Derivation of per-residue thermodynamic parameters for ligand-protein interactions

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Introduction

The Human Genome Project is providing a wealth of information on nucleic acid and protein sequences. However, if this information is to be of value for rational drug design, it is necessary to obtain a deeper understanding of the molecular basis of ligand-protein interactions. The key to understanding the affinity of a ligand for its receptor lies in the dynamics and thermodynamics of the association rather than a simple static picture.

With isothermal titration calorimetry (ITC), it is possible to obtain the global thermodynamic parameters governing a biomolecular association, however, from the point of view of ligand optimization, it would be of immensurable benefit to obtain these thermodynamic parameters on a per-residue basis. Recently, new methodologies have been described by which these thermodynamic parameters can be derived on a per-residue basis from NMR relaxation data, thus offering a means by which the thermodynamics of the interactions can be characterized at a level of detail that has, until now, not been possible. Our aim is to use both techniques to rationalize differences in the thermodynamics of binding in structural terms.

NMR studies of ABP

The chosen system is the L-arabinose-binding protein (ABP), a 33 kDa protein derived from *E. coli* that binds L-arabinose and D-galactose with similar affinities in the μM range. This system has been selected because the ligands are simple monosaccharides and excellent crystal structures are available for ABP in complex with some monossacharides.

Crucial to the success of this project is the availability of the resonance assignments of ^{1}H, ^{13}C and ^{15}N signals. To achieve that, we decided to prepare a sample with essentially complete enrichment with ^{13}C and ^{15}N and a level of ~50% random deuteration. It is known that deuteration of protein improves the resolution and sensitivity of NMR experiments. This labeling pattern optimizes the sensitivity of experiments used to assign sidechain ^{1}H and ^{13}C resonances by correlating them with the resonances from backbone nuclei whilst still being a good compromise for recording NOESY experiments. In addition, we have used the TROSY versions of the experiments that offer significant additional improvements in resolution and sensitivity in ^{1}H-^{15}N correlation experiments.

Due to the size of the protein, a set of three independent 3D triple resonance experiments [HNCA/HN(CO)CA, HNCO/HN(CA)CO, HN(CA)CB/HN(COCA)CB] and the ^{15}N-HSQC-NOESY are being used for backbone assignment. Side-chains will be assigned from HN(CA)CB/HN(COCA)CB, ^{15}N-TOCSY-HSQC and H(CACO)NNH experiments.

ITC studies of ABP

In addition to the 'natural' ligands (L-arabinose and D-galactose) we are using all the possible deoxy-analogues of D-galactose to delineate the precise thermodynamic contribution to binding of the individual hydroxyl groups. The binding affinities of this panel of deoxy analogues are being determined by isothermal titration calorimetry, which additionally provides the ΔG^{o}, ΔH^{o} and ΔS^{o} of binding for each analogue. These parameters will be examined in order to decompose the 'global' thermodynamic parameters into contributions on a per-residue basis.

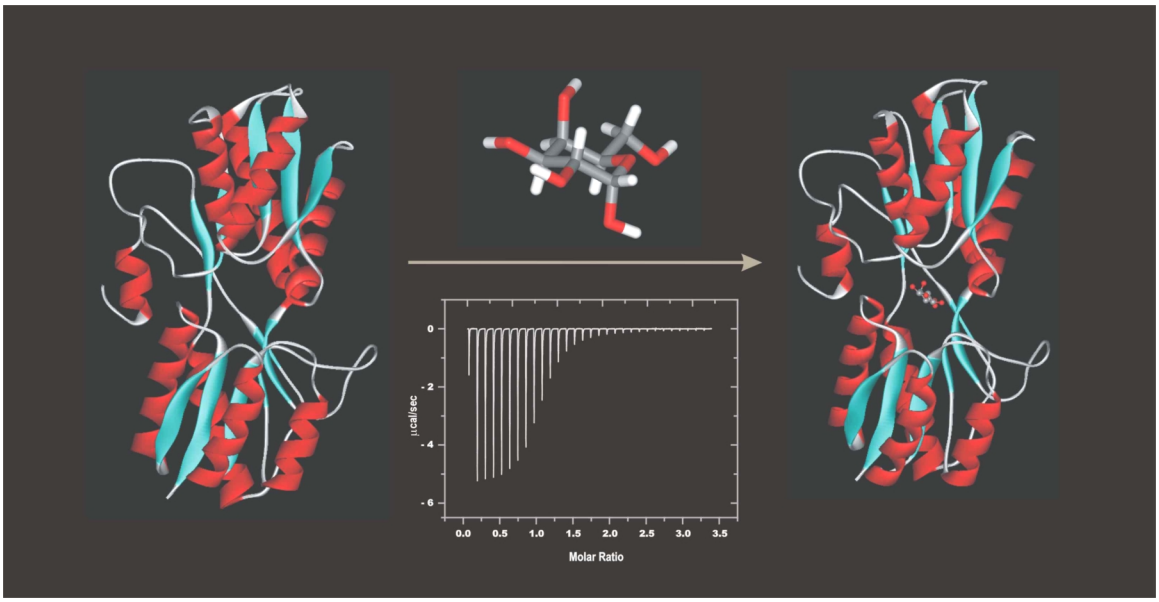

Figure. Titration of ABP with D-galactose on a typical ITC experiment. At specific time intervals, a small volume (10 µL) of the ligand solution is injected into the cell containing the protein, giving rise to the titration heat effects. Analysis of the data yields the global thermodynamic parameters ΔG, ΔH and ΔS.

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