utility of molecular dynamics for NMR three-dimensional structure determination/refinement is reviewed. Molecular dynamics emerges as a universal tool to explore the conformational space of the molecule while taking into account an empirical energy function in combination with experimental information, such as interproton distances from NOE measurements. In principle, molecular dynamics can be used to overcome the local minimum problem of nonlinear optimization. However, the success of molecular dynamics refinement depends on the choice of the annealing protocol to cool the system from a high-temperature or highly strained state to physiological temperatures or to a relaxed state. The annealing protocols that have been developed by us and others will be discussed.

CA 022 NMR STRUCTURAL ANALYSIS FROM THE PERSPECTIVE OF A PROTEIN CRYSTALLOGRAPHER, Wayne A. Hendrickson, Howard Hughes Medical Institute, Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY 10032.

The characteristics of atomic models derived from 2D NMR data differ considerably from those of atomic models derived from diffraction data. In the NMR case, results are typically presented as a set of independent solutions obtained by direct fitting to the NOE distance constraints and, possibly, to spin-spin coupling constants. Conformational flexibility and positional uncertainty are then evident as diversity in the cluster of multiple models. In the x-ray case, an initial model is fitted to an experimental electron-density map and then this single model is refined directly against the diffraction data. Conformational flexibility is taken into account through parameters that represent atomic mobility (mean square displacements of atoms from their rest positions) and exceptionally flexible regions, for which interpretable density is absent, are left out of the model. The effectiveness of refinement in protein crystallography improved markedly when programs were introduced for optimization directly against the primary diffraction data instead of against the electron-density distribution, a derived quantity. From this perspective, one would wish to develop analogous NMR methods by which a single model that incorporates mobility parameters could be refined directly against the 2D spectra instead of against distance constraints. The theory for NOE intensities and profiles is less well defined than in the diffraction counterpart. This could limit the proposed approach, but simulations with appropriate normalizations might be computationally effective and would certainly simplify the structural presentation.

CA 023 DATA ANALYSIS METHODS - Panel Discussion.
David Cowburn, The Rockefeller University, NY, NY, 10021, with K. Wüthrich, ETH Honggerberg, J. Prestegard, Yale U., A. Brunger, Yale U., J. Hoch, Rowland Inst., T. James, UCSF, M. Clore, NIH.

The panel will consider the state-of-the-art, major unanswered questions, and areas of research opportunity in data analysis methods. Areas to be addressed include methods of spectral analysis; automation of spectral analysis; possible applications of 'Expert Systems' approaches; the use of Distance Geometry methods, and their combination with experimental data, and with theoretical calculations of Molecular Mechanics and Dynamics; the calculation of corrections for spin diffusion; and considerations of the accuracy of force fields used in molecular calculations.

In addition to addressing technical questions arising from these topics, the panel will discuss the limits of precision and accuracy available from these analytical methods, whether analytical methods need major improvements in order to increase their utility to applications in molecular biology, and applications to other biopolymers and less rigid proteins.

CA 024 **DETERMINATION OF DNA AND PROTEIN STRUCTURES IN SOLUTION VIA COMPLETE RELAXATION MATRIX ANALYSIS OF 2D NOE SPECTRA,** Thomas L. James and Brandan A. Borgias, Departments of Pharmaceutical Chemistry and Radiology, University of California, San Francisco, CA 94143.

The 2D NMR experiment is now routinely used in a vast array of specialized experiments for spectrum assignment and structural characterization of nucleic acids and proteins. Here we will principally examine the capability of using the homonuclear 2D NOE experiment; it has the potential for providing numerous interproton distances. The use of 2D NOE spectra in macromolecular structure determination is now becoming widespread. But, while considerable success has been achieved using this technique to provide distance constraints for distance geometry or molecular dynamics calculations leading to structures, there are certain limitations and precautions that are not always appreciated.

The 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 or drug recognition. These subtle variations demand detailed knowledge of the structure and, therefore, accurate internuclear distance determinations. But one can probably 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 the 2D NOE spectrum is often all that is needed to obtain the information necessary for calculation of a modestly high-resolution protein structure in solution. But, in proteins possessing less common structural features, it may be especially valuable to have more accurate interproton distances for use with the computational techniques. And, even more importantly, we will want better defined structures at ligand binding sites (with and without ligand bound). Use of a complete relaxation matrix approach (CORMA) to ascertain interproton distances form 2D NOE peak intensities offers the opportunity of determining protein solution structure with greater accuracy and resolution.

We will compare different methods of analyzing 2D NOE spectra for internuclear distance and structural information. The most effective techniques employ an iterative method entailing CORMA in concert with molecular mechanics, molecular dynamics or distance geometry calculations.

Future Perspectives

CA 025 NMR AND GENETIC ENGINEERING: HOW GOOD IS THE MATCH?[a] John L. Markley, Andrei T. Alexandrescu, Prashanth Darba, Jasna Fejzo, Andrew P. Hinck, Andrzej M. Krezel, David M. LeMaster,[b] Stewart N. Loh, Slobodan Macura, Ed S. Mooberry, Ross J. Reedstrom, Eldon L. Ulrich,[c] Jin-Feng Wang, William F. Walkenhorst, William M. Westler, and Zsolt Zolnai, National Magnetic Resonance Laboratory at Madison, Biochemistry Department, University of Wisconsin, 420 Henry Mall, Madison, WI, 53706.

The short answer to the title question is, "excellent--in some cases." NMR requires large amounts of protein (1-10 μmole, depending on the NMR experiment), and the most attractive strategies for NMR analysis of larger proteins (10 kDa and above) require stable isotope labeling. Protein engineering methods provide for the large scale production of interesting proteins and facilitate the introduction of isotopic labels into appropriate sites. Mutagenesis methods can be used to produce proteins modified to overcome poor spectral resolution arising from protein aggregation or low thermal stability or poor spectral sensitivity arising from low solubility. As a tool for conformational analysis, NMR avoids the major bottleneck of single-crystal diffraction studies, namely the growth of suitable crystals. The amounts of time required to obtain a new structure of a small protein by NMR and X-ray diffraction (once crystals are in hand) appear comparable. NMR methods can be used to determine a limited number of interproton distances rather quickly and precisely. It remains to be seen how effective NMR will be as an approach to global structure with proteins larger than 20 kDa. With appropriate stable-isotope labeling, however, it should be possible in many cases to obtain site-specific NMR assignments with proteins in the 50 kDa range. Studies of these assigned resonances can then provide a wealth of useful information such as answers to the questions: is the protein covalently or conformationally homogeneous; are two protein samples folded in the same way; how large an effect does a mutation have on the structure of the protein; does a fragment of the protein have the same structure it adopts in the intact protein; what are the pK_a values of particular groups in the protein? Illustrations will be drawn from studies of naturally occurring single amino acid variants of turkey ovomucoid third domain which have different properties as proteinase inhibitors and staphylococcal nuclease mutants overproduced in *E. coli* which have altered thermal stability.

a. Support from the NIH and NSF is gratefully acknowledged.
b. Present address, Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60201.
c. Present address, Upjohn Co., Kalamazoo, MI, 49001.

CA 026 CONFORMATIONAL ANALYSIS OF POLYPEPTIDES AND PROTEINS, Harold A. Scheraga, Baker Laboratory of Chemistry, Cornell University, Ithaca, New York 14853-1301

Two-dimensional NMR spectroscopy, plus distance geometry, provides a family of closely-resembled protein structures. These provide good starting conformations for energy minimization to obtain a low-energy structure. In the absence of the large number of distance restraints obtainable from NMR spectroscopy, other methods are required to help overcome the multiple-minima problem in computing low-energy structures of proteins. The most promising of these are: (a) build-up procedure, (b) optimization of electrostatics, (c) Monte Carlo plus minimization, (d) electrostatically driven Monte Carlo, (e) inclusion of a limited number of distance restraints, (f) adaptive importance sampling Monte Carlo, (g) relaxation of dimensionality, and (h) pattern recognition. These procedures have been applied to a variety of polypeptide structural problems, and the results of such computations will be presented. Present efforts are being devoted to scaling up these procedures from small polypeptides to proteins, to try to compute the three-dimensional structure of a protein from its amino acid sequence.

Theoretical Analysis of Proteins (joint)

CA 027 EXPLORING ENZYME CATALYTIC MECHANISM BY CAD APPROACHES, Arieh Warshel, Department of Chemistry, University of Southern California, University Park, Los Angeles, CA 90089-1062

The origin of enzyme catalysis is explored by Computer Simulation approaches and the feasibility of Computer Aided Design of enzymes is examined. . The CAD program used (referred to as ENZYMIX) combines the EVB method and a free energy perturbation method[1] in evaluating the free energy of enzymatic reactions. The simulations explore both the effect of the mutations and the more challenging aspect of the overall storage of catalytic free energies. Several test cases are presented and discussed. It is found in agreement with our early studies[2] that the electrostatic free energies of the reacting system is the key factor in structure-function correlation of enzymatic reactions.

(1) A. Warshel, F. Sussman and J-K. Hwang, J. Mol. Biol., 201, 139 (1988).
(2) A. Warshel, Act. Chem. Res., 14, 284 (1981).

Structural Analysis of Protiens (joint)

CA 028 PROTEIN STRUCTURES IN SOLUTION: COMPARISON WITH CORRESPONDING CRYSTAL STRUCTURES, Kurt Wüthrich, Eidgenössische Technische Hochschule-Hönggerberg, CH-8093 Zürich, Switzerland.

Compared to the data available on amino acid sequences, relatively few three-dimensional protein structures have been determined. Furthermore, until recently three-dimensional protein structure determination was limited to the crystalline state. This scarcity of experimental data on protein conformations is one of the principal bottle-necks in protein design and protein engineering. The introduction of nuclear magnetic resonance spectroscopy as a second technique for protein structure determination besides X-ray diffraction in single crystals (K. Wüthrich, NMR of Proteins and Nucleic Acids, Wiley, New York, 1986) promises to improve the situation both by providing complementary information to that contained in crystal structures and by the addition of new structures. New structures will cover proteins which could either not be crystallized, or for which the molecular architecture in noncrystalline states is different from that in single crystals. A complementation of the crystal structure may be obtained when the same molecular architectures are preserved in the different states, but the structures vary by subtle, local differences. With regard to all these different possible situations detailed comparisons of protein structures in solution and in single crystals are of fundamental interest. Such comparisons will be presented, with special emphasis on the variability of conformational ordering along the amino acid sequence in the crystal structures and in solution.

Late Additions

CA 029 STUDIES OF THE 3D STRUCTURE OF COMPLEMENT PROTEIN C5A AND C5A MUTANTS BY 2D AND 3D NMR, Erik R.P. Zuiderweg, David G. Nettesheim, Stephen W. Fesik, Edward T. Olejniczak, Wlodek Mandecki, Karl W. Mollison, Jonathan Greer and George W. Carter, Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL 60064.
C5a, a 74 amino acid protein, is an inflammatory mediator derived from the serum complement system. The protein interacts with receptors on polymorphonuclear leukocytes. It is the goal of the investigations to obtain the identities, locations and conformations of C5a residues interacting with the leukocyte receptor to aid in the design of compounds interfering with this interaction. Such compounds would be therapeutic agents for the treatment of inflammatory diseases.

The solution tertiary structure of human C5a was calculated from a large number of distance constraints derived from NOESY data sets using the DISMAN algorithm. By performing calculations with different target functions, it was demonstrated that contiguous regions of slowly exchanging amide protons identify helices in this protein. Additional intra residue distance constraints were defined from a heteronuclear 3D NOESY-HMQC experiment using a 0.7 mM solution of C5a uniformly labeled with N15. In this experiment the heavily overlapping amide proton region of the NOESY spectrum was greatly simplified by editing with respect to the frequencies of the isotopically labeled amide nitrogens. Quantitative NOESY/ROESY Overhauser effect build up rates were obtained to show that the rotational correlation time of the C-terminal part of the molecule is much shorter than in the remainder of the molecule. Therefore, independent evidence was obtained to show that this part of the molecule is unstructured in solution. The solution structure of C5a is compared with crystal data of the related C3a protein and with NMR solution conformational data on C3a, which are at variance with the crystal data.

Site specific mutant proteins were prepared to identify the location of residues which interact with the C5a receptor on polymorphonuclear leukocytes. 2D NMR spectroscopy was used to characterize the conformation of the mutant C5a molecules. Using fully interpreted NOESY datasets, examples were found for cases where mutants with reduced receptor affinity showed only very limited conformational changes in the immediate environment of the mutagenesis site thus identifying the mutated residues as located in the C5a-receptor interface. Furthermore, a mutant protein with reduced receptor affinity has been identified where extensive conformational changes occurred at a location remote from the mutagenesis site. The results of this joint biological assay/NMR structure mutagenesis study are used to propose the location of the C5a receptor binding site.

