Nuclear Magnetic Resonance

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One of the great challenges of contemporary Nuclear Magnetic Resonance (NMR) spectroscopy is the application of the technique to highly complex problems in biology. No other form of spectroscopy can contribute to the elucidation of the structure, function, and dynamics of biomacromolecules at the atomic level. Our research is focused on the following three broad areas: the structure of complexes between DNA and anticancer antibiotics; the structure of unusual forms of DNA that have biological significance; and the structure and function of moderately sized proteins. Work is continuing on structural studies of a large protein–protein complex, the structures of small chaperone proteins, and the investigation of the interaction of spermine with various forms of DNA. As our expertise in macromolecular structure determinations increases we intend to tackle more demanding structural problems. In the near future, we will attempt the structure determination of the *N*-terminal and *C*-terminal domains of a 42 kDa protein that is overexpressed in the cells of early breast cancer tumours. The ultimate goal of this work is to use the structure of the protein to design drugs that may be used to block the progression of the tumour cells. The major theme of our work is to deduce the function of biological molecules and complexes from knowledge of their structure and dynamics at the atomic level.

Interaction of the □ Subunit and the □ Subunit of DNA Polymerase III

The catalytic core of *Escherichia coli* DNA polymerase III contains three tightly associated subunits (□, □ and □). The refinement of the three-dimensional structure of the □ subunit was completed by the NMR group. The □ subunit has three □-helices in the *N*-terminal two thirds of the protein that fold to form a triangular shape. The surface of □ is bipolar; with one face containing most of the acidic residues and the other face most of the long-chain basic residues. The C-terminal section of □ has many charged and hydrophilic amino acid residues, but has no well-defined secondary structure, and exists in a highly dynamic state. This dynamic conformational exchange in the helices hindered the completion of a satisfactory three-dimensional structure of □. As part of a program aimed at understanding the molecular mechanism of the core, we have set out to investigate the association of the □ and □ subunits. Preliminary experiments, in which ^{15}N, ^{13}C-labelled □ is bound to the unlabelled *N*-terminal domain of the □ subunit, have demonstrated that more ^{1}H–^{15}N crosspeaks with a greater dispersion are observed in the ^{15}N HSQC spectrum of the complex. A partially refined structure of the □ subunit bound to □ has been determined. The basic structure of □ has not changed but two of the helices that were poorly defined in the uncomplexed □ subunit are properly formed in the complex. We have recently mapped the binding surface of □ on □ using advanced NMR techniques. Not surprisingly, the surface corresponds to a hydrophobic patch on □ formed on one of the previously ill-formed □-helices. Work is now in progress to align the refined NMR structure of □ with the X-ray structure of the *N*-terminal half of □. This will constitute a rare combination of these two powerful structural technologies *(with E.M. Bulloch, N.E. Dixon, S. Hamdan, T.K. Ronson, S.E. Brown* [Entomology CSIRO*])*

Structures of Small Chaperone Proteins

Small heat shock proteins stabilise other proteins under conditions of stress, for example, heat, oxidation, or the presence of heavy metals. The nature of the chaperone action of these proteins is poorly understood. Although these proteins are small in mass, they exist as large oligomeric species and, hence, present a challenge to NMR techniques. Two small heat shock proteins, hsp18.1 and hsp16.9, have been overexpressed and uniformly labelled with ^{15}N in the laboratories of Associate Professor John Carver and Professor Elizabeth Zierling. The spectra of hsp18.1 were disappointing, but the results with hsp16.9 are very encouraging and analyses of the spectra are now in progress. *(with J.A. Carver, R.A. Lindner [U. Wollongong], E. Zierling [U. Arizona, Tucson])*

NMR Studies of the Interaction of Spermine with Oligonucleotides

Spermine, an aliphatic polycationic molecule found in all cells, has an essential role in cell growth and differentiation. At present, there is no thorough understanding of how polyamines exert their physiological effects. Spermine is known to interact with both DNA and with proteins, yet the details of these interactions and the molecular basis of the biological function of spermine are poorly understood. There is evidence in the literature that spermine interacts with different forms of DNA in distinct and divergent modes. We have confirmed this and have characterised the complexes of spermine with duplex B-DNA and G-DNA using a specifically ^{13}C-labelled spermine and advanced NMR techniques to take advantage of the specific isotope label on spermine. ^{13}C T_1 and T_2 relaxation times and changes in the ^{1}H-^{13}C dipolar coupling of ^{13}C-labelled spermine are used to characterise the dynamics of spermine in the presence of different forms of DNA. Spermine is bound more tightly to G-DNA than B-DNA. We have recently located this unique interaction of spermine with G-DNA to the loop regions of folded DNA quadruplexes. *(with J. Coughlan)*

ESX, a Protein Overexpressed in the Early Stages of Epithelial Breast Cancer

ESX is a protein that belongs to the *Ets* family of transcription factors. *Ets* proteins exhibit diverse roles in development, cell differentiation and tissue-specific gene expression and are implicated in cancers such as acute myeloid leukemia and Ewings sarcoma. The ESX transcription factor may have a role in the activation of the HER2/neu oncogene, which is overexpressed in over 40% of breast tumours. We are interested in determining the structure of ESX using X-ray crystallography and NMR. To this end we have overexpressed the C-terminal end of ESX containing the two DNA-binding domains. Attempts will be made to crystallize this fragment. A ^{15}N-labelled form of the C–terminal fragment of ESX is also available and NMR studies will commence shortly. The long-term goal is to determine the complete structure of ESX and studies to optimize expression and folding of the *N*-terminal fragment and the complete protein are envisaged. This project is supported in part by a Yamagiwa-Yoshida travel grant from the International Union against Cancer. *(with C.C. Benz, G. Scott [Buck Institute for Age Research, USA])*

http://rsc.anu.edu.au/keniry.html