THE POSSIBILITY OF DIRECT INTERACTION BETWEEN THE HEMES IN HEMOGLOBIN

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The Problem.—Heme-heme interaction in hemoglobin may be defined briefly as the dependence of the ligand-binding properties of a particular subunit heme upon the state of liganding of other subunit hemes in the same tetramer. The existence of such dependence is clearly demonstrated by a comparison of the equilibrium ligand-binding properties of the isolated α and β subunits with that of hemoglobin.1 The nature of heme-heme interaction has not yet been elucidated, although it has been the subject of much consideration. Pauling2 attempted with limited success to implement the concept of direct ligand-dependent interactions between the hemes themselves. The currently more popular approach is to assume that the actual subunit interactions arise at the interface between the adjacent subunits and that their ligand dependence stems from ligand-induced configurational changes in the individual subunit.3

This latter approach gains appeal from its generality, since no particular property of a heme need be invoked. However, this very generality is an enormous obstacle to the formulation of a quantitative description of protein function in terms of structure. Recent attempts4, 5 to quantify or partially quantify this allosteric model, as it is called, and to apply it to hemoglobin utilize assumptions, made in the interest of mathematical tractability, that would appear to rob the model of physical meaning.

In the hope of discovering a simpler alternative to this situation, Libby4 began, in 1964, to re-examine the possibility of long-range interaction between the hemes themselves, utilizing the new knowledge of hemoglobin structure obtained since the appearance of the Pauling model.6, 7 Since Wyman9 had shown that the diheme produced by the splitting of hemoglobin in concentrated urea displays nearly all the cooperative ligand binding characteristic of the tetraheme, Libby assumed that two of the six possible heme pairs interact much more strongly than do the other four. Wyman’s value for the ligand-dependent interaction between the two hemes in diheme (−3.5 kcal/mole of dihemes) was employed as an approximate value for the interaction between the two closest heme pairs. X-ray studies had revealed that the two hemes in these close pairs are approximately 25 Å apart and nearly coplanar.7

Various mechanisms by which two such hemes could directly interact with the required energy were examined for feasibility. Monopole interactions were excluded because the heme carries no net charge in either the liganded or unliganded configuration. Permanent electric dipole interactions were excluded because the near-$D_{4h}$ symmetry of the heme would seem to rule out the existence of permanent dipoles of the requisite size. Magnetic dipoles were excluded because unrealistically large ring currents in the conjugated pi-electron system of the heme would have to be invoked. Electron exchange bonding between the
hemes was ruled out because an infinitesimally small overlap between the electronic wave functions of the two hemes would be expected.

The possibility of induced-dipole (London dispersion) interaction was closely investigated. It was found that a ligand-dependent interaction of the requisite energy would be possible if there happened to exist in the plane of the heme an anomalously large electronic polarizability (static value \( \approx 10^4 \text{ Å}^3 \)) resulting from a highly allowed low energy \( \pi - \pi^* \) transition in the region of 5–20 \( \mu \). The ligand dependency of such an interaction would result from the withdrawal of electrons from the highest filled pi-orbital upon liganding. Although this state of affairs would be unique, to our knowledge there exists no theoretical objection to, or conclusive experimental evidence against, the presence of such a transition. Therefore an experimental search was undertaken.

_Attack and Results._—Because there are major technical difficulties associated with a search for the infrared absorption corresponding to the hypothetical transition, it was decided to determine the effect of such a transition upon the dielectric properties at a frequency considerably below that of the transition. A modified Kirkwood equation\(^{10} \) was employed to calculate the effect of the predicted change in heme polarizability upon the real part of the dielectric constant (\( \varepsilon' \)). It was found that an easily measurable difference in the dielectric constant of concentrated solutions of oxy- and deoxyhemoglobin would follow from such a transition. In order to isolate the effect of ligand-dependent changes in the electronic polarizability of heme from other possible ligand-dependent changes in the orientational polarizability of hemoglobin, it was decided to perform the measurements at a frequency well above the orientation dispersion region of hemoglobin,\(^{11} \) thus eliminating contributions from orientation polarization of permanent dipoles in the hemoglobin molecule. (This assumes no substantial volume shrinkage to occur when the protein is oxygenated, an apparently reasonable assumption since the requisite shrinkage to make the loss in electronic polarizability from the theory would be 16,000 Å\(^3\) or about 15\% of the molecular volume.)

Using a coaxial microwave bridge method, the details of which will be described elsewhere,\(^{12} \) the real and imaginary parts of the dielectric constant of 30 per cent (by weight) solutions of human oxy-deoxyhemoglobin were measured at 3 GHz at two temperatures, 1° and 24°C (Table 1). The absolute accuracy of the measurement was estimated at ±1.5 per cent, but the reproducibility, which is indicated in Table 1, is a more important criterion for reliability in a difference measurement. At both temperatures, the observed difference between

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Solution</th>
<th>Dielectric constant ( \varepsilon' )</th>
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</thead>
<tbody>
<tr>
<td>24°C</td>
<td>Deoxy</td>
<td>51.8 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Oxy</td>
<td>51.7 ± 0.3</td>
</tr>
<tr>
<td>1°C</td>
<td>Deoxy</td>
<td>52.0 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>Oxy</td>
<td>51.9 ± 0.2</td>
</tr>
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For an assumed polarizability change of \( 10^4 \text{ Å}/\text{heme} \), the modified Kirkwood equation predicts

\[ \Delta \varepsilon' = \varepsilon'_{\text{deoxy}} - \varepsilon'_{\text{oxy}} = 3.3. \]
the dielectric constant of oxy- and deoxyhemoglobin was less than 10 per cent of the predicted difference. This consequently rules out the possibility of a polarizability change large enough to support the London force hypothesis.

Since higher-order multipole interactions are even shorter in range than London force interactions, these were not studied. It is concluded that cooperative behavior in hemoglobin is not a result of induced dipole (London dispersion) interactions between the binding sites.

This report is abstracted from a dissertation submitted by one of us (A.M.) in partial satisfaction of the requirements for the Ph.D. degree at the University of California, Los Angeles.

Note added in proof: It has just come to our attention that the difference in the dielectric constant of 10.7% solutions of horse deoxy- and oxyhemoglobin has been measured at 9.4 GHz (von Casimir, W., N. Kaiser, F. Keelmann, A. Mayer, and H. Vogel, Biopolymers, in press). Their value for the difference was 0.02 ± 0.005 which extrapolates to 0.05 ± 0.005 at our concentration. The higher precision obtained in their study strengthens the conclusions drawn here. We thank Prof. A. Mayer and his co-workers for advance notice of these results.

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