CA 030 DNA STRUCTURES WITH HOOGSTEEN BASE PAIRS, Juli Feigon, Dara Gilbert, and Ponni Rajagopal, Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90024. Alternatives to Watson-Crick base pairing of nucleotides, such as Hoogsteen base pairs, have been recognized since the first crystal structures of nucleotides, but until recently they have been thought to be biologically irrelevant for DNA. We will present data on two DNA structures which contain Hoogsteen base pairs: (a) an echinomycin-DNA complex and (2) a triple-stranded DNA. Echinomycin is a cyclic octadepsipeptide antibiotic which bis-intercalates into DNA. Crystal structures of echinomycin-DNA complexes show Hoogsteen base pairs formed adjacent to echinomycin binding sites. We have found that Hoogsteen base pairs for similar echinomycin-DNA complexes are also formed in solution. However Hoogsteen base pairs which form in the interior of a duplex are much less stable than those formed at the ends of a duplex, and at physiological temperatures they are exchanging between a Hoogsteen and an open-base paired state. We have also investigated the structures formed from the homopurine:homopyrimidine sequences d(GAGAGAGA) and d(TCTCTCTC). Under appropriate conditions (pH <6, addition of Mg^{+2}, excess $d(TC)_4$) the predominant conformation observed is a triple-stranded helix in which a $d(GA)_4$ and a $d(TC)_4$ strand are Watson-Crick base paired and a second $d(TC)_4$ strand is Hoogsteen base paired in the major groove to the $d(GA)_4$ strand.

CA 031 STRUCTURE OF A RETROVIRAL ZINC FINGER: 2D NMR SPECTROSCOPY AND DISTANCE GEOMETRY CALCULATIONS ON A SYNTHETIC FINGER FROM HIV-1 NUCLEIC ACID BINDING PROTEIN, p7,
Michael F. Summers, Dennis Hare, Terri L. South and Bo Kim, Chemistry Department, University of Maryland Baltimore County, Baltimore, MD 21228, and Hare Research, Inc. (D.H.), 14810 216th Ave., N.E., Woodinville, WA 98072.

Two-dimensional ^{1}H-^{1}H and ^{1}H-^{13}C correlation NMR spectroscopic results are reported for studies of the 1:1 Zn^{+2} adduct with an 18-residue, synthetic peptide with amino acid sequence of the first finger region (residues 13-30) of the HIV-1 nucleic acid binding protein (NABP), p7. Data analysis afforded complete assignment of the ^{1}H spectrum and partial assignment (upfield region) of the ^{13}C spectrum of $Zn(p7^{13-30})$. The metal binding mode was determined unambiguously for the ^{113}Cd adduct ($^{113}Cd(p7^{13-30})$) using one-dimensional ^{1}H-^{113}Cd spin echo difference NMR spectroscopy. A high resolution three-dimensional structural study on the zinc adduct was carried out by an iterative approach employing the distance geometry program, DSPACE, in combination with 2D NOE back calculations. A family of refined structures was generated using a bounds matrix containing more than 140 distance constraints. Most of the interproton distances of 3.5 Å or less were constrained using bounds of $\pm$ .05 Å. Diagonal peak relaxation curves and NOE build-up curves generated using the refined structures matched the experimental curves to within 20% error. The total penalty (squared sum of the bounds violations) was less than 0.4 $Å^2$, and the largest violation was less than 0.1 $Å^2$.

Recent site-directed mutagenesis studies suggest that the non-conserved amino acid residues of the zinc finger may be involved directly in retroviral gene recognition [Gorelick et al., Proc. Natl. Acad. Sci. USA, in press]. The implications of our structural findings on retroviral gene recognition processes and the potential utility of these findings for development of new chemotherapeutic treatments for AIDS and cancer will be discussed.

DNA and RNA; Peptides

CA 100 **COMPARATIVE NMR STUDIES OF OLIGO-N-METHYLPYRROLECARBOXAMIDE• d[CGAAATTTCG] COMPLEXES.** Xiaolian Gao[1], Renzhe Jin[2], Scott Younguist[3], Peter B. Dervan[3], Kenneth J. Breslauer[2] and Dinshaw J. Patel[1], [1]Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY 10032, [2]Department of Chemistry, Rutgers, The State University of New Jersey, New Brunswick, NJ 08903, [3]Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125. The binding of tri-N-methylpyrrolecaboxamide (P3) and tetra-N-methylpyrrolecarboxamide (P4) to the d[CGAAATTTCG] duplex in aqueous solution were examined by one and two dimensional NMR. Most of the drug and DNA protons were assigned in the complex. Structural features of the complex were elucidated from intermolecular NOEs between the drug and DNA protons. Several resonances of thymidine sugar protons in the complex exhibit unusual upfield shifts and their assignment assisted the alignment of the P3 and P4 ligands in the minor groove at the A•T rich sites. Molecular models of P3•DNA and P4•DNA complexes which satisfy the NMR distance constraints were constructed and energy minimizated. These NMR studies on two distamycin analogs, which differ in the number of N-methylpyrrolecarboxamide units, provide insights into the molecular basis for the sequence specific recognition of the minor groove in DNA.

CA 101 SOLUTION STRUCTURES OF NUCLEIC ACID FRAGMENTS DETERMINED BY NMR AND RESTRAINED MOLECULAR DYNAMICS, A.M. Gronenborn, Laboratory of Chemical Physics, Building 2, room 123, NIDDK, National Institutes of Health, Bethesda, MD 20892

The solution structures of several DNA and RNA oligonucleotides will be discussed. The methodology to determine such structures involves NMR measurements and restrained molecular dynamics. First all nonexchangable and exchangeable proton resonances are assigned in a sequential manner. Subsequently a large set of approximate interproton distance restraints is derived from 2D NOESY spectra at short mixing times. These distances are used as the basis for refinement using restrained molecular dynamics in which the interproton distances are incorporated into the total energy function in the form of effective potentials. Two sets of calculations are carried out for each oligonucleotide starting from the classical A-form and the classical B-form. The final structures are obtained after convergence for all calculations. Details of the various structures will be presented.

CA 102 THE STRUCTURE OF AN ACETYLAMINOFLUORENE MODIFIED DODECAMER Thomas R. Krugh and Guanjin. J. Huang Department of Chemistry University of Rochester Rochester, N.Y. 14627

The complementary dodecamers, d(CACACGCACACA) and d(TGTGTGCGTGTG) were synthesized by the solid phase phosphoramidite method and purified by reverse phase HPLC. The single guanine of d(CACACGCACACA) was reacted with the carcinogen acetoxy-acetylaminofluorene and purified by HPLC giving the acetylaminofluorene (AAF) modified dodecamer d(CACAC^{AAF}GCACACA) with the acetylaminofluorene moiety attached to the C8 position of guanine. The purified adduct was mixed in a 1:1 ratio with the complementary strand to give the d(CACAC^{AAF}GCACACA)• d(TGTGTGCGTGTG) modified duplex. Under the conditions used for the two-dimensional NMR experiments the T_m of the modified duplex is close to 60 °C. The proton resonances of both the modified and unmodified duplexes were assigned by NOESY and TOCSY experiments at various temperatures and mixing times. The addition of the AAF moiety results in structural distortions near the modified guanine. ^{31}P NMR data also reflect the structural distortions. Imino proton spectra on the modified duplex indicate that the imino protons of all base pairs are shielded from rapid solvent exchange, with the exception of the modified guanine N_1 proton which exhibits faster exchange than adjacent guanine N_1 protons. The analysis of the data in terms of the structure of the modified and unmodified duplexes will be presented. Supported by NCI grant CA-35251.

CA 103 **^{1}H and ^{31}P NMR Studies of Abasic Sites in DNA Duplexes.** Matthew W. Kalnik[a], Chien-Neng Chang[b], Francis Johnson[b], Arthur P. Grollman[b], and Dinshaw J. Patel[a], [a]Department of Biochemistry & Molecular Biophysics, Columbia University, New York, NY, 10032, [b]Department of Pharmacological Sciences, SUNY, Stony Brook, NY, 11794. Three similar complementary nonanucleotide duplexes containing abasic sites with the general sequence d(C-A-T-G-A-G-T-A-C)•d(G-T-A-C-X-C-A-T-G), where X is either a tetrahydrofuran moiety (isosteric with 2-deoxyribofuranose), an n-propylene moiety, or an ethylene moiety, have been investigated in solution by proton and phosphorus NMR. The majority of the exchangeable and nonexchangeable proton resonances have been assigned by analysis of two-dimensional homonuclear COSY and NOESY experiments. The most significant results are that the residue opposite the abasic site stacks into the helix and that the integrity of the helix is maintained in the absence of the base, the cyclic ribose moiety, and one of the carbon atoms of the carbon-phosphodiester backbone. Several phosphorus resonances are shifted downfield of the chemical shift range typical of phosphodiester groups in B-DNA. These resonances have been assigned by a combination of 2-D heteronuclear proton-phosphorus correlation experiments and specific ^{17}O labelling experiments, indicating that the conformation of the phosphodiester backbone is different in each of the three nonanucleotide duplexes.

CA 104 **NMR STUDIES OF THE EXOCYCLIC 1,N^2-PROPANO-2'-DEOXYGUANOSINE ADDUCT IN A DNA DUPLEX: DISTINCT CONFORMATIONS AT LOW AND HIGH pH.** Michael Kouchakdjian[1], Edward Marinelli[2], Frances Johnson[2], Arthur Grollman[2], and Dinshaw Patel[1], [1]Department of Biochemistry and Molecular Biophysics, College of Physicians and Surgeons, Columbia University, New York, NY 10032, [2]Department of Pharmacological Sciences, SUNY, Stony Brook, NY 11794. Exocyclic nucleic acid adducts result from the covalent modification of DNA by various carcinogenic and mutagenic agents. The exocyclic 1,N^2-propano-2'-deoxyguanosine lesion was studied opposite adenosine in a nonanucleotide duplex by two dimensional NMR. The steric constraints resulting from the exocyclic modification are resolved in the DNA helix through the formation of two distinct pH dependant conformations. At pH 5.8, the guanine base containing the exocyclic adduct has a distorted syn glycosidic torsion angle with the exocyclic ring directed into the major groove. The modified base is stabilized by using its Hoogsteen edge for the formation of two hydrogen bonds with the ring protonated adenosine on the partner strand. At pH 8.9, the exocyclic base adopts an anti configuration and is oriented into the minor groove. This work emphasizes the importance of protonation at low pH and the need for two hydrogen bonds in the stabilization of purines oriented with a syn glycosidic torsion angle.

CA 105 THE STRUCTURE OF AN ACETYLAMINOFLUORENE MODIFIED DODECAMER Thomas R. Krugh and Guanjin. J. Huang Department of Chemistry University of Rochester Rochester, N.Y. 14627
The complementary dodecamers, d(CACACGCACACA) and d(TGTGTGCGTGTG) were synthesized by the solid phase phosphoramidite method and purified by reverse phase HPLC. The single guanine of d(CACACGCACACA) was reacted with the carcinogen acetoxy-acetylaminofluorene and purified by HPLC giving the acetylaminofluorene (AAF) modified dodecamer d(CACAC^{AAF}GCACACA) with the acetylaminofluorene moiety attached to the C8 position of guanine. The purified adduct was mixed in a 1:1 ratio with the complementary strand to give the d(CACAC^{AAF}GCACACA)• d(TGTGTGCGTGTG) modified duplex. Under the conditions used for the two-dimensional NMR experiments the T_m of the modified duplex is close to 60 °C. The proton resonances of both the modified and unmodified duplexes were assigned by NOESY and TOCSY experiments at various temperatures and mixing times. The addition of the AAF moiety results in structural distortions near the modified guanine. ^{31}P NMR data also reflect the structural distortions. Imino proton spectra on the modified duplex indicate that the imino protons of all base pairs are shielded from rapid solvent exchange, with the exception of the modified guanine N_1 proton which exhibits faster exchange than adjacent guanine N_1 protons. The analysis of the data in terms of the structure of the modified and unmodified duplexes will be presented. Supported by NCI grant CA-35251.

CA 106 THE CONFORMATION OF A CONSENSUS SEQUENCE FOR LOOP E OF 5S RIBOSOMAL RNA. G. Varani, B. T. Wimberly & I. Tinoco, Jr., Department of Chemistry and Laboratory of Chemical Biodynamics, University of California, Berkeley, CA, 94720
The solution structure of an RNA oligonucleotide (26-mer) containing the consensus sequence for loop E of eukaryotic 5s RNA has been investigated by one- and two-dimensional NMR. The exchangeable proton spectrum indicates that an internal loop separates two stems of 4 and 5 base-pairs. This observation rules out alternative structures containing mismatched base-pairs proposed from chemical and enzymatic studies. The non-exchangeable proton spectrum has been assigned by 2-dimensional NMR. Interproton distances from NOESY at short mixing times and scalar couplings derived from 2QF-COSY and 2-quantum experiments have been used to determine glycosidic angles, sugar puckers and other conformational features. The conformation of the stems is very close to standard A-form RNA, and extensive stacking is conserved in the loop. Unusual structural and dynamic features are localized in the nucleotides connecting the loop to the stems. It is likely that some of these features are specifically recognized by transcription factor IIIA and by ribosomal proteins.

