

NMR facility

Arnout Kalverda and Steve Homans

Overview of facility

The NMR facility is equipped with a 750 MHz, a 600 MHz and two 500 MHz Varian Inova NMR spectrometers. All instruments are setup to use ^1H , ^{13}C , ^{15}N and ^2H during normal operation. In the spring of 2005, a cryoprobe will be added to the 750 MHz NMR spectrometer that will enhance the sensitivity of the system by up to a factor of 3.

Dynamics and thermodynamics of ligand binding

The binding of a ligand to a protein often results in changes in the dynamics of the protein upon binding. These dynamic changes are reflected in the thermodynamics of binding through their contribution to the entropy of the system. Using Mouse Urinary Protein, changes in backbone and side-chain dynamics have been probed with ^{15}N and ^2H relaxation methods. Comparisons were made between the apo-protein and two closely related ligands. The changes in dynamics upon ligand binding indicate a complex network of dynamic compensation. Whereas methyl groups that are located in the binding pocket become more rigid, other side-chains located in a shell further away from the ligand binding site become more mobile. This hints that there is a shift in the most prevalent dynamic modes upon ligand binding. Measurements of the changes in dynamics are being continued with site directed mutants of the protein.

The interplay between dynamics and thermodynamics in ligand binding is also being studied in a second system: arabinose binding protein which binds to galactose and deoxy-derivatives of galactose. As a step towards using NMR measurements with this protein, the sequential assignments have been completed and NMR relaxation studies are underway.

Identification of a methyl-thio sugar substituent in the *M. tuberculosis* cellwall

Lipoarabinomannan is a major component of the *Mycobacterium tuberculosis* cell wall. An unusual sugar substituent has been found as part of the mannosyl capping structure on Lipoarabinomannan. Using a combination of NMR, mass spectrometry and chemical synthesis, this sugar substituent was determined to be a 5-deoxy-5-methylthio- α -xylofuranosyl structure. It is reasonable to assume that it may be biosynthetically derived from 5'-methyl-thioadenosine, which is a byproduct of polyamine biosynthesis. The presence of this unusual sugar may provide an angle on potential new targets for the development of antituberculosis drugs.

NMR of protein folding

In collaboration with the group of Prof. S.E. Radford, native state hydrogen exchange has been used with the Colicin Immunity protein Im7 to show that the hydrogen exchange data provide information on the secondary structure of an intermediate state. To demonstrate whether hydrogen exchange occurred from the intermediate state, local fluctuations or global unfolding, data were compared between the native protein and a mutant (I72V). The mutation significantly destabilises the intermediate state relative to the unfolded state, but only slightly destabilises the native state. The hydrogen exchange rates reflect the free energy difference with the state from which exchange occurs, and the hydrogen exchange patterns shift with the changes in free energy upon mutation. Thus residues that exchange from the intermediate have decreased hydrogen exchange rates, while residues that exchange through global unfolding have increased hydrogen exchange rates in this case. This method, using site directed mutagenesis to identify the state from which hydrogen exchange occurs,

could have more widespread use in identifying the presence of secondary structure in intermediate states.

Publications

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