A SURFACE FREE ENERGY MODEL FOR PROTEIN STRUCTURE IN SOLUTION

Hemoglobin Equilibria

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INTRODUCTION

How much information is required to evaluate the equilibrium constants of structural changes in biopolymers? Hemoglobin structures are known in great detail, but their complexity limits our ability to extract information about the energetics of interaction in solution and the equilibria involving structural changes. We have found that it is possible to account for a number of aspects of hemoglobin equilibria by minimizing detail and using only two molecular parameters, the surface area and the surface charge. The surface area, the interfacial contact area between the molecule and the solution, can be estimated from x-ray structures (Muirhead et al, 1967), while the surface charge can be calculated from titration data (German and Wyman, 1937).

In this brief presentation we shall outline our general approach and some results of our calculations. It appears that the two molecular parameters, when combined to evaluate a surface free energy, provide enough detail for physically meaningful energy calculations.

RESULTS AND DISCUSSION

By considering hemoglobin as a colloidal system and using only the molecular surface area and the surface charge, the structural changes during reactions have been evaluated in terms of changes in surface free energy. Changes in the equilibrium constants have then been calculated in terms of the free energy (Blank, 1973). Some of the unusual properties of hemoglobin and the complex interrelations between properties can be demonstrated, as summarized in Table I. This simple model accounts for the appearance of dissociation at certain pH's and for the variation of the dissociation constant with oxygenation (Blank, 1980b). It also shows that the oxygenation equilibrium constant varies with pH, as in the acid and alkaline Bohr effects, and with the ionic strength (Blank, 1975). Furthermore, the model

TABLE I
PROPERTIES CALCULATED BY THE SURFACE FREE ENERGY MODEL

Physical property	Calculated dependence
Tetramer dissociation constant	Onset at acid and alkaline pH's Order of magnitude of constant Variation with oxygenation Variation with ionic strength
Oxygenation equilibrium constant	Bohr effect (acid and alkaline) Variation with ionic strength
Hill coefficient in oxygen binding equation	Variation with binding—it is not constant! Behaves as a Gibbs surface excess
Linkage (dissociation and ligand binding) functions	Linked through joint effect on surface free energy

shows that the empirical Hill coefficient in the oxygen binding equation is not a constant, as has been demonstrated by Roughton et al (1955). The coefficient is simply related to and behaves as a Gibbs surface excess (Blank, 1976, 1980b).

The qualitative success of the approach emphasizes the importance of surface charge and surface area as properties that can lead to estimation of the free energies of some proteins in solution. The surface free energy appears to take into account the hydrophobic as well as the electrostatic interactions that are known to play a major role in determining the shape of proteins in solution.

Thus far, our work has been limited to hemoglobin, but other molecules show similar properties. For example, the haemocyanins (Bannister, 1977) have many more (48) subunits and the molecular weights of the molecules are much higher ($\sim 3 \times 10^6$), but they show the same kinds of dissociation reactions with changes of pH and salt as found in hemoglobin. There are also parallels in the oxygen binding reactions of these copper bearing pigments. It is very likely that our simplified approach to free energy calculations for macromolecules will prove useful in dealing with these cases too.

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CHARGE TRANSFER STABILIZATION OF HEMOGLOBIN STRUCTURES

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The porphyrin macrocycle in heme proteins is embedded in a hydrophobic pocket. Although it is clear that many functional properties of heme proteins are governed by the interaction between the heme and its local environment, the detailed nature of these forces has not been

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