CA 200 ANTIBODY-ANTIGEN INTERACTIONS AND BOUND ANTIGEN CONFORMATION IN COMPLEXES OF THREE ANTIPEPTIDE ANTIBODIES STUDIED BY 2D TRNOE DIFFERENCE SPECTROSCOPY.
Jacob Anglister*, Michael Levitt**, Barbara Zilber*, *Polymer Research Department, The Weizmann Institute of Science, Rehovot 76100, ISRAEL and Department of Cell Biology, Stanford University, Stanford CA 94305, USA.

We have calculated 2D TRNOE difference spectra which pinpoint cross peaks due to intermolecular transfer NOE between antibodies and peptide antigen and cross peaks due to intramolecular magnetization transfer in the bound peptide further transferred to the free. Three monoclonal antibodies have been studied; two of them cross reactive with cholera toxin while the third does not bind the toxin. In particular, we have been studying the interactions between the antibodies' aromatic amino acids and peptide residues. Preliminar models for the combining sites were calculated based on the crystallographic data available for other antibodies. The models allow us to identify the interacting antibodies residues. Model building and the 2D difference spectra indicate that the two antibodies cross reactive with cholera toxin recognize a loop structure while the one that does not bind the toxin recognize a totally different conformation.

CA 201 STRUCTURAL STUDIES OF PEPTIDES CONTAINING ALPHA-METHYL AMINO ACIDS, Denise D. Beusen[1], William C. Hutton[2], William B. Wise[2], Miroslaw T. Leplawy[3], Janusz Zabrocki[3], and Garland R. Marshall[1]. Department of Pharmacology[1], Washington University School of Medicine, St. Louis, MO 63110; Monsanto Company[2], St. Louis, MO 63198; and Institute of Organic Chemistry[3], Politechnika, Lodz, Poland
The incorporation of alpha-methyl amino acids (MeX) dramatically reduces the conformations available to a peptide, thereby making these residues useful tools in drug design and in the study of peptide conformation. Unfortunately, the absence of an alpha proton means that sequential resonance assignment using straightforward COSY and NOESY methods is not possible, particularly when several MeX residues are linked together. Using indirect detection methods (HMQC and HMBC) in conjunction with COSY and NOESY experiments, we have assigned ^{1}H and ^{13}C resonances in two MeX-containing peptides: Ac-Val-MeF-Ile-NMe (derived from positions 3-5 of angiotensin) and Ac-Phe-MeA-MeA-MeA-Val-Gly-Leu-MeA-MeA-OH (segment 1-9 of emerimicins III & IV). These assignments have enabled us to do structural determinations for both peptides.

CA 202 **Solution Structure of Transforming Growth Factor-alpha (TGF-α) by NMR Methods,** Stephen C. Brown, Luciano Mueller, Frank Brown, Judy Hempel, and Peter W. Jeffs, Smith Kline & French Laboratories, Mail Code L-940, P. O. Box 1539, King of Prussia, PA 19406-0939.

Transforming growth factor alpha (TGF-α) has been implicated as an autocrine agent regulating the growth of several types of cancer. The proton NMR resonances of human TGF-α have been completely assigned and three dimensional structures were generated from the NOE data using the distance geometry algorithm DSPACE. The distance constraints and structures were analyzed by several geometrical methods designed to detect inconsistencies in the input data and resulting local structural distortions. In addition, NOESY spectra were back-calculated from the refined structures to ascertain the match between the original data and the calculated structures.

CA 203 **SEQUENCE-SPECIFIC CARBON RESONANCE ASSIGNMENTS FOR MURINE EPIDERMAL GROWTH FACTOR DETERMINED BY PROTON-DETECTED HETERONUCLEAR CORRELATED SPECTROSCOPIES AT NATURAL ^{13}C ABUNDENCE,** Bernardo Celda[a], Gaetano T. Montelione[b] & Gerhard Wagner[b], [a] Departmento de Quimica Fisica, Facultad de Quimicas, Univ. de Valencia, 46100-Burjassot (Valencia), Spain; Institute of Science and Technology, [b] Biophysics Research Division, Univ. of Michigan, Ann Arbor, MI 48109.

Heteronuclear 2D NMR experiments provide infomation about protein structure and dynamics which is complementary to that obtained from homonuclear proton experiments. In order to access this information, we have determined a nearly complete set of protonated-carbon resonance assignments for the murine epidermal growth factor (mEGF; 53 amino acids) at pH 3.0, 28° C., and protein concentration of ca 5mM. These ^{13}C-NMR assignments were obtained by proton-carbon correlation from the previously determined sequence-specific ^{1}H-NMR assignments[1] of mEGF. The 2D-NMR spectra used in determining these carbon assignments include double-DEPT z-filtered and multiple quantum zz-filtered heterocorrelated spectroscopies[2,3] obtained with proton-detection at natural ^{13}C abundence. Features of these spectra provide information about internal molecular dynamics, particularly with regard to the relative motion of the two domains which constitute the mEGF structure.

1. Montelione, G.T., Wüthrich, K. & Scheraga, H.A. Biochemistry 27:2245 (1988).
2. Nirmala, N.R. & Wagner, G. J. Am. Chem. Soc. in press
3. Montelione, G.T. & Wagner, G. J. Am. Chem. Soc. submitted.

CA 204 NMR OF ANTIBODY - ANTIGEN INTERACTIONS : EPITOPE MAPPING AND CONFORMATIONAL STUDIES OF THE "LOOP" PEPTIDE. Janet C. Cheetham, Christina Redfield, Robert E.Griest, Christopher M. Dobson and Anthony R. Rees, Laboratory of Molecular Biophysics, South Parks Road, Oxford, OX1 3QU, England.

The "loop" comprises residues 57 - 84 of the hen egg-white lysozyme (HEL) sequence. A panel of monoclonal antibodies was raised against the peptide immunogen and five that also showed cross-reactivity to the native protein selected. For one, Gloop1, two-dimensional nmr has been used to study binding of the peptide antigen, and in particular to identify the regions of the "loop" immobilised at the antibody combining site surface. The epitope assigned in this way is larger than that suggested by serological studies of the protein antigen, lysozyme, twenty of the twenty-eight "loop" residues showing some perturbation in their nmr spectrum between the "free" and "bound" states. The possible structural origins of this difference are examined and its implications for the use of linear peptides as "mimics" of protein epitopes discussed. Preliminary results of nmr studies of the solution conformation of the "loop" peptide are also reported. NOE measurements on the disulphide-bridged or "closed" form of the "loop" are being used to probe structural similarities between this region in the peptide and protein antigens.

CA 205 SOLUTION STRUCTURES OF GROWTH HORMONE RELEASING FACTOR ANALOGS AS DETERMINED BY 2-D NMR AND CONSTRAINED MOLECULAR DYNAMICS: SYNTHETIC HELIX STABILIZATION. David Fry, David Greeley, Vincent Madison, Arthur Felix, and Edgar Heimer. Departments of Physical Chemistry and Peptide Research, Hoffmann-La Roche, Inc., Nutley, NJ 07110.

NMR was used, along with molecular dynamics and CD, to determine structures for GRF(1-29)-NH_2 and three analogs: [Ala^{15}]-GRF(1-29)-NH_2; [Sar^{15}]-GRF(1-29)-NH_2; and cyclo$^{8-12}$[Asp^8,Ala^{15}]-GRF(1-29)-NH_2. Assignments were made from 2-D experiments, including COSY, relay-COSY, DQ, TQ, HOHAHA, and NOESY. NOEs were converted into distance constraints, and applied in the molecular dynamics program CHARMM, to obtain low-energy structures which satisfied the NMR data. The structures were further evaluated in terms of agreement with CD.

In 75%CD_3OH/25%H_2O pH 6, GRF(1-29)-NH_2 is α-helical from residues 4-29, with some kinks. The Ala^{15} and cyclo$^{8-12}$ analogs are very similar, each in an α-helix of nearly ideal geometry, from residues 4-29. The 8-12 lactam bridge seems fully compatible with an α-helix. The structures do not change significantly at pH 3. The Sar^{15} analog, soluble only at pH 3, is extended at residues 1-17, with some irregular helix at 18-27.

In 90%H_2O/10%D_2O pH 3, GRF(1-29)-NH_2 has α-helical structure only at residues 9-14 and 24-28. The Ala^{15} analog has α-helices at residues 9-15 and 21-26. The cyclo$^{8-12}$ analog retains more α-helical structure, comprised of residues 7-17 and 21-25. Therefore, it appears that the asp_i-lys_{i+4} lactam bridge represents a synthetic means of stabilizing an α-helix.

CA 206 **Environmental dependancy of the conformation of bradykinin as it might relate to the biologically active species.** R. P. Hicks, P. R. Blake, D. J. Kyle, W. J. Rzeszotarski, Nova Pharmaceutical Corporation. 6200 Freeport Centre, Baltimore MD 21224.

Bradykinin is a nonapeptide hormone (Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-Pro^7-Phe^8-Arg^9) involved, either directly or indirectly, in a variety of physiological responses including inflammation, regulation of blood pressure, pain, and symptoms associated with the common cold. Without question, the conformation of a small linear peptide such as bradykinin will be rigorously dependent on it's local chemical environment. This concept can be extrapolated to support the notion that, while random in solution, the conformation of bradykinin becomes ordered in the non-polar biophase of the cell membrane prior to it's occupation of the receptor. As an approach toward quantifying this environmental dependancy, we have examined bradykinin in DMSO solution as well as in 90% dioxane/water, the latter of which is a non-solvating environment.

Sequence specific resonance assignments were made using a combination of modern NMR techniques including COSY, HOHAHA, NOESY, and ROESY. The experimentally observed inter-proton distances were subsequently incoprorated as restraints into a series of molecular dynamics calculations as a means of obtaining reasonable solution conformations. Comparisons and contrasts pertaining to the conformation of bradykinin in these two distinct chemical environments will be discussed in terms of relevance to a biologically active conformation.

CA 207 SPECTROSCOPIC ANALYSIS OF ZINC FINGER DOMAINS OF YEAST ADR1. Grace Parraga, E. T. Young and Rachel E. Klevit. Department of Biochemistry, University of Washington, Seattle, WA 98195.

The 'zinc finger' DNA-binding motif was first proposed based on sequence analysis, partial proteolysis and zinc content of *Xenopus* transcription factor IIIA. Other eukaryotic proteins have subsequently been found to contain contiguous repeats of this postulated DNA binding domain. The yeast protein ADR1, a positive transcription regulator of ADH2, contains two tandemly repeated finger domains. Point mutations in ADR1 showed that specific conserved residues within the finger domains were essential for protein function. To obtain structural information, single and double zinc finger peptides were chemically synthesized and the metal and DNA-binding properties of these peptides were assessed as well as the solution structure of the metal stabilized domains. An experimentally determined model of a single finger is proposed that is consistent with circular dichroism, visual and 2DNMR spectroscopy of the peptide reconstituted in the presence of zinc. In particular 1D and 2D NOEs confirm the existence of a hydrophobic core, (involving conserved Phe, Leu and His residues) which was predicted to form stabilizing the small globular domain. As well, zinc binding is coincident with the formation of an α-helix that is amphiphilic which may be important in the facilitation of DNA binding by the peptide. Our data support the hypothesis that a single zinc finger can fold into an independent structure sufficient for DNA binding.

CA 208 SYSTEMATIC SEARCH IN ANALYSIS OF NMR CONSTRAINTS OF PEPTIDES,
Garland R. Marshall, Denise D. Beusen, Hiroshi Iijima, S. F. Karasek, Berkeley Shands and Richard A. Dammkoehler, Departments of Pharmacology and Computer Science, Washington University, St. Louis, MO 63110

Algorithms for the systematic exploration of conformational space to determine the set of conformers consistent with distance constraints derived from NMR have been developed. As a test case, the data of Kessler et al. on cyclosporin, a cyclic undecapeptide, was analyzed using a 10^{o} grid search on the backbone. Over 2 million conformers were consistent which were of two classes. One class was that seen in the crystal structure, while the other class differed in the type of beta turn. Only the class in the crystal structure was found in published work when the data was analyzed by either distance geometry or constrained molecular dynamics, suggesting inadequate sampling in the applications of these approaches. Several other peptides have been analyzed by this approach and new algorithms developed whose speed make this approach viable for structures with sufficient NMR constraints.

CA 209 **SOLUTION STRUCTURES OF MURINE EPIDERMAL AND HUMAN TYPE-α TRANSFORMING GROWTH FACTORS DETERMINED BY NMR AND DISTANCE GEOMETRY**,
G.T. Montelione[1], M.E. Winkler[2], A.W. Burgess[3], K. Wüthrich[4], H.A. Scheraga[5], & G. Wagner[1], [1]Institute of Science and Technology, Biophysics Research Division, Univ. of Michigan, Ann Arbor, MI 48109; [2]Dept. of Medicinal and Biomolecular Chemistry, Genentech, Inc., S. San Francisco, CA 94080; [3]Ludwig Institute, Melbourne Tumour Biology Branch, Victoria 3050, Australia; [4]ETH-Hönggerberg, CH-8093 Zürich, Switzerland; [5]Baker Laboratory of Chemistry, Cornell Univ., Ithaca, NY 14850.

