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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explored. We have recently developed a highly sensitive Raman difference spectroscopy (RDS) (1) to assess the importance of such forces by studying perturbations on the heme due to changes in this protein environment. Application of this technique to ferrous deoxyhemoglobins with high affinity quaternary structures stabilized by chemical modification of the proteins yields Raman frequency differences which correlate with stabilization of the high affinity conformation (2). Similarly, changes are observed in certain Raman modes when the allosteric effector, IHP, is added to methemoglobins (3). These data provide evidence for an electronic stabilization between the heme and the protein.

In RDS measurements on native and chemically modified deoxyhemoglobins stabilized in either the R or the T structure, frequency differences were detected in several Raman modes (see Fig. 1) which are known to be sensitive to the electron density in the antibonding orbitals of the porphyrin macrocycle. The shifts to lower frequencies provide evidence for an increase in electron density in the antibonding orbitals when the protein is transformed to the R structure. This increase may result from a charge transfer interaction in which the porphyrin macrocycle serves as an electron acceptor and a near heme residue serves as an electron donor. From consideration of possible electron donors and pathways, it appears from x-ray structural analyses that phenylalanine CD-1 has the appropriate stereochemical properties to account for the observed RDS data.

The study of quaternary structural transitions in various methemoglobins provides an additional experimental test of the influence of the protein on the liganded heme. As

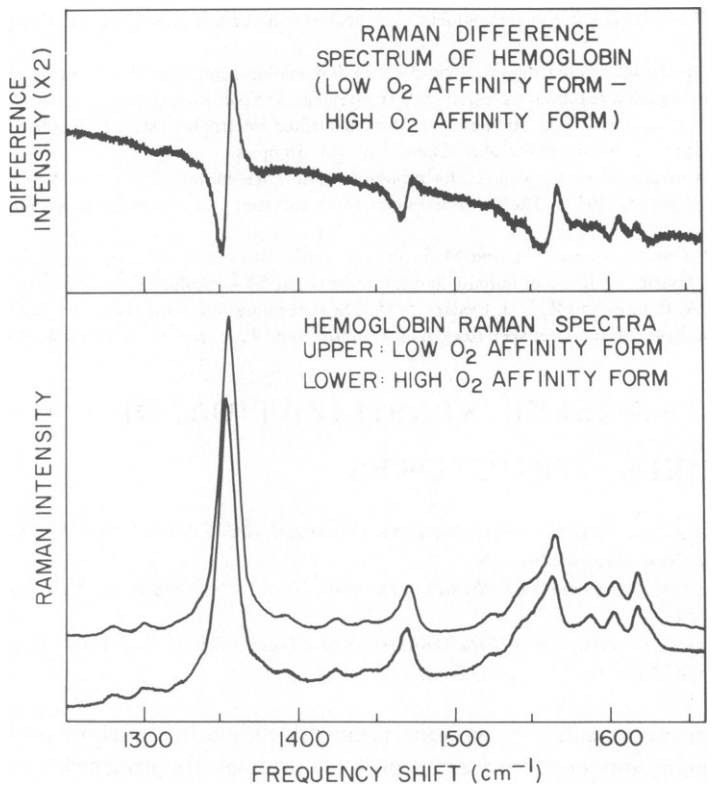

Figure 1 Raman (bottom) and Raman difference spectra (top) of native (low O_2 affinity) and chemically modified (high O_2 affinity) deoxyhemoglobins.

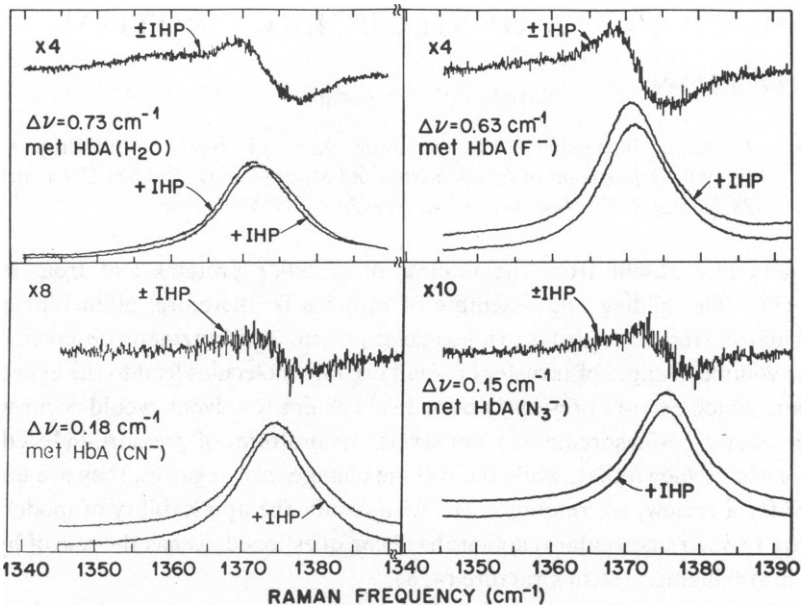

Figure 2 Raman spectra and Raman difference spectra for a series of methemoglobins with and without IHP.

predicted from the charge transfer model derived from the deoxyhemoglobin experiments, the Raman frequencies of the electron density mode in the 1,350–1,380 cm^{-1} region increase with the stability of the R structure in the various ligated species. Furthermore, as shown in Fig. 2, addition of IHP to liganded methemoglobins causes a frequency shift of >0.5 cm^{-1} in that mode when a quaternary structure change occurs (H_2O and F^-) and a smaller frequency shift of <0.2 cm^{-1} when IHP induces only tertiary changes (CN^- and N_3^-).

The evidence for charge transfer stabilization provided by these data makes an electronic interaction model for hemoglobin cooperativity an attractive possibility. Although the frequency differences which we observe correlate with the biochemical properties of hemoglobin and may be interpreted within the framework of this model, quantitative calculation of the stabilization energy depends on several parameters whose values have not yet been determined. Only when these become known will we be able to determine if the protein-heme charge transfer interaction plays a significant or a minor role in the conformational stabilization of hemoglobin.

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