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 with a special focus on proteins that bind to DNA and RNA. 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 determination 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.

NMR Studies of the Interaction of Spermine with Oligonucleotides

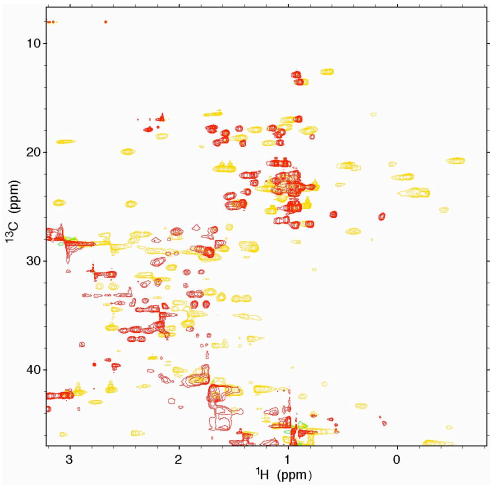
Spermine, an aliphatic polycationic molecule found in all cells, has an essential role in cell growth and differentiation. At present, there is no thorough understanding of how polyamines exert their physiological effects. Spermine is known to interact both with DNA and with proteins, yet the details 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 homonuclear and heteronuclear NOEs are used to characterise the dynamics of spermine in the presence of different forms of DNA. Spermine is bound more tightly to folded forms of G-DNA than B-DNA or linear G-DNA. Work is in progress to determine detailed dynamical models of the interaction of spermine with DNA to expand this work to the effect of spermine on protein-DNA complexes *(with J. Coughlan)*.

Novel Antibiotics and DNA

Calothrixin A and B are novel pentacyclic metabolites from cyanobacteria that exert growth-inhibitory effects at nanomolar concentrations against rapidly proliferating cell cultures. The binding properties of the calothrixins and their synthetic analogues with various structural forms of DNA are under investigation by NMR, circular dichroism and fluorescence. *(with E.A. Owen, R. Rickards, and C. Chai, M. Waring [Chemistry, ANU], G.D. Smith [BAMBI, ANU])*

Interaction of the θ Subunit and the ε Subunit of DNA Polymerase III

The catalytic core of *Escherichia coli* DNA polymerase III contains three tightly associated subunits (α, ε, and θ). The refinement of the three-dimensional structure of the θ subunit was completed by the NMR group. The θ subunit has three α-helices in the *N*-terminal two thirds of the protein that fold to form a triangular shape. As part of a program aimed at understanding the molecular mechanism of the core, we have set out to investigate the association of the θ and ε subunits. The structure of the θ subunit bound to ε has been refined using an innovative technique that combines NOE restraints with distance and orientation restraints calculated from a paramagnetic centre located in the active site of ε. The basic structure of θ has not changed but two of the helices that were poorly defined in the uncomplexed θ subunit are properly formed in the complex. We have recently mapped the binding surface of ε on θ using advanced NMR techniques. Not surprisingly, the surface corresponds to a hydrophobic patch on θ formed on one of the previously ill-formed α-helices. Work is now in progress to align θ with ε using the same paramagnetic restraints that were used to refine the structure of θ. *(with E.M. Bulloch, N.E. Dixon, S. Hamdan, T.K. Ronson, G. Otting, G. Pintacuda, and S.E. Brown [Entomology, CSIRO])*



^{13}C-HSQC NMR spectrum of the θε complex showing unshifted peaks (red) and paramagnetic shifted peaks (gold) due to a lanthanide ion located at the ε active site

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/research/keniry.php