Three dimensional structures for natural murine epidermal (mEGF) and recombinant human type-α transforming (hTGFα) growth factors in aqueous solution have been determined by ^{1}H-NMR spectroscopy. Both mEGF and hTGFα have two small β-sheets, which are structurally homologous. For mEGF, the ^{1}H-NMR data were used as input for DISMAN distance geometry calculations to determine the polypeptide chain fold. This solution structure was subsequently refined by incorperating many more NOE-derived distance constraints (particularly between the two β-sheet domains) and spin-spin vicinal coupling constants. These additional data determine more precisely the relative orientations of the two domains and the side-chain conformations. Distance geometry calculations have also been used to determine the polypeptide chain fold of hTGFα. Despite their overall three-dimensional structural homology, amide proton exchange studies reveal differences in the structural dynamics of mEGF and hTGFα at pH 3.5 and 28° C. Our progress in completing ^{13}C and ^{15}N assignments for these growth factors, which are needed for more detailed dynamic studies, are also described.

CA 210 THE SOLUTION CONFORMATION OF TYPE I COLLAGEN α-1 CHAIN N-TELOPEPTIDE
Albin Otter,Xiaohong Liu,George Kotovych and Paul G. Scott,Departments of Chemistry and Oral Biology,University of Alberta,Edmonton/Alberta,Canada

The N-terminal telopeptide of type I α-1 collagen directs linear growth of the fibrils in collagen fibrillogenesis. This function presumably depends on specific interactions between the telopeptide and the triple helices of collagen monomers adjacent to it in the growing fibrils. Because such interactions are likely to be strongly conformation dependent, we studied the structures of this important region of the collagen monomer by 600 MHz ^{1}H NMR spectroscopy. Based on various two-dimensional, phase-sensitive techniques such as COSY, RELAY and ROESY, the conformation of the backbone of the 19 amino acid peptide could be established. In H_2O, pH 7, a completely extended structure is observed. In CD_3OH/H_2O (60/40) the conformation is significantly different, and the spectroscopic evidence clearly indicates that the D^{7}-E^{8}-K^{9}-S^{10} fragment forms a β-turn, stabilized by a hydrogen bond between NH(S^{10}) and CO(D^{7}). The sections preceding and succeeding the turn exist in a non-random, extended conformation with no inter-action between the two parts of the peptide, thus excluding a β-sheet. Our data indicate that type I and type II β-turns coexist. The nature of the β-turn was studied in more detail in a NMR analysis of the D-E-K-S fragment. The results clearly indicate that a charge interaction between the opposite charges of the E^{8} and K^{9} side-chains is essential for the formation of the β-turn.

CA 211 CONFORMATIONAL ANALYSIS BY NUCLEAR MAGNETIC RESONANCE OF SYNTHETIC PEPTIDES INHIBITING HERPES SIMPLEX VIRUS RIBONUCLEOTIDE REDUCTASE, Paul R. Rosevear, George Krudy, Suman Rakhit, Jean-Marie Ferland, Shafi Hosain and Sylvie Goulet, Department of Biochemistry and Molecular Biology, University of Texas Health Science Center Houston, Houston, TX 77225, and Bio-Mega Quebec, Canada

Two dimensional proton nuclear magnetic resonance spectroscopy at 500 MHz has been used to characterize several peptides known to specifically inhibit herpes simplex virus type I and type 2 ribonucleotide reductase. With the use of sequential assignment methods, chemical shifts were obtained for all backbone and side-chain protons in both aqueous and nonaqueous, DMSO/sulfolane solvents. Temperature dependence of the amide proton chemical shifts, coupling constants and magnitude of observed nuclear Overhauser effects suggest a preferred conformation for several of these peptides in solution. Determination of the preferred solution structure and dynamics of these peptides are under investigation using a combination of distance geometry and molecular dynamics calculations. These results are used to propose an explanation for the inhibitory activity of these antiviral peptides.

CA 212 2D AND MULTINUCLEAR NMR STUDIES OF Zn AND ^{113}Cd ADDUCTS WITH A ZINC FINGER FROM HIV-1 NUCLEIC ACID BINDING PROTEIN, P7, Michael F. Summers, Terri L. South, Bo Kim, and Dennis Hare, Chemistry Department, University of Maryland Baltimore County, Baltimore, MD 21228, and Hare Research Inc. (D.H.), 14810 216th Avenue, N.E., Woodinville, WA 98072.

110 MHz ^{113}Cd and 500 MHz ^{1}H NMR results are presented for the 1:1 Cd^{2+} and Zn^{2+} adducts with an 18-residue, synthetic peptide with the amino acid sequence of the first finger region (residues 13 through 30) of nucleic acid binding protein (NABP), p7, from HIV-1 (the causative agent of AIDS). The ^{113}Cd NMR chemical shift of the $^{113}Cd^{2+}$ adduct ($^{113}Cd(p7^{13-30})$; δ = 653 ppm) is consistent with 3Cys, 1His coordination and is in excellent agreement with the shift of 638 ppm ($\Delta\delta$ = 15 ppm) observed for Cd bound by 3Cys and 1His in Cd-substituted gene 32 protein (g32p) (D. Griedrac, J.E. Coleman, in preparation). 2D ^{1}H-^{1}H and ^{1}H-^{13}C (HMQC) correlation experiments on the zinc adduct afforded complete assignment of the ^{1}H spectrum and partial assignment (δ < 100 ppm) of the ^{13}C spectrum. Whereas the ^{1}H signals of the amide protons in metal-free $p7^{13-30}$ are broad and do not exhibit NH-CH_α scalar coupling, 15 of the 17 backbone amide protons in $Zn(p7^{13-30})$ appear as resolved multiplets, reflecting the presence of a single, highly stable tertiary structure. From analysis of 2D ROESY, 2D NOESY, and scalar coupling data, a preliminary tertiary structure has been determined for the zinc adduct. Structure refinements based on quantitative treatment of NOE data are underway. Our results provide support for earlier predictions (Berg, J.M. Science (1986), 232, 485) regarding the mode of metal binding to retroviral NABPs.

CA 213 **Two Dimensional Proton NMR Studies of Arc Repressor Mutants: Resonance Assignments and Secondary Structure Predictions.** Michael G. Zagorski[1], James U. Bowie[2], Robert T. Sauer[2], and Dinshaw J. Patel[1], [1]Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY 10032, [2]Department of Biology, MIT, Cambridge, MA 02139. Arc, a small, 53 amino acid protein produced by the *Salmonella* phage P22, binds as a dimer to its DNA operator site and represses transcription from the P_{ant} promoter of P22. Previous work using missense mutations demonstrated that the N-terminal residues are important for operator recognition but do not affect the ability of Arc to fold into a stable three-dimensional structure, thus suggesting that Arc may not possess the conventional helix-turn-helix DNA binding structural motif. Extensive 2D NMR work was performed on a mutant (PL8) having leu instead of pro at position 8 which had a higher melting temperature and therefore permitted data to be recorded at higher temperatures. Analysis of NOESY data recorded in H_2O solution indicates that PL8 is predominately helical with two separate α-helical segments separated by a reverse or half turn. Residues located at the N-terminus, however, form part of a anti-parallel β-sheet arrangement thus further suggesting that indeed DNA binding of Arc does not involve a conventional helix-turn helix structure.

Protiens/Metalloproteins

CA 300 H NMR STUDIES ON PARAMAGNETIC METALLOPROTEINS - LUCIA BANCI, IVANO BERTINI, AND CLAUDIO LUCHINAT, Department of Chemistry University of Florence and Institute of Agricultural Chemistry, University of Bologna, Italy

NMR spectroscopy on paramagnetic systems is a powerful technique in the study of metalloproteins. The understanding of the factors affecting the NMR parameters, i.e. isotropic shift and nuclear and electron relaxation, for the different metal ions naturally or artificial present in the protein is the basis for the structural characterization of the active site and for the understanding of the structure-function relationship. In particular we have rationalized the NMR parameters for magnetically coupled dimers, metal ion centers often present in metalloproteins. The comprehension of the effect of magnetic coupling on the isotropic shifts as far as on nuclear and electron relaxation is necessary for the assignment of the NMR spectra in these systems and for their structural characterization. Furthermore NOE measurements allow us to design the active cavity and to understand the role of the various aminoacid residues during the enzymatic catalysis. These advanced techniques may take the advantage of side directed mutagenesis, that allows the substitution of key residues in the active cavity.

The ^{1}H NMR characterization of the active cavity of superoxide dismutase (SOD) and carbonic anhydrase (CA) will be presented and the results will be discussed in terms of reaction mechanism. Spectroscopic studies on a large number of mutants of SOD, in which residues inside the active cavity have been changed, have allowed us to clarify the role of some residues during the enzymatic reaction and to answer to some open questions on the reaction pathway.

CA 301 TOWARDS THE STRUCTURAL BASIS FOR REGULATION BY Ca-BINDING PROTEINS USING 2D 1H NMR, Johan Kordel, Nick Skelton, Torbjorn Drakenberg, Sture Forsen & Walter J. Chazin, Dept. of Molecular Biology, Research Institute of Scripps Clinic, 10666 N. Torrey Pines Road, La Jolla, CA 92037

The calcium ion (Ca2+) is ubiquitous in biological systems, performing a variety of roles which include functioning as a "second messenger" in cell division and growth, muscle contraction, secretion, ion transport and certain metabolic processes. A central role in this messenger system is played by a family of highly homologous regulatory proteins (eg., calmodulin, troponin C) that undergo conformational changes upon binding of Ca2+, thereby translating transient influxes of Ca2+ into metabolic or mechanical responses. Despite great structural similarities, these proteins exhibit a wide range of Ca2+ affinities and selectivities. One of the principal objectives of research on this family of proteins is to understand the role of protein conformation and dynamics in determining function and metal binding properties. We address these issues using site-directed mutagenesis and 1H NMR. Our initial studies have focussed on Calbindin D9k because its small size (75 amino acids) makes it readily amenable to 1H NMR. We report on our progress with the comparative analysis of the conformation and dynamics of calcium-free and calcium-loaded forms of a binding domain from this family of proteins.

CA 302 NMR STUDIES OF PROTEIN STRUCTURE - APPLICATIONS TO SMALL AND NOT QUITE SO SMALL PROTEINS, Paul C. Driscoll, Paul J. M. Folkers, G. Marius Clore and Angela M. Gronenborn, Laboratory of Chemical Physics, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892, U.S.A.

The structure of a small 43 amino acid polypeptide from a sea anenome has been determined to high resolution from NMR data alone. We shall show how the inclusion of stereospecific assignments of the β-methylene protons into the determination of interproton distances results in a significant improvement in the 'quality' of the structures obtained by hybrid distance-geometry/dynamical simulated annealing calculations. We are also investigating the usefulness of ring current shift calculations in the identification of stereospecific assignments of a slowly flipping tyrosine ring and surrounding prochiral groups, which would enable further refinement of the structures obtained. Similar improvement in the definition of the structure of the anticoagulant protein Hirudin has been also been obtained, though in this case additional information was obtained by a comparison of the spectra of the wild type protein with a point mutant. Experience shows that improvement of the definition of protein structures comes best with the maximisation of the number of experimental data.

We are also endeavouring to extend the NMR technique to proteins that are large on the NMR scale. We shall show experiments performed on the important lymphokine interleukin-1β protein (M.W. 17,377 Da). We are applying an assignment strategy based on the use of nitrogen-15 and deuterium labelled Il-1β, specifically with heteronuclear multiple quantum correlation and relay experiments, including 3D NMR. Additionally the availability of a number of single residue mutants has aided greatly in the attempt to assign the NMR spectrum of this 153 residue recombinant protein.

CA 303 STRUCTURAL FEATURES OF A MEMBRANE PROTEIN DETERMINED BY ^{1}H NMR: THE DCCD-BINDING SUBUNIT OF THE F_1F_0 ATPASE, Mark E. Girvin and Robert H. Fillingame, Department of Physiological Chemistry, University of Wisconsin, Madison, WI 53706

F_1F_0 ATPases couple the transmembrane flow of protons to ATP synthesis or hydrolysis. The F_0 sector is the transmembrane proton translocating portion of the enzyme, and is composed of 3 kinds of subunits: a, b, and c. Subunit c in E. coli contains 79 residues, and has been postulated to span the membrane as a hairpin of two α-helices. Proton translocation is abolished by the specific binding of DCCD to Asp_{61} of the c subunit. This acidic residue is positioned in the middle of the proposed hydrophobic C-terminal helix.

Subunit c of the ATPase appears to be a good candidate for a structural study of a membrane protein by NMR because of its small size and the availability of a large number of point mutations to aid with assignments. We are determining the structure of purified subunit c in organic solvent. Biochemical studies on mutant and wild type forms of the protein showed that the solubilized protein retains properties seen in the native complex. Using COSY and HOHAHA spectra, 48 of the side chains of subunit c have been assigned to amino acid type. Fourteen residues have been assigned specifically (Tyr_{10}, Gly_{18}-Ala_{20}, Ala_{24}, Lys_{34}, Gly_{38}-Ala_{39}, Thr_{51}, and Gly_{71}-Met_{75}). Our structural conclusions to date, that Ala_{24} and Asp_{61} are in close proximity, and that at least the ends of the membrane spanning domains of the c subunit are α-helical, agree with the models proposed for this protein. We are now extending our assignments using mutants, isotopic labelling, and a synthetic peptide.

