Biochemical Pharmacology, Vol. 40, No. 1, pp. 3–5, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00
Pergamon Press plc

PREFACE

AN OVERVIEW OF RECENT PROGRESS IN LIGAND–RECEPTOR RESEARCH BASED ON NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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An understanding of the structural factors that control interaction between ligands and their receptors in solution is now possible through two- and three-dimensional nuclear magnetic resonance (2D- and 3D-NMR) spectroscopy. Of particular value is the ability to examine these interactions in solution and follow the kinetics of reactions that involve equilibria amongst isoforms of ligand or protein. The conference reported in this volume was convened to examine the latest developments in this technique and their application to the design of new therapeutic agents.

In the first session, chaired by A. Bax, current methodology with particular emphasis on new pulse trains and the several approaches to analysis was reviewed. The evolution of 3D-NMR spectroscopy employing ^{15}N-labeling of the peptide bond and the potential as well as the limitations of its application to macromolecules were considered. As a contrast to these approaches to macromolecular structure, R. G. Shulman presented an overview with examples of the application of NMR spectroscopy to follow the kinetics of interactions in a metabolic pathway in cellular or whole animal systems.

Returning to the central theme, J. H. Prestegard added a new dimension to the formulation of protein conformation. He documented the existence of a dynamic equilibrium between two conformers in an acyl carrier protein from spinach, a circumstance unlike the enzyme from *Escherichia coli* in which 2D-NMR indicates a single rigid structure. These results provide support for other evidence suggesting that proteins may exist in more than one distinct conformation, a phenomenon that affords potential new insights into control mechanisms. Structural analyses of this nature require sophisticated computation algorithms to permit construction of a model with appropriate distance and angular constraints. D. A. Case reviewed current procedures and introduced some new approaches based on chemical shifts and nuclear Overhauser effect (NOE) intensities that may permit development of models with greater precision and resolution. In a related paper, D. L. Turner addressed means to explore the range of conformations that small to intermediate size proteins may assume. A protocol was presented based on energy estimation of members of each class of conformers, and a range of experimental conditions and computational methods was outlined. The potential structures of an antigenic *N*-acetyl nonapeptide from influenza virus were presented based on stepwise application of refinement methods that permit approximation of a minimal energy conformation. Also discussed was the application of this energy approach to estimation of the energetic requirements to assume other conformations that may be necessary for biological interactions.

The second session was devoted to application of the above structural methods to bioactive peptides. K. Wüthrich, a germinal contributor to much of current methodology, noted the relative advantages of the three-dimensional structural models constructed from NMR data as contrasted to the rigid structures obtained by X-ray crystallography. He also discussed the ability of NMR methods to provide evidence of short-lived transient conformers that could be critical to explaining the interactions of such proteins in biological systems. A specific example of conformer interconversion in the case of bradykinin was presented by R. E. London. Analysis of the *cis–trans* interconversion around a proline residue revealed a bistable equilibrium that may control biological activity. To investigate factors that affect this interconversion and its equilibrium, the effect of peptide substitutions on either side of the prolyl bond has been evaluated.

The development of solution structures for human epidermal growth factor (hEGF) and human transforming growth factor-α (hTGF-α) by I. D. Campbell indicated the applicability of these methods to proteins in the <10 kD range. The availability of modified proteins through site-directed mutagenesis coupled with bioactivity determinations has made possible the direct correlation between three-dimensional structural elements and biological activity.

The binary complex between L-Lys-D-Ala-D-Ala and vancomycin was employed as a model for the interaction of this antibiotic with intact peptidoglycan in the bacterial cell wall. D. H. Williams delineated the structural elements that affect the substrate affinity characteristics and kinetics of this interaction. He also extended these concepts and methodology to explore on a theoretical basis the

interactions of other antibiotics with DNA. An adjacent site on the bacterial cell wall is the target site of lysozyme. K. D. Kopple has employed a paramagnetic nitroxide spin label to explore the surface of this and other enzymes. Comparison of the *N*-acetyl glucosamine complex with the free enzyme permits identification of the ligand binding residues by this method.

A higher level of macromolecular organization is represented by proteins associated with membranes. R. W. Behling demonstrated the role of the membrane as an initial contact surface for acetylcholine prior to its interaction with the acetylcholine receptor. It is hypothesized that the initial interaction with the membrane permits the definition of the probable bioactive conformation of acetylcholine for its interaction with the receptor. Another level of resolution was presented by E. Hawrot. Selected peptide fragments of the acetylcholine receptor site exhibit high-affinity binding to α-bungarotoxin. The sequence specific assignment for these 12- to 18-residue peptides was presented with a proposed extrapolation to their conformation at the binding site of the intact protein.

