02.1-34 THEORETICAL CALCULATION OF RELATIVE AFFINITIES IN PROTEIN-SUBSTRATE BINDING. Hartmut Luecke & Florante A. Quiocho, Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas, USA.

Our laboratory has been engaged in the determination of the three-dimensional structures of several binding proteins from Gram-negative bacteria. These proteins, which are located in the periplasmic space, serve as essential components of osmotic shock-sensitive active transport systems for a large variety of carbohydrates, amino acids and ions. Several of the sugar-binding proteins also act as initial receptors for chemotaxis. With the highly refined structure of the L-arabinose-binding protein (ABP) and experimental values of free energy changes of binding for L-arabinose, D-fucose, D-galactose and various deoxy-D-galactoses, we compute the difference in the relative free energy change for binding of different substrates using the thermodynamic cycle-perturbation method. This technique provides quantitative information about the contribution of individual hydroxyl-protein interactions to the overall binding energy of the sugar substrate, which can be compared with values obtained experimentally.

The thermodynamic cycle of interest is

$$\begin{array}{ccc} ABP:S_1 & \rightarrow & ABP:S_2 \\ \uparrow & & \uparrow \\ ABP+S_1 & \rightarrow & ABP+S_2 \end{array}$$

with

$$ABP+S_1 \rightarrow ABP:S_1 \quad \Delta A_1 \quad [1]$$
$$ABP+S_2 \rightarrow ABP:S_2 \quad \Delta A_2 \quad [2]$$
$$ABP+S_1 \rightarrow ABP+S_2 \quad \Delta A_3 \quad [3]$$
$$ABP:S_1 \rightarrow ABP:S_2 \quad \Delta A_4 \quad [4]$$

where [3] and [4] denote hypothetical reactions. The relative free energy difference between binding S_1 and S_2 is

$$\Delta\Delta A = \Delta A_1 - \Delta A_2 \quad [5]$$

or, hence A, the Helmholtz free energy, is a thermodynamical state function,

$$\Delta\Delta A = \Delta A_3 - \Delta A_4 \quad [6]$$

where ΔA_3 refers to the relative free energy of hydration for molecules S_1 and S_2.

The perturbation technique is used to compute ΔA_3 and ΔA_4. First, potential energy functions V_1 for the ABP/S_1/solvent system and V_2 for the ABP/S_2/solvent system are defined. Next, one defines a "mixed" potential energy function V_λ such that

$$V_\lambda = \lambda V_2 + (1-\lambda) V_1 \quad [7].$$

The free energy is then computed by performing stepwise perturbation of the ABP/S_1/solvent system to the ABP/S_2/solvent system by changing the potential energy function parameters (atomic radii and partial charges) of S_1 in discrete increments to those of S_2. For each step, λ_i, a dynamical simulation yields the free energy for perturbation parameter values λ about simulation step values λ_i

$$A_3(\lambda) - A_3(\lambda_i) = -kT \ln \langle \exp(-(V_\lambda - V_{\lambda_i})/kT \rangle_{\lambda_i} \quad [8]$$

where $\langle \rangle_{\lambda_i}$ is a canonical ensemble simulation average for V_{λ_i}. The simulation stepsize, $\lambda_{i+1} - \lambda_i$, is chosen to assure adequate statistics in the region of perturbation parameter λ where the the results $A_3(\lambda)$ from simulation i overlap those from i+ 1, and $\Delta A_3 = A_3(\lambda = 1) - A_3(\lambda = 0)$ is obtained by piecing together the individual simulation steps. The potential energy V_1 ($V_{\lambda=0}$) is computed for a large number of system configurations to obtain a statistically significant canonical simulation average [8]. Then the "mixed" potential energy, V_λ, is computed for each configuration saved from the simulation for discrete values of the perturbation parameter λ.

The computations were carried out on a Cray X-MP using molecular dynamic algorithms of the program system AMBER with a dynamic step size of 2 fs. The L-arabinose-binding protein, the primary ligand and 182 highly ordered waters from the X-ray structure consist of 3,406 atoms. In order to simulate substrate binding in solution, the protein-substrate complex has been immersed in a box of nearly 2,200 water molecules obtained from a previous liquid state simulation, raising the total number of atoms in the system to over 10,000.

The first calculation for the perturbation of D-galactose (S1) to 1-deoxy-D-galactose (S2) yielded 19.77 kcal/mol for ΔA_3 and 24.11 kcal/mol for ΔA_4, or a $\Delta\Delta A$ of -4.34 kcal/mol. This value differs only 3% from the experimental value of -4.21 kcal/mol.

02.1-35 IRON-SULFUR CLUSTER IN ACONITASE AT 3.0A RESOLUTION. By A.H. Robbins and C.D. Stout, Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

Aconitase in the inactive form contains a [3Fe-4S] cluster (H. Beinert, et al, Proc. Natl. Acad. Sci., 1983, 80, 393-396). The enzyme, MW 80,000 daltons, from pig heart crystallizes in space group $P2_12_12$ with a=173.7, b=72.0, c=72.8A and one molecule in the asymmetric unit. The sites of the three Fe atoms were determined from the anomalous difference Patterson map using film data at 2.7A resolution (A.H. Robbins and C.D. Stout, J. Biol. Chem., 1985, 260, 2328-2333). We have recently calculated a 3.0A resolution electron density map. X-ray diffraction data were collected on the area detector diffractometer at UCSD for the native protein and three isomorphous derivatives (Hg, Pt and the apo-protein). For 350,000 total observations on six crystals Rsymm(I) was 3-6%. Double isomorphous replacement phase calculations at 3.0A using the Hg and Pt derivatives alone without contribution from Fe for 19,400 reflections gave a figure of merit of 0.76 following iterative solvent flattening and phase refinement (B.C. Wang, Methods Enzymol., 1985, 115, 90). Prior to model building, the map shows that two of the Fe sites have one protein ligand each, and that the third site has one or two ligands. The native anomalous difference Fourier map shows a compact peak at the cluster site, confirming the earlier interpretation of three Fe sites with Fe-Fe separations less than 3.0A, and consistent with the expected enhancement of Fe in this map (f"(Fe)=3.4 vs. f"(S)=0.6). The native vs. apo isomorphous difference Fourier map shows a larger, broader peak at the cluster site, consistent with the relative contribution from sulfur (f(Fe)=26 vs. f(S)=16), and indicating S-S separations longer than 3.0A. The uniformity of the electron density among the Fe sites in each of Fourier maps indicates that the Fe occupancy is roughly equal at each site, i.e. the cluster is cooperatively depleted in the apo-protein. A fourth derivative prepared with $PbCl_2$ (3.0A film data) has a single partial occupancy site adjacent to the cluster which represent the Fe site incorporated upon activation to a [4Fe-4S] cluster (M.H. Emptage, et al., Proc. Natl. Acad. Sci., 1983, 80, 4674-4678). These maps are being used to test the predicted cubane structures for the [3Fe-4S] and [4Fe-4S] forms of the enzyme. The electron density of the protein shows extensive α-helical and β-sheet structure and is presently being modeled. Supported by NIH grant GM-36325.