CA 304 CONFORMATIONAL CHANGES DURING BINDING OF THE SNAKE NEUROTOXIN, α-BUNGAROTOXIN, TO SYNTHETIC PEPTIDES CORRESPONDING TO THE BINDING DOMAIN OF THE NICOTINIC ACETYLCHOLINE RECEPTOR. Edward Hawrot, Guo-qiang Song, and Ian Armitage, Departments of Pharmacology, Molecular Biophysics and Biochemistry and Diagnostic Radiology, Yale University School of Medicine, New Haven, CT 06510

NMR studies reveal that significant conformational changes occur upon the binding of the snake neurotoxin, α-bungarotoxin (BGTX), to either of two synthetic peptides derived from the primary sequence of a portion of the binding domain located on the α subunit of the nicotinic acetylcholine receptor. One synthetic peptide, a 12mer corresponds to residues 185-196, and the other, an overlapping 18mer, extends from 181-198 of the α subunit. In both cases, upon formation of the binding complex the C4 proton resonance from His-4 of BGTX is converted to two resonances of equal intensity, in apparent slow exchange with each other. With the 18mer, the two peaks can be immediately observed, whereas with the 12mer, a longer time of incubation is required to resolve the two peaks, although other resonance changes in the 12mer are observed immediately upon mixing. The interpretation that the two resonances represent chemical exchange between two conformers of the bound complex is supported by variable temperature studies, by varying the ratio of components, as well as by saturation transfer and selective inversion experiments. Changes in other aromatic resonances also are consistent with this interpretation. Upon complex formation, a number of new resonances appear in the downfield region possibly corresponding to the C2 protons of the three histidines in the complex. Preliminary studies suggest that some of these peaks also are modulated by chemical exchange. In a control study, BGTX was added to a 14mer comprised of an unrelated peptide sequence. No perturbations were observed in any of the resonances in the aromatic region of the spectrum suggesting that the changes observed with the 12mer and the 18mer were due to "specific" binding interactions.

CA 305 ^{1}H NMR STUDIES ON BOVINE CYCLOPHILIN: STRUCTURAL CHARACTERIZATION OF THE PROTEIN AND ITS COMPLEX WITH CYCLOSPORIN A, Sarah L. Heald*, Matthew W. Harding¶, Robert E. Handschumacher¶ and Ian M. Armitage*,§, Department of Molecular Biophysics and Biochemistry*, Diagnostic Radiology§ and Pharmacolgy¶, Yale School of Medicine, New Haven, CT 06510. Cyclophilin (CyP, 163 residues, Mr 17,737 Daltons) is a cytosolic protein that specifically binds the potent immunosuppressant cyclosporin A (CsA). The native form of the bovine thymus isoform has been analyzed by 2D NMR methods in aqueous media, as well as following exhaustive D_2O exchange. Spectral analysis of the NH/NH, NH/αCH and αCH/αCH regions using a semi-rigorous form of the Main-Chain Directed (MCD) strategy indicates that the residual amides in D_2O form a coherant hydrophobic domain which yields 2D NMR features suggestive of beta-sheet. The drug-free and CsA-bound CyP form two discrete protein structures which are in slow exchange on the NMR timescale. Based on chemical shift changes observed for assigned sidechain and/or mainchain resonances, only a few of the amino acid residues identified to date are perturbed by complex formation. The regions of the protein affected by complexation can be divided into two categories: a hydrophobic and a H_2O accessible domain. The amino acids perturbed by complexation in the hydrophobic domain include Leu1, Val1 and Phe12 which appear to be a part of an anti-parallel beta-pleated sheet. The amino acids in the H_2O accessible region of the protein include the one Trp, Ala7, Ala8 and L10 residues.

CA 306 ASSIGNMENTS IN THE CARBON NMR SPECTRUM OF A LARGE PEPTIDE: BOVINE PANCREATIC POLYPEPTIDE (BPP). Ann H. Hunt and Ronald E. Chance, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285

Bovine pancreatic polypeptide, BPP, is a 36-residue hormone first isolated during the purification of insulin and glucagon:

1 10 20
H.Ala.Pro.Leu.Glu.Pro.Glu.Tyr.Pro.Gly.Asp.Asn.Ala.Thr.Pro.Glu.Gln.Met.Ala.Gln.Tyr.
30
Ala.Ala.Glu.Leu.Arg.Arg.Tyr.Ile.Asn.Met.Leu.Thr.Arg.Pro.Arg.Tyr.NH_2

Heteronuclear correlation experiments are being applied to obtain ^{13}C NMR assignments for BPP in H_2O solution.

CA 307 NMR HYDROGEN EXCHANGE STUDIES OF CRAMBIN IN NONAQUEOUS SOLVENTS, Jack Hwang and Frances H. Arnold, Division of Chemistry and Chemical Engineering 206-41, California Institute of Technology, Pasadena, CA 91125.

The small protein crambin is soluble and stable in a wide variety of polar nonaqueous solvents and provides an excellent opportunity for studying solvent effects on protein structure and dynamics. Proton resonance assignments have been completed for a number of residues in crambin. To investigate solvent effects on the dynamics of crambin in different organic solvents, 1D and 2D NMR was used to measure exchange rates of long-lived amide protons distributed along the crambin backbone.

CA 308 ^{1}H-NMR ANALYSIS OF BRANCHED TYPE 2 CHAIN LACTO-SERIES GANGLIOSIDES FROM HUMAN ERYTHROCYTES, S.B. Levery, N.H. Andersen and S. Hakomori, The Biomembrane Institute and Univ. of Washington Dept. of Chemistry, Seattle, WA 98119.

Branched blood-group active type 2 chain lacto-series gangliosides isolated from human erythrocyte membranes have been proposed to have the general structure (Watanabe et al., J. Biol. Chem. 253:8962, 1978; Kannagi et al., Carbohydr. Res. 120:143, 1983):

$R_1 \rightarrow 3$
$R_2 \rightarrow 2\,\beta$Gal$\rightarrow$4βGlcNAc$\searrow 6$
$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad 3\,\beta$Gal$\rightarrow$4$\beta$GlcNAc$\rightarrow$3$\beta$Gal$\rightarrow$4$\beta$Glc$\rightarrow$1Ceramide
$R_3 \rightarrow 3\,\beta$Gal$\rightarrow$4βGlcNAc$\nearrow$

1, R_1=OH, R_2=αFuc, R_3=αNeuAc (well characterized)
2, R_1=αGal, R_2=αFuc, R_3=αNeuAc (uncharacterized)
3, R_1=αGalNAc, R_2=αFuc, R_3=αNeuAc (partially char.)

A 2-D ^{1}H-NMR strategy (PS-DQF-COSY, RECSY, TOCSY, TQF-COSY, NOESY) has been used to confirm these structures. In addition, data relevant to questions of three-dimensional structure have been obtained and analyzed. The results can be compared with those of Dabrowski et al. (Biochemistry 27:5149, 1988) on related neutral glycosphingolipids from rabbit erythrocytes.

CA 309 ^{1}H-NMR STUDIES ON T-PA KRINGLE 2, Miguel Llinás, Robert F. Kelley† and In-Ja L. Byeon, *Department of Chemistry, Carnegie Mellon University, Pittsburgh, PA 15213,* and †*Biomolecular Chemistry Department, Genentech, Inc., South San Francisco, CA 94080.*

Tissue-type plasminogen activator (t-PA), a blood plasma glycoprotein of ~70 kD, activates plasminogen to plasmin and thus plays a crucial role in fibrinolysis. It is structured by five, covalently linked, modular units consisting of a finger, a growth factor, two kringles and a protease domains. There is good evidence indicating that the finger and the second kringle (K2) are involved in t-PA binding to fibrin. The K2 domain, $M_r \tilde{<} 10{,}000$, has been cloned and expressed in *E. coli* and is currently being characterized by 1D and 2D ^{1}H-NMR spectroscopy at 300, 500 and 620 MHz. Preliminary results indicate a folded, compact structure for K2 which is analogous to that of plasminogen (PLGN) kringles. Assignments are proposed for the aromatic K2 resonances based on comparison with the PLGN K4 spectrum and site specific mutants. As is the case for the PLGN K4, the lysine-binding site of the t-PA K2 is highly aromatic in character. We notice that the His^{64} side chain is most sensitive to ligand presence and, in conjunction with Trp^{62} and Trp^{72}, is likely to be at the binding site. Data will be presented concerning pH and ligand titratability of aromatic side chains. Experiments are in progress to totally assign the K2 ^{1}H-NMR spectrum and derive its solution conformation.

CA 310 SOLUTION STRUCTURE DETERMINATION AND REFINEMENT OF PLASTOCYANIN FROM PHASEOLUS VULGARIS BY 2D NMR, Jonathan M. Moore, David A. Case, Walter J. Chazin, Garry P. Gippert and Peter E. Wright, Department of Molecular Biology, Department of Molecular Biology, Research Institute of Scripps Clinic, 10666 North Torrey Pines Road, La Jolla, California 92037.

Solution structures for plastocyanin (MW 10,000) from Phaseolus vulgaris (French bean) have been generated by NMR and distance geometry methods. Structures were determined to the highest resolution possible by inclusion of a complete set of NOE distance constraints, torsion angle constraints derived from $^3J_{HN\alpha}$ and $^3J_{\alpha\beta}$ coupling constant values, and hydrogen bonding constraints obtained by observation of slowly exchanging amide protons. NOE distance constraints were obtained after careful analysis of NOE buildup curves for all resolved resonances in NOESY spectra obtained at 15 mixing times in the range 10-400 milliseconds. In addition, stereospecific assignments have been pursued for several spin system types through correlation of coupling constants and intraresidue contacts observed in the COSY and NOESY spectra, respectively. Structures obtained by NMR methods for this protein may be compared with those previously reported for plastocyanin from the green alga, *Scenedesmus obliquus* (Moore et al., Science 240, 314 (1988)) for which NMR assignments were not as extensive, as well as the structure determined for the highly homologous poplar protein by X-ray crystallographic methods (Guss & Freeman, J. Mol. Biol. 169, 497 (1986)). The resolution of the energy minimization structures, with particular regard to local structure and surface side chain conformation, are assessed as a function of the number and types of distance and dihedral angle constraints included as input for structure determination.

CA 311 **SELECTIVE AND NON-SELECTIVE T_1 MEASUREMENTS OF BINDING CONSTANTS OF ACETYLCHOLINE AND ITS ANTAGONISTS TO SYNTHETIC AND GENETICALLY ENGINEERED PEPTIDES OF THE ACETYLCHOLINE RECEPTOR.** Yigal Fraenkel*, Gil Navon*, Ami Aronheim† and Jonathan M. Gershoni.† *School of Chemistry, Tel Aviv University, Ramat Aviv, Tel Aviv 69978; †Department of Biophysics, Weizmann Institute of Science, Rehovot 76100, Israel.

The nicotinic acetylchholine receptor (AChR) is a ligand-regulated ion channel, composed of five subunits. Binding of acetylcholine to its α-subunits triggers channel opening. By systematically proteolyzing the α-subunit and checking fragments for toxin binding it was found that the region, α180-200 binds the inhibitor α-bungarotoxin(α-BTX). However due to the inadequacy of the methods for the detecting binding with K_d values exceeding 0.1 mM, binding of acetylcholine and other agonists to fragmented a subunits has not been directly demonstrated. In the present work we report such measurements, using selective and non-selective T_1 relaxation times of the ligands.

The amino acid sequence α184-200 was constructed and expressed by genetic engineering. Thus a bacterial fusion protein capable of binding α-BTX (trpE+α184-200) and a control bacterial protein with no binding capacity (trpE alone, Aronheim et al (1988) J. Biol. Chem. 263:9933) were subjected to NMR analysis. Binding constants for acetylcholine, nicotine, gallamine and d-tubocurarine to the recombinant α184-200 fusion protein were found to be: 2.4, 3.6, 0.15, and 0.24 mM respectively. For the non binding, control proteins, K_d values were always greater than 10 mM, and were regarded as background. Measurements were also performed for ligand binding to synthetic peptides corresponding to sequences α1-20, α128-143, and α184-200. Synthetic α184-200 showed similar binding constants to those obtained for its recombinant analogues, the other peptides showed only background binding.

CA 312 STEREOSPECIFIC ASSIGNMENT AT PROCHIRAL CENTERS IN PROTEINS, Michael Nilges, G. Marius Clore, Paul C. Driscoll and Angela M. Gronenborn, Laboratory of Chemical Physics, NIDDK, National Institutes of Health, Bethesda MD 20892, U.S.A.

A new method for stereospecific assignment at prochiral centers in proteins has been introduced recently (Billeter, M., et. al., XIII ICMRBS, abstract S22-6). It involves a systematic search of the conformational space compatible with intraresidue and sequential NOEs as well as $^3J_{N\alpha}$ and $^3J_{\alpha\beta}$ coupling constants. Using a similar approach, we have investigated the extent to which stereospecific assignments can be obtained from NMR data, and the factors that may prevent an assignment. Additionally, the results obtained from a systematic search are compared with those from calculations using a "floating assignments" approach.