The third session addressed methods that would permit identification of the structural elements that control target recognition. Since this involves through space interaction, transfer nuclear Overhauser effects play a pivotal role in the analysis. A. M. Gronenborn gave a detailed and lucid review of the potential and limitations of this method with specific examples of nucleotide triphosphate binding to deoxyhemoglobin and the interaction of a tetrapeptide with elastase.

Another detailed example of the value of the transfer NOE method was given by A. S. Mildvan. The interproton distances of nucleotide triphosphate substrates when bound to a large fragment of DNA polymerase were examined in the presence of RNA templates. This analysis was performed on two large peptides that are involved in nucleotide binding.

Antibody–antigen recognition constitutes a very specific and accessible model for ligand–macromolecule interaction. P. E. Wright prepared the Fv fragment of an immunoglobulin with specific isotopic labeling for NMR analysis. He also employed NMR methods to extract conformational information on appropriately labeled immunogenic peptides as they bind to the Fab fragment of specific immunoglobulins. A residue-directed focus on this same topic was provided by N. G. J. Richards. Prolines in an immunogenic nonapeptide were replaced with 2-methyl prolines to generate altered conformations. Sharply different reactivity with immunoglobulins was demonstrated and the extension of this concept to the triprolyl peptide bradykinin discussed.

Although protein–ligand interactions were the initial focus of much NMR structural research, studies of the interactions of ligands with nucleic acids by these methods are now common. R. Kaptein used a *lac* repressor N-terminal peptide (56 residues) to examine the interaction between the *lac* operator and DNA. He concludes that most of the points of contact between the protein and DNA reside in the second helix of the *lac* headpiece and DNA bases in the major groove. This is contrary to the orientation found in other repressor–operator complexes analyzed by X-ray crystallography. The role of Zn^{2+} "fingers" in the attachment of proteins to DNA was examined by M. F. Summers in a retroviral system. He demonstrated the application of NMR techniques to the elaboration of the critical three-dimensional constraints in both the peptide and the nucleic acid. It would appear that the retroviral Zn^{2+} fingers differ considerably from other classical DNA Zn^{2+} finger motifs. L. G. Marzilli presented evidence for the distinct structural features that characterize the complex between the therapeutic agent *cis*-platinum and a 12 and 14 oligomeric nucleotide. Using platinum, phosphorous and proton NMR, he was able to identify the favored structure of the loop region from four potential model structures.

The final session specifically addressed the application of the NMR method to drug and macromolecule interactions. I. M. Armitage employed several of the methods discussed in previous sessions to delineate the solution conformation of the cyclosporine receptor, cyclophilin, and changes in this protein consequent to cyclosporine binding. The global properties of the binding site, including a hydrophobic patch, have been established and a model proposed for features of the structure that interact with cyclosporine. Additionally, evidence for several new conformations of cyclosporine in mixed solvents was presented. A fulsome analysis of cyclosporine conformations was presented by H. Kessler. His refinement of these structures by molecular dynamics considerations was contrasted to the crystal structure. The effects of more polar solvents on both the peptide backbone and side chains were elucidated rigorously.

M. Ikura demonstrated the value of recombinant expression with uniform ^{15}N-labeling in the establishment of the three-dimensional structure of calmodulin, a frequent target for drug development. Complementary to this approach was the use of specific ^{15}N-labeled amino acids which, when combined with ^{13}C-labeled amino acids, permits sequential assignment of selected residues.

The enzyme dihydrofolate reductase has provided an important testing ground for comparison of drug interactions with a macromolecular target. J. Feeney presented an overview of their groups' studies of the drug–receptor site using a host of different probing strategies to monitor NMR signals from both the ligands and protein.

A synthesis of several of the techniques discussed in the symposium was provided by S. W. Fesik to achieve a structural definition of the macromolecular site to which drugs bind. He employed isotope editing methods to filter extraneous information and used an elegant three-dimensional NMR technique to establish the structure of large enzyme–inhibitor complexes.

The symposium concluded with a proposal by G. R. Marshall of a new general method for ensuring completeness in sampling conformational space, a limitation inherent in the several computational methods used for the generation of atomic coordinates. The approach consisted of the systematic evaluation of all torsional variables, and it was illustrated for the ligand cyclosporin A.

The convenors of this meeting wish to express their appreciation to the staff of Pergamon Press that provided financial and organizational support. We would also like to thank the many fine speakers, most of whose papers are included in this volume.

An overview of the subjects covered makes it clear that nuclear magnetic resonance spectroscopy will be a major tool for future drug design research based on the three-dimensional definition of target receptor or catalytic sites.