Molecular dynamics in mouse urinary protein by NMR methods.

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Introduction

Central to the understanding of binding affinity of a ligand for its receptor are the dynamics and thermodynamics of the association. Despite the vast accumulation of X-ray crystallographic, static data of bio-molecular structures of proteins that are enzymes and receptors, relatively little is known about the dynamic behaviour of these proteins.

To perform their function, proteins exhibit a significant degree of flexibility on a wide range of time scales from femtoseconds to seconds. Detailed descriptions of dynamics of the specific protein-ligand contacts and their changes upon establishment of these contacts will help in promoting a better understanding of biologically important processes. Data obtained on a residue-by-residue basis greatly enhances opportunities in protein engineering and ligand optimisation in rational drug-design. NMR relaxation phenomena offer a great potential by which standard free energies and entropies can be derived on a per residue basis - a level of detail crucial for the understanding of molecular basis of ligand-protein interactions.

MUP as a model system

The mouse major urinary proteins (MUPs) are a class of highly homologous, pheromone-binding proteins having molecular weights of about 19kDa. MUP-I is a member of group 1 gene products (30 genes) that are expressed in the liver under hormonal control and are excreted in the urine of male mice at high levels. MUPs bind to several small hydrophobic pheromone molecules and their function is to carry them through the aqueous environment, protecting them from decomposition, and regulating their release from urine. The high resolution X-ray crystal structure available indicates a conserved beta-barrel of eight beta-strands and an alpha helix. A preliminary isothermal calorimetric (ITC) study in our laboratory indicated that two of the ligands to MUP, 2-isopropyl, 3-methoxypyrazine and 2-isobutyl, 3-methoxypyrazine display an order of difference in binding affinity to MUP despite similarity in their chemical structures. MUP and its small hydrophobic ligands serve as a suitable model system in which to conduct NMR relaxation studies and to rationalise differences in relaxation parameters between different protein-ligand complexes in terms of binding specificities.

Amide and methyl dynamics from ^{15}N and ^{13}C relaxation

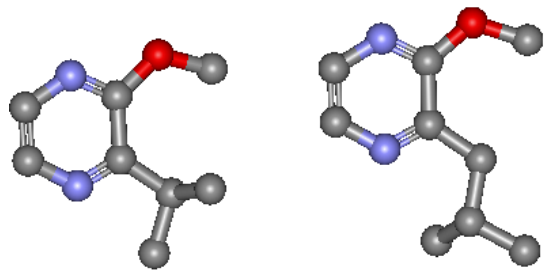
As part of our composite strategy employing NMR spectroscopy, ITC and theoretical free energy perturbation (FEP) calculations to study thermodynamics of binding of a panel of related ligands in MUP-I, backbone and side chain (NH and CHD_2) spin-lattice (T1), spin-spin (T2) relaxation times and heteronuclear nOe (nuclear Overhauser effect) are measured at backbone amide and side chain methyl sites in uniformly incorporated ^{15}N and ^{13}C samples respectively (partial deuteration at methyl groups in the latter). Fractionally deuterated methyl groups (CHD_2) offer an advantage in studying side chain dynamics in that they avoid difficulties arising from interference effects between multiple ^{13}C-^{1}H bond vectors as ^{13}C is relaxed solely by a single proton. The experiments involve recording a series of high-resolution ^{15}N-^{1}H (for backbone) or ^{13}C-^{1}H (for sidechain) correlation maps with the intensity of each correlation attenuated by ^{1}H relaxation as a function of delays characterising the appropriate relaxation parameter (T1 or T2). The decay of intensities were fitted monoexponentially to obtain the relaxation time constants T1 and T2. For the measurement

of heteronuclear nOe, two spectra in each case were collected with proton saturation and one without. The correct ratio of the respective peak intensities provides steady state nOe.

The relaxation parameters (T1, T2 and nOe) sensitive to the picosecond-nanosecond time scale probe the amplitude of bond vector fluctuations. These parameters can be expressed in terms of an order parameter (S^2) that varies between 0 for an unrestricted internal motion to 1 for rigidity. S^2 can be related to the entropy of the corresponding molecular degrees of freedom under the 'model-free' formalism described by Lipari and Szabo. The data obtained both in the absence and presence of ligand will be interpreted in terms of free energies and free entropies of binding on a per-residue basis.

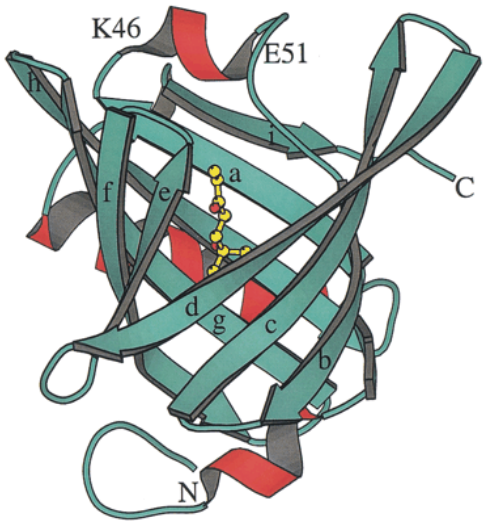
The analysis of data on free and different bound states of MUP is underway.

MUP ligands



2-Isopropyl, 3-methoxypyrazine
*K*a=1.7±0.2x10^6 M^{-1}

2-isobutyl,3-methyoxypyrazine
*K*a=1.5±0.2x10^7 M^{-1}



X-ray Crystallographic Structure of MUP

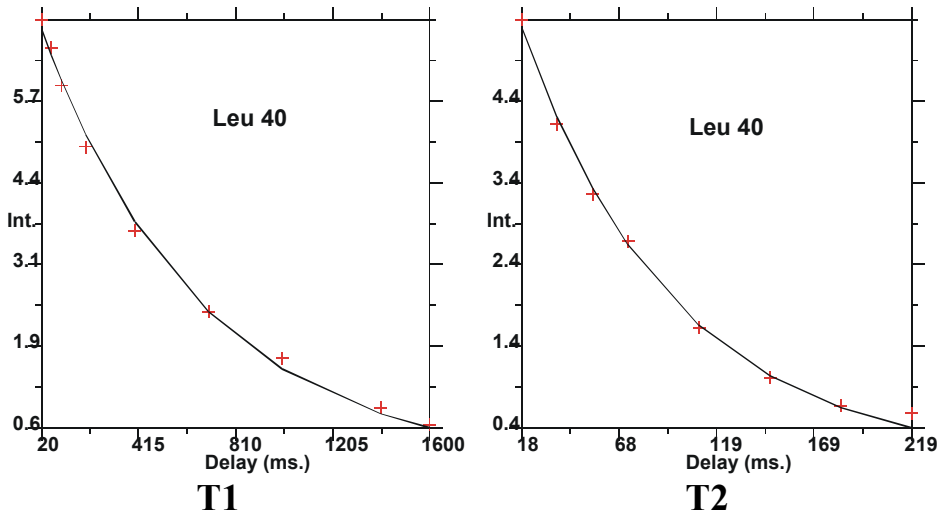


Figure: ^{15}N-^{1}H Relaxation Profiles of an active site residue

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