CA 313 MECHANISMS OF MOLECULAR RECOGNITION IN HEME PROTEINS, Stephen G. Sligar, Departments of Chemistry and Biochemistry, University of Illinois, Urbana IL 61801.

Equilibrium and dynamic structures of heme proteins dictate the mechanisms and specificity of polypeptide chain folding, macromolecular association, electron transfer physics, recognition of substrates, and the chemistry of catalysis. In order to probe the structure-function correlations in heme proteins we have utilized site-directed mutagenesis of cloned or totally synthetic genes of sperm whale myoglobin, Aplysia myoglobin, rat liver cytochrome b_5, putidaredoxin, cytochrome $P\text{-}450_{cam}$ and the four-helical bundle cytochrome b_{562}. In order to precisely understand the physics and chemistry of the fundamental processes described above, concerted structural, catalytic, dynamic, spectroscopic and biochemical information must be obtained. In the case of sperm whale myoglobin, for instance, we have obtained gram quantities of the following mutants. For examining distal pocket effects, Histidine E7 (H64) was replaced by Gly, Cys, Lys, Tyr, Val, Met, Arg, Phe, Asp, and Thr. Valine E11 (V68) has been replaced with Ile, Phe, Ala, and Glu. E7/E11 double mutants Gly/Ile, Gly/Phe, and Gly/Ala have been constructed and over-expressed. The contribution of Arg 45 to the salt linkage at one pocket access channel has been examined by replacing this residue with Gly, Asp, Ser, and Asn. The role of axial ligands in the bio-inorganic chemistry of myoglobin has been realized by removing the normal histidine at F8 (H93) in favor of Cys and Tyr. In my presentation I will describe 1D and 2D proton NMR data defining the effect of these various mutations on the structure of myoglobin, linking this to defined kinetic and equilibrium data related to the mechanisms of molecular recognition in this system.

CA 314 STRUCTURAL CHARACTERIZATION OF THE INTERACTIONS BETWEEN CALMODULIN AND SKELETAL MUSCLE MYOSIN LIGHT CHAIN KINASE (576-594)G: EFFECT OF PEPTIDE BINDING ON THE Ca^{2+}-BINDING DOMAINS, A. Joshua Wand and Steven H. Seeholzer, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111

The calcium binding protein calmodulin (CaM) and its complex with a peptide derived from the sequence of the calmodulin binding domain of skeletal muscle myosin light chain kinase (skMLCK(576-594)G) have been studied by one and two dimensional ^1H NMR techniques. Resonances arising from the residues comprising the antiparallel sheet structures associated with the calcium binding domains of calmodulin and their counterparts in the CaM-skMLCK(576-594)G complex have been assigned. The assignment was initiated by application of the main chain directed assignment strategy. It is found that, despite significant changes in chemical shifts of resonances arising from residues in this region of CaM upon binding of the peptide, the antiparallel beta sheets have virtually the same structure in the complex as in CaM. Hydrogen exchange rates of peptide NH within the beta sheet structures are significantly slowed upon binding of peptide. These data, in conjunction with the observed nuclear Overhauser effect (NOE) patterns and relative intensities and the downfield shifts of associated amide and alpha resonances upon binding of peptide, show that the peptide stabilizes the Ca^{2+}-bound state of calmodulin. The observed pattern of NOEs within the β-sheets and the symmetry between them corresponds closely to that predicted by the crystal structure. These findings imply that the apparent inconsistency of the crystal structure with recently reported low angle X-ray scattering profiles may lie within the putative central helix spanning the two globular domains.

CA 315 SEQUENTIAL AMINO ACID RESIDUE ASSIGNMENTS BY ONE-BOND SCALAR COUPLINGS: APPLICATION OF $^{13}C\{^{15}N\}$ SINGLE BOND CORRELATION AND $^{13}C\{^{13}C\}$ DOUBLE QUANTUM CORRELATION TO ISOTOPICALLY LABELED PROTEINS, William M. Westler, Eldon L. Ulrich and John L. Markley, National Magnetic Resonance Facility at Madison, Department of Biochemistry, University of Wisconsin-Madison, Madison, WI 53706.

Recent applications of two-dimensional (2D) heteronuclear [$^{1}H\{^{13}C\}$, $^{1}H\{^{15}N\}$ and $^{13}C\{^{15}N\}$] and homonuclear [$^{13}C\{^{13}C\}$] NMR spectroscopy to proteins have shown the utility of selective and uniform ^{13}C and ^{15}N labeling of proteins for the assignment of specific amino acid residues in proteins. We present here a methodology for the sequence specific assignment of proteins uniformly enriched in ^{13}C and/or ^{15}N by using the scalar coupling between nuclei separated by a single chemical bond. $^{13}C\{^{13}C\}$ double quantum correlation ($^{13}C\{^{13}C\}$ DQC) is used to identify pairs of carbonyl and alpha carbons that belong to a particular amino acid. The identification of the amino acid to a specific type utilizes $^{13}C\{^{13}C\}$ connectivities of the side chain and ancillary information from other heteronuclear or $^{1}H\{^{1}H\}$ homonuclear experiments. By using $^{13}C\{^{15}N\}$ single bond correlation ($^{13}C\{^{15}N\}$ SBC), the alpha carbon can be connected to the amide nitrogen within an amino acid residue. $^{13}C\{^{15}N\}$ SBC is then used to span the peptide bond via the scalar coupling between the amide ^{15}N of residue i with the carbonyl ^{13}C of residue i-1. The use of this method will be demonstrated with the cytochrome c_{555} from Anabaena 7120 that is labeled with ^{13}C and/or ^{15}N. [Supported by National Institutes of Health Grant RR02301 from the Biomedical Research Technology Program, Division of Research Resources and Grant 88-37262-3406 from the U. S. Department of Agriculture.]

CA 316 PARVALBUMIN: METAL-ION INDUCED CONFORMATIONAL CHANGES AS PROBED BY NMR-MONITORED LANTHANIDE EXCHANGE, Thomas C. Williams* and Brian D. Sykes, MRC Group in Protein Structure and Function, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7, and *Department of Pharmacology, Medical University of South Carolina, Charleston, SC 29425. Within the *helix-loop-helix* class of calcium-binding proteins, the correlation between structure and function is best understood for parvalbumins' (108 residues) two high affinity calcium/magnesium sites. In order to evaluate the charge-density specificity of such sites, the largest lanthanide analogue of calcium, La(III), and the lanthanide analogue of magnesium, Lu(III), have been used to prepare two diamagnetic analogues of Ca-pike(III)parvalbumin. By using one- and two-dimensional proton NMR spectroscopy, we have assigned 66 of the 91 detectable mainchain HN-C(alpha)H correlations in Ca-pike(III)parvalbumin and both metal-binding sites of each lanthanide form. Local conformational perturbations arising from calcium-to-lanthanide metal-ion exchange have been evaluated from the changes in the COSY and NOESY spectra. To better understand the global conformational changes associated with metal-ion exchange in parvalbumin and larger proteins of this class, site-selective exchange by paramagnetic lanthanides has also been used. Paramagnetic contributions to the chemical shift and relaxation of several assigned NMR resonances have been determined in order to begin to evaluate paramagnetically shifted spectra of parvalbumin in terms of its solution conformation.

CA 317 STRUCTURE OF HPr, A PHOSPHOCARRIER PROTEIN, IN ITS PHOSPHORYLATED AND UNPHOSPHORYLATED FORMS. Michael G. Wittekind, Jon R. Herriott, Dennis R. Hare, E. Bruce Waygood, and Rachel E. Klevit, Department of Biochemistry, University of Washington, Seattle, WA 98195.

HPr is one of the protein components in the phosphoenolpyruvate:sugar phosphotransferase system in bacteria, and is phosphorylated on a histidine residue along the pathway in which a phosphoryl group from phosphoenolpyruvate is transferred to hexoses. Complete resonance assignments for the 85-residue HPr in its unphosphorylated form have been presented and its structure is being determined and refined using distance geometry methods. The assignments and models serve as a basis for studies on the phosphorylated form of HPr (PHPr). Although PHPr is normally hydrolyzed rapidly, conditions have been found that stabilize PHPr and a regeneration system has been designed to prolong the lifetime of PHPr to the duration of 2DNMR experiments. The COSY spectrum revealed only a limited number of chemical shift perturbations, allowing the original assignments to be used. NOESY spectra revealed both the loss and gain of NOE peaks, indicating a conformational change induced by phosphorylation. Details of the conformation of PHPr will be presented.

CA 318 RECOMBINANT HUMAN TRANSFERRIN N-TERMINAL HALF-MOLECULE; CHARACTERIZATION AND NMR STUDIES. Robert C. Woodworth, Department of Biochemistry, University of Vermont, Burlington, VT 05405; and Ross T. A. MacGillivray and Walter Funk, Department of Biochemistry, University of British Columbia, Vancouver, BC, Canada V6T 1W5.

Insertion of two stop codons in the middle of the 2.2 kb cDNA for human serum transferrin (hTF) and excision of the 1.1 kb downstream segment gave a 1.1 kb segment coding for the N-terminal half (hTF/2N) and the signal peptide. Insertion of this fragment into the vector pNUT, in place of the gene for human growth hormone, gave a construct with a mouse metallothionein promoter for hTF/2N and an SV40 promoter for dihydrofolate reductase. Transformed baby hamster kidney (BHK) cells with a high copy number of the plasmid were selected in medium containing 0.5 mM methotrexate. Within a week after induction by 0.08 mM Zn(II), the medium contained 20 to 50 mg/L of recombinant hTF/2N. The isolated recombinant hTF/2N was substantially identical to proteolytically-derived wild-type hTF/2N by SDS-PAGE, immunoreactivity, iron binding, uv-vis spectroscopy and proton NMR. Substitution of selected aromatic amino acids, deuterated in the side chain, in the BHK medium has allowed production of recombinant hTF/2N preparations with simplified NMR spectra in the low field region.

Supported by USPHS Grant DK21739, a Visiting Scientist Award from the Medical Research Council of Canada and a grant from the BC Health Care Research Foundation.

Isotopic Methods; Larger/Ordered Systems

CA 400 ^{13}C CHEMICAL SHIFTS AND DEUTERIUM ISOTOPE EFFECTS FOR STRUCTURE DETERMINATION OF PROTEINS, Poul Erik Hansen and Erik Tüchsen, Institute for Life Sciences and Chemistry, Roskilde University, DK-4000 Roskilde, Denmark.
Genetic engineering calls for fast methods to determine conformational changes caused by specific residue changes. ^{13}C NMR seems a way to achieve this goal, as genetic engineering makes it feasable to ^{13}C enrich proteins. Hydrogen-bonding as well as other weak interactions such as NH-π bonds are essential in determining the tertiary structure. One of the parameters to monitor is hydrogen-bonding. The carbonyl carbons seem ideal as a gauge for hydrogen-bonds. Deuterium isotope effects over two bonds have been shown to be a measure of hydrogen-bond strength in intra-molecularly hydrogen-bonded systems. As most of the carbonyl carbon resonances have been assigned for BPTI, this is used as a model. The paper describes how two- and three-bond isotope effects on ^{13}C chemical shifts of amide carbonyl carbons may be used to evaluate the strength and the geometry of hydrogen-bonds using BPTI deuteriated at specific amide positions. Ideally ^{13}C chemical shifts of carbonyl carbons should be able to tell about hydrogen-bonding as the carbonyl bond is strongly effected. The paper demonstrates how ^{13}C chemical shifts in a qualitative fashion may be used to classify the residues in terms of α-sheet, random coil or β-helix and how chemical shifts of carbonyl carbons may be correlated to those of the α-carbons. The direct use of ^{13}C chemical shifts to monitor conformational changes is a step forward.

CA 401 MEASUREMENT OF BACKBONE J COUPLINGS IN PROTEINS BY TWO DIMENSIONAL HETERONUCLEAR MULTIPLE QUANTUM NMR, Lewis E. Kay*, B. Brooks[a], D. Torchia[b], S. Sparks[b] and Ad. Bax*, Laboratory of Chemical Physics*, National Institute of Diabetes and Digestive and Kidney Diseases*, Division of Computer Research and Technology[a] and National Institute of Dental Research[b], National Institutes of Health, Bethesda, Maryland, 20892.

Several new techniques, requiring ^{15}N incorporation, are described for measuring NH-$C^{\alpha}H$ J couplings in proteins. High sensitivity heteronuclear ^{1}H-^{15}N multiple quantum correlation spectra retain the homonuclear J coupling information in the F_1 dimension. Because of the favorable relaxation properties of ^{15}N-^{1}H zero and double quantum coherences, significant line narrowing occurs in the F_1 dimension compared to the regular NH ^{1}H line width, permitting high accuracy measurements, even for medium sized proteins. Methods for convenient analysis of such coupling information are described, correcting for line width and dispersion mode contributions. The new approaches are demonstrated for the protein staphylococcal nuclease (18kD). The ϕ backbone angles derived from the J couplings are compared with coupling values obtained from a molecular dynamics simulation of the protein. A discussion of the relaxation properties of ^{15}N-^{1}H multiple quantum coherence in proteins will also be presented.

CA 402 DEUTERIUM NATURAL ABUNDANCE NMR SPECTROSCOPY: MONOTERPENE BIOSYNTHESIS, AN OVERVIEW

M.F. Leopold, S. James Gordon, Art Edison, Jimmy Lee Seidel, W.W. Epstein and D.M. Grant, Department of Chemistry, University of Utah, Salt Lake City, Utah, 84112. Recent work in this lab has focused on the study of monoterpene biosynthesis by natural abundance (N.A.) deuterium NMR. A high-field instrument (400MHz) coupled with an internal fluorine lock gives accurate and reproducible measurement of site-specific deuterium intensities. Information derived from N.A. deuterium spectra suggests that the biosynthesis of limonene in citrus involves the regioconversion of the α-terpinyl cation to limonene. Further study of the acyclic precursor linalyl acetate has demonstrated the continuity of relative deuterium intergration and supported regiospecific hydrogen abstaction to give limonene. α- and ß-pinene have been isolated from broomweed and various *Pinus* species. Both optically pure compounds and mixtures were obtained . The isotopic partitioning of the pinyl cation by the cyclase enzyme is examined and compared to the theoretical value. The irregular monoterpene chrysanthemol (isolated from sagebrush) is also examined to study the mechanism of the suggested condensation of two molecules of dimethylallyl pyrophosphate (DMAPP). Secondary isotope effects cause enzyme preference of one molecule to ionize. This enzyme selection is reflected in non-symmetric intergration intensities in the product. Comparison of data collected on various monoterpenes suggests several trends which are also discussed.

CA 403 ASSIGNMENT OF THE BACKBONE ^{15}N AND ^{1}H NMR RESONANCES OF T4 LYSOZYME BY ISOTOPIC LABELLING AND THE MAIN-CHAIN-DIRECTED ALGORITHM,

L.P.McIntosh[$], D.F.Lowry[#], A.J.Wand[*], A.G.Redfield[#], and F.W.Dahlquist[$]; $Inst. of Mol. Biol., Univ. of Oregon, Eugene OR 97403; *Inst. for Cancer Res., Fox Chase Cancer Center, Philadelphia PA 19111; #Dept. of Biochem., Brandeis Univ., Waltham MA 02254.

We are assigning the backbone ^{1}H and ^{15}N NMR resonances of T4 lysozyme (18.7kD) using the complementary approaches of isotope-editted NMR and the Main-Chain-Directed (MCD) algorithm. We have resolved and classified over 140 amide ^{1}H-^{15}N resonances into 16 amino acid classes by selective and uniform ^{15}N enrichment of T4 lysozyme. Many of these peaks have been specifically identified by multiple isotope labelling, mutations, and ^{15}N-directed NOE measurements. Through a variety of heteronuclear experiments we are mapping amide ^{1}H-^{15}N peaks to NH-CαH-CßH spin systems defined by 2D ^{1}H-NMR and are resolving COSY and NOESY amide crosspeaks by ^{1}H and ^{15}N shift. The MCD algorithm is used to assign NH-CαH-CßH units by recognition of characteristic proton NOESY connectivity patterns. The applications of these assignments include the investigation of saturation transfer from water, comparison of the folded and unfolded states of the protein, and analysis of the structural and dynamic effects of point mutations by $^{1}H/^{2}H$ exchange.

CA 404 STRUCTURE IN SOLUTION OF A CALCIUM BINDING PARVALBUMIN FROM THE ALPHA PHYLOGENETIC SERIES AS ESTABLISHED BY TWO-DIMENSIONAL ^{1}H NMR,

André Padilla, Adrien Cavé and Joseph Parello, Centre CNRS-INSERM Pharmacologie-Endocrinologie and Unité Associée N°1111, CNRS Pharmacy School, Montpellier, France.

The structure of alpha pike 5.0 parvalbumin in its Ca-loaded form has been studied in solution by two-dimensional proton NMR at 360 MHz using a conventional strategy of sequential assignments. In order to overcome the problem of spectral overlapping due to the presence of 108 residues in the protein, experiments were performed at different pH and temperature values, either in H_2O or in D_2O solutions. The amino acid sequence of pike 5.0 parvalbumin is thus completely characterized by nearly the totality of its NH, $C^{\alpha}H$ and $C^{\beta}H$ resonances (421 protons assigned among 429 in total). When associated with the remaining side-chain resonances, such sequence-specific assignments provide the basis for establishing the secondary organization and the tertiary folding of the polypeptide chain. The 3D structure of pike 5.0 parvalbumin in solution has been established by using the "distance geometry" or DISGEO procedure on the basis of about 450 interproton NOE connectivities (short-, medium- and long-range) in conjunction with a selection of phi and chi dihedral constraints. The exactness of the inferred structure in solution has been assessed by comparison with the polypeptide folding previously established in the crystalline state for two parvalbumins of the beta phylogenetic series (i.e. carp 4.25 and pike 4.10). Pike 5.0 parvalbumin was selected as a prototype of the alpha phylogenetic series for which no crystalline structure is presently available. Although very similar to a beta parvalbumin by its folding, the alpha parvalbumin studied here markedly differs from a beta parvalbumin by the length of its C-terminal helix domain, which includes 11 residues instead of 10 in the latter. Such an observation is likely to be related to the enhanced conformational stability of alpha parvalbumins. A parallel conformational analysis has been carried out by NMR in solution with the parvalbumin molecule in its Mg-loaded form thus establishing the type of conformational rearrangements, which are associated with Ca-Mg exchange in parvalbumins.

CA 405 COMPARISON OF HYDROGEN EXCHANGE BEHAVIOR WITH SUBNANOSECOND DYNAMICS IN THE PROTEIN UBIQUITIN Diane M. Schneider, Martin J. Dellwo and A.Joshua Wand, Institute for Cancer Research, Phila. PA. 19111.

Ubiquitin is a 76 residue protein (MW = 8565) found in all eukaryotic cells examined to date. It is a crucial component of the ATP-dependent proteolytic mechanism. In a complex series of enzymatic events, proteins are targeted for destruction through covalent conjugation to the C-terminus of ubiquitin. The molecular nature of this signalling system is not understood. As part of a comprehensive study of the structure, dynamics and energetics of this protein and its conjugates we are evaluating the relationship between the hydrogen exchange behavior of the exchangeable protons in ubiquitin and the subnanosecond dynamics at these sites. Hydrogen exchange rates for 25 amide protons have been measured at two pH values in a series of 2D COSY experiments. These amides map primarily to the 5-strand β-sheet and the α-helix as expected.

Hydrogen exchange measurements reflect the structural, energetic and dynamical properties of the hydrogen bonds in the ubiquitin molecule. In order to evaluate the dynamical component in this data, we are measuring the relaxation parameters in a ubiquitin sample which has been isotopically enriched in ^{15}N. The results from these experiments are interpreted in terms of the model independent theory of Lipari and Szabo. This theory allows us to determine a generalized order parameter and effective correlation time for each site investigated. These order parameters, which reflect the amplitude of high frequency motion, can be compared with hydrogen exchange rates in order to determine the extent to which exchange at a particular atomic position is influenced by small amplitude fluctuations.

CA 406 INTERACTIONS BETWEEN ANABAENA 7120 APOFLAVODOXIN AND FLAVIN MONONUCLEOTIDE DETERMINED BY HYDROGEN-1, CARBON-13, AND NITROGEN-15 NMR SPECTROSCOPY, Brian J. Stockman and John L. Markley, Department of Biochemistry, 420 Henry Mall, University of Wisconsin-Madison, Madison, WI 53706. Uniformly carbon-13 (26%) and nitrogen-15 (95%) enriched flavodoxins have been purified from Anabaena 7120 grown on $^{13}CO_2$ and $K^{15}NO_3$ as sole carbon and nitrogen sources. A concerted approach to hydrogen-1, carbon-13, and nitrogen-15 resonance assignments [Stockman et al., Biochemistry, in press] was used to assign many of the amino acid resonances in the oxidized protein. Isotope-directed NOE's obtained by using a sample of non-enriched apoflavodoxin reconstituted with carbon-13 labeled FMN identify a tryptophan residue overlapping the benzene subnucleus of the flavin isoalloxazine ring. Carbon-13 chemical shifts, assigned using $^{13}C\{^{13}C\}$ double quantum correlation spectroscopy, indicate that both carbonyl groups of the bound flavin are only weakly hydrogen bonded and that the flavin ring is only slightly polarized by interactions with the protein. Nitrogen-15 chemical shifts, indicative of strong hydrogen bonding to N3 and N5 but weak hydrogen bonding to N1, also support this interpretation. The chemical shift of N5 resembles that of free FMN in aqueous solution, suggesting that the N5 position is hydrogen bonded to water and that this edge of the cofactor is solvent accessible. [Supported by USDA grant 88-37262-3406, NIH grants RR02301, RR02781, and GM07215, and NSF grant DMB-8415048].

CA 407 SEQUENTIAL AMINO ACID RESIDUE ASSIGNMENTS BY ONE-BOND SCALAR COUPLINGS: APPLICATION OF $^{13}C\{^{15}N\}$ SINGLE BOND CORRELATION AND $^{13}C\{^{13}C\}$ DOUBLE QUANTUM CORRELATION TO ISOTOPICALLY LABELED PROTEINS, William M. Westler, Eldon L. Ulrich and John L. Markley, National Magnetic Resonance Facility at Madison, Department of Biochemistry, University of Wisconsin-Madison, Madison, WI 53706.

Recent applications of two-dimensional (2D) heteronuclear [$^{1}H\{^{13}C\}$, $^{1}H\{^{15}N\}$ and $^{13}C\{^{15}N\}$] and homonuclear [$^{13}C\{^{13}C\}$] NMR spectroscopy to proteins have shown the utility of selective and uniform ^{13}C and ^{15}N labeling of proteins for the assignment of specific amino acid residues in proteins. We present here a methodology for the sequence specific assignment of proteins uniformly enriched in ^{13}C and/or ^{15}N by using the scalar coupling between nuclei separated by a single chemical bond. $^{13}C\{^{13}C\}$ double quantum correlation ($^{13}C\{^{13}C\}$ DQC) is used to identify pairs of carbonyl and alpha carbons that belong to a particular amino acid. The identification of the amino acid to a specific type utilizes $^{13}C\{^{13}C\}$ connectivities of the side chain and ancillary information from other heteronuclear or $^{1}H\{^{1}H\}$ homonuclear experiments. By using $^{13}C\{^{15}N\}$ single bond correlation ($^{13}C\{^{15}N\}$ SBC), the alpha carbon can be connected to the amide nitrogen within an amino acid residue. $^{13}C\{^{15}N\}$ SBC is then used to span the peptide bond via the scalar coupling between the amide ^{15}N of residue i with the carbonyl ^{13}C of residue i-1. The use of this method will be demonstrated with the cytochrome c_{555} from Anabaena 7120 that is labeled with ^{13}C and/or ^{15}N. [Supported by National Institutes of Health Grant RR02301 from the Biomedical Research Technology Program, Division of Research Resources and Grant 88-37262-3406 from the U. S. Department of Agriculture.]

CA 500 SOLID STATE CP/MAS NMR STUDIES OF TRANSMEMBRANE PROTON PUMPING IN BACTERIORHODOPSIN, Edward A. Dratz, Koji Nakanishi* and Sam Belgerson, Department of Chemistry, Montana State University, Bozeman, MT 59717, *Department of Chemistry, Columbia University, New York, NY 10027.

Bacteriorhodopsin (bR) is a light driven transmembrane proton pump. The bR protein is able to create a 150 mV gradient across a 3.0 nm membrane hydrocarbon, which is equivalent to 500,000 volts/cm. We have prepared carbon-13 labeled bR in its native purple membrane form by reconstitution with retinals isotopically enriched at several sites in the chromophore. Other preparations are prepared by biosynthetic incorporation of aspartic acid C-13 labels on the carboxylic acid group. These carboxylic acid groups are believed to be principal sites of transmembrane proton transfer. We are investigating the mechanism of proton pumping by monitoring changes of the chemical shift tensor when bR pumping intermediates are trapped at low temperatures. The K intermediate stores a major fraction of the photon energy trapped at -150°C. In addition, binding of metal ions to the surface of bR modulates the chromophore environment and changes the color of the protein from blue to purple. The structural basis of this color change is being investigated. Experiments are in progress with C-13 labeling of a specific, single aspartic acid residues by in vitro peptide semi-synthesis (in collaboration with Dr. Robert Renthal, University of Texas, San Antonio). The current status of these measurements will be discussed.

CA 501 SOLID STATE ^{15}N NMR OF THE ACTIVE SITE HISTIDYL RESIDUE OF A CRYSTALLINE SERINE PROTEASE INHIBITED BY A PEPTIDE BORONIC ACID

Shauna Farr-Jones*, Steven O. Smith¶†, Charles A. Kettner§, Robert G. Griffin¶, William W. Bachovchin*, *Department of Biochemistry, Tufts University, Boston MA 02111; ¶Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, MA 02139; §Central Research & Development Dept., E.I. DuPont de Nemours Co., Experimental Station, Wilmington DE 19898 †Present address, Department of Molecular Biophysics & Biochemistry, Yale University, New Haven, CT 06510

^{15}N NMR solution studies of the active site histidyl (His-57) residue of α-lytic protease (α-lp), inhibited by a series of peptide boronic acids in solution showed that two types of complex could be formed with α-lp. Analogs of good substrates formed type 1 complexes viz. Ser-195 adducts in which the His-57 was protonated from pH 4 to 10.5. Both imidazole N-H protons are strongly H bonded. Analogs of non-substrates form complexes (type 2) in which the boron atom of the inhibitor is covalently bonded to the $N^{\epsilon 2}$ of His-57 while $N^{\delta 1}$ is protonated and H-bonded, presumably to Asp-102. In type 2 complexes, inhibitors are bound from pH 4 to 10 and $N^{\delta 1}$ is protonated over this range. Solid state ^{15}N NMR spectroscopy of crystalline α-lp shows that α-lp inhibited by a non-substrate analog (MeOSuc-Ala-Ala-Pro-boroPhe) forms a type 2 complex, the same as in solution. This is the first example of a crystalline serine protease inhibited with a boronic acid forming a histidine rather than serine adduct.

CA 502 CP-MAS ^{13}C NMR STUDIES OF ISOTOPICALLY LABELED BACTERIORHODOPSIN

Metz G.,Siebert F.,Engelhard M.,Hess B., Institut für Biophysik, Freiburg,MPI für Ernährungsphysiologie,Dortmund F.R.G

Solid-state ^{13}C MASS NMR spectra of 4-^{13}C Asp labeled bacteriorhodopsin (bR), the major protein in the purple membrane of halobacterium halobium, reveal enhanced resonances in the carbonyl (170-177 ppm) and the aromatic region (110-114 ppm). Also the deionized (blue) membrane and the C-terminal deprived bR were investigated. Spectra were recorded with and without cross polarisation with different recycle delays between pulses.
The NMR data provide evidence for at least two protonated aspartic acids in the hydrophobic region of the protein, which experience a different environment in the blue bR. On removal of the C-terminus a downfield shift of Asp resonances indicates a change from a salt like to a more aqueous environment. Apparently the C-terminus folds onto the membrane covering certain regions of the surface. As expected the internal Asp experience no change by this removal.
The new aromatic resonances in the labeled sample can be correlated to Trp residues, indicating an unknown biosynthetic pathway in halobacteria. The enrichment of Trp was proven by tracing the label by concomitantly adding 4-^{14}C Asp.

CA 503 TWO-DIMENSIONAL CHEMICAL SHIFT CORRELATION SPECTROSCOPY

Mark H. Sherwood, D. W. Alderman, David M. Grant, Department of Chemistry, University of Utah, Salt Lake City, Utah 84112

A new two-dimensional (2D) NMR technique has been developed for measuring chemical shift tensors in complex single crystals thus opening the possibility of using these tensors as structural and conformational probes in large molecules. The basis of the technique is to obtain 2D spectra in which the peaks are located by the chemical shifts at two different orientations of the crystal with respect to the magnetic field (1). From these spectra, the chemical shifts of a given nucleus at two widely separated orientations can be correlated and measured. The spectra are obtained by rapidly moving the crystal from an initial orientation to a final orientation during the mixing time of a chemical exchange sequence. A mechanism which allows movement between any two of six well chosen orientations is employed in order to measure six frequencies which fully characterize the chemical shift tensor. The geometry of this mechanism will be explained.

This technique has been used to measure the 12 carbon-13 chemical shift tensors in single crystal sucrose. The six 2D spectra used in this determination will be displayed along with the resulting tensors.

The possibilities of employing this technique to measure tensors in much more complicated molecules will be discussed.

(1) C. M. Carter, D. W. Alderman, and D. M. Grant, J. Magn. Reson. 65, 183 (1985) and 73, 114 (1987).

CA 600 **A COMPUTER PROGRAM FOR VIEWING AND INTERPRETING** 2-DIMENSIONAL NMR SPECTRA, Mark R. Day, Irwin D. Kuntz, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143

Modern computer workstations have the potential to be a powerful tool in the interpretation and assignment of 2-dimensional NMR spectra. Current display technology, coupled with increased CPU performance, is now capable of supplementing and even replacing the unwieldy plots and reams of paper that are often used to trace out assignments in 2D spectra. We describe a software package that utilizes a network-based windowing system to provide the researcher with a simple method for inspecting and annotating 2D NMR data sets on a variety of workstations. Mouse based tools allow the user to conveniently interact with the spectra, carrying out such tasks as tracing connections between peaks, and labeling spectra. Normal windowing operations, such as scrolling and zooming are fully supported. Multiple windows allow the simultaneous display of several different spectra; arbitrary areas of any of these spectra can be displayed at a variety of magnification levels. This visualization package is among the first in our continuing efforts to computerize many of the tedious tasks that are now a part of 2D NMR structure determination.

CA 601 AUTOMATED QUANTIFICATION OF 2D NMR SPECTRA: APPLICATIONS TO BIOMOLECULES, F. Delaglio, D. N. Master, K. Swanson, S. Wang+, K. Bishop+, P. N. Borer+, New Methods Research, Inc., Syracuse, NY, 13210, +NIH NMR Resource, Syracuse University, Syracuse, NY, 13244-1200.

Using an eclectic approach, including the tools of non-linear optimization, cluster analysis, and logic programming, we demonstrate schemes for automated information extraction and presentation, oriented for use on modern scientific workstations. Examples include the computationally and graphically challenging tasks of 2D-deconvolution, rule-based coupling constant extraction, and automated NOE volume extraction by surface fitting. Applications to the DNA duplex $[TAGCGCTA]_2$ and the peptide Tyr-Gly-Gly-Phe-Leu are illustrated.

CA 602 BUILDING A SUPERVISED SYSTEM FOR SPECTROSCOPIC CHEMICAL DATA-ANALYSIS BASED ON MULTIVARIATE ANALYSIS AND LOGIC PROGRAMMING, **Hans Grahn[1], Frank Delaglio[2], Ulf Edlund[3], Yvon Th. van den Hoogen[4], Cornelis A.G. Altona[4], George C. Levy[5], M. W. Roggenbuck[5] and Phil N. Borer[5]**

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A challenging task in data-processing of spectroscopic and chemical data is to develop automated methods for the examination and interpretation of the large data arrays often resulting from modern instruments. Often these procedures are referred to as "pattern recognition" (ParC) methods. From the point of view of a supervised or computer-assisted analysis, the pattern recognition procedure is only a small part of an overall scheme. A complicated problem like the interpretation of a two-dimensional NMR spectrum of a macromolecule is not likely to be completely solved only by using a conceptually simple approach like ParC. We here present a comprehensive framework for automated analysis of two-dimensional NMR spectra. Multivariate analysis and logic programming techniques are used to address the problems of overall spectral assignment with an innovative scheme that also includes coordination of the best aspects from several currently existing reduction and assignment methods. Results are presented including automated spin system separation for the peptide Tyr-Gly-Gly-Phe-Leu, using double quantum filtered COSY data. Results are also shown from a multivariate study of a NOESY spectrum of an RNA octanucleotide duplex [CACAUGUG]2. A region in the spectrum was chosen to build a training set that later can be used for assigning similar regions in more complex RNA molecules. The methodology may also assist in developing rules that allow assignments in other regions of the NOESY spectrum for short RNA oligomers.

CA 603 DATA PROCESSING IN NMR SPECTROSCOPY: A LOOK TO THE FUTURE, ***George C. Levy, F. Delaglio, P. Sole, *H. Grahn, J. Begemann, and *R.E. Hoffman, New Methods Research, Inc. and *NIH Resource, Bowne Hall, Syracuse University, Syracuse, New York 13244-1200**

The methods and applications of advanced data processing in NMR spectroscopy are developing at a very rapid rate. Despite this, there are significant challenges ahead as NMR spectroscopists begin to use very large 2-dimensional arrays for studies of large biomolecule structure and further, in the offing, lies the advent of practical 3- and 4-dimensional spectroscopy studies. The latter will challenge current and even projected computer hardware technology with respect to data input and output as well as computational speed. Moreover, the chemist will be challenged by extraordinary difficulties in feature recognition in these complex multi-dimensional datasets. Advances in signal analysis, pattern recognition, 3-dimensional graphics, and large data flow are *all* required to make this important new tool valuable for biomolecular research. Advances in computer hardware and software technology are required to meet this challenge, as well as the challenge of 1-dimensional spectroscopy in difficult cases such as *in vivo* spectroscopy. This paper will summarize anticipated trends.

CA 604 STRATEGIES FOR NMR STRUCTURE REFINEMENT. Peter A. Mirau, AT&T Bell Laboratories, 600 Mountain Ave, Murray Hill, NJ 07974

Three dimensional structures can be determined from distances measured in 2D NMR experiments. However, for accurate structure determination, it is necessary to compare the structures with the experimental 2D NOE data. One strategy for NMR structure refinement begins with the simulation of an experimental 2D NOE spectrum. When the experimental and calculated spectra are the same, the peak volumes used in the simulation can be solved for the complete matrix of relaxation rates from which distances may be determined at a single mixing time. From measurements at several mixing times the distances and their uncertainties can be measured, and the structure determined by distance geometry or restrained molecular dynamics. The quality of the structures can be determined by comparison of the experimental rate matrix (with its uncertainties) with the rate matrix and 2D NOE spectrum calculated from the proposed structure. NMR structure refinement will be illustrated for the double-stranded DNA fragment $d(CTTCGAAG)_2$.

CA 605 ***IMPROVING THE RESOLUTION OF A PROTEIN'S NMR DETERMINED SOLUTION STRUCTURE: A Simulation using Spin Label-Derived NMR Distances.*** *John F. Thomason, Irwin D. Kuntz, Department of Pharmaceutical Chemistry, University of California San Francisco, San Francisco, CA 94143*

The use of spin label-derived NMR distances, in conjunction with NOE data, can improve the quality with which the solution structure of a protein can be modeled. Simulated spin label distances taken from the crystal structure of Bovine Pancreatic Trypsin Inhibitor are used as input into a modified Distance Geometry alogrithm with improved sampling characteristics. One to five spin labels are used, each of which provides 50-250 spin label distances. The spin labels are placed at sites on the protein where spin label modification has been shown to be feasible. These sites are the amino terminus and the amino groups of the lysine side chain of which there are four (residues: 14, 26, 41 and 46). The spin label data sets were used with and without an NOE data set. In all cases the modeled structures in which the spin label data were used significantly reduced the root-mean-square deviations as compared to the NOE data set alone. The primary cause of this effect is that the long distances from the spin labels greatly reduce the available conformation. This study verifies that the use of spin labels can improve the quality with which the solution structure of a protein can be determined.

Late Additions

CA 700 HIGH-RESOLUTION NMR APPROACH TO SPATIAL STRUCTURE OF BACTERIORHODOPSIN, A.S.Arseniev, A.G.Sobol, I.V.Maslennikov, G.V.Abdulaeva, I.L.Barsukov, V.F.Bystrov, Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Moscow, USSR. Bacteriorhodopsin (BR) is a light-driven proton pump of the purple membrane of *Halobacterium halobium*. As an approach to the BR spatial structure, use was made of an artificial milieu which (i) preserves protein conformation formed in the membrane and (ii) provides high-resolution NMR spectra. BR and its large fragments initially denatured and then redissolved in a methanol-chloroform mixture possess multiple conformational states. However, the samples purified by gel chromotography in methanol-chloroform have single conformations as detected by ^{19}F-NMR and 2D ^{1}H-NMR spectra. Moreover CD and ^{19}F-NMR revealed that solubilized BR and its fragments retain a native-like secondary structure and specific tertiary structure. The tertiary structure of BR was probed by use of double labeled derivatives obtained via spin labeling with stable nitroxyl radicals of biosynthetical 5-fluorotryptophan- or 3-fluorophenylalanine-labeled BR. Spatial structures of isolated BR fragments were studied by 2D ^{1}H-NMR spectroscopy. The structural analysis was greatly facilitated and accomplished by use of synthetic peptides.

CA 701 DETERMINATION OF PROTEIN LOCAL STRUCTURE FROM TWO-DIMENSIONAL ^{1}H NMR DATA, A.L.Lomize, A.G.Sobol, A.S.Arseniev, V.F.Bystrov, Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Moscow, USSR A method was suggested to determine conformations of amino acid residues of a protein and effective correlation time τ_c from cross-peak intensities of NOESY spectra. The method consists in fit of complete relaxation matrix of dipeptide unit protons to experimental cross-peak intensities by varying $\phi,\psi,\times$ torsional angles and τ_c, longitudinal relaxation times being accounted. To verify the method, model calculations of the BPTI local structure at mixing times τ_m=25-300 ms and τ_c=4 ns were carried out. The method works well with $\tau_m \leq$200ms. As a result the backbone ϕ,ψ torsion angles were uniquely determined at τ_m=100 ms for all but Gly residues of BPTI with mean deviation of 14 and 7°, respectively, from crystallographic values of those angles, and $\times'$ angles were uniquely determined for the majority of side chains.