Calorimetric Studies of Hemoglobin Function, the Binding of 2,3-Diphosphoglycerate and Inositol Hexaphosphate to Human Hemoglobin A

(Received for publication, January 10, 1974)

DENNIS P. NELSON, W. DANIEL MILLER, AND LUTZ A. KIESOW
From the Experimental Medicine Division, Naval Medical Research Institute, Bethesda, Maryland 20014

SUMMARY

Calorimetric titration curves demonstrate the heat of binding of organic phosphates to oxy- and deoxyhemoglobin at pH 7.4 in 0.05 M 2,2-bis(hydroxymethyl)-2',2'-2''-nitroethanol buffer and 0.1 M CI-1. An absence of binding heat was noted at this pH value for both the 2,3-diphosphoglycerate (2,3-DPG) and inositol hexaphosphate (IHP) interactions with oxyhemoglobin. At pH 6.0, an exothermic heat of reaction is observed between both oxy- and deoxyhemoglobin and 2,3-DPG, the deoxy complex being the more stable. The binding ratio of 2,3-DPG and IHP to deoxyhemoglobin at all pH values studied was found to be 1:1. A strong pH dependence in the ΔH for these reactions suggests a proton involvement. The pH coefficient of binding is markedly different between the two forms of reduced hemoglobin. Mg2+ is shown to be a competitive inhibitor of 2,3-DPG-deoxyhemoglobin binding through formation of a nonbinding 2,3-DPG-Mg2+ complex. IHP forms a more stable complex than 2,3-DPG with deoxyhemoglobin at pH 7.0 to 7.4, and it shows reactivity at pH 7.4 with methemoglobin. This complex seems to be quite stable with a small heat of formation and involves all hemoglobin molecules, not just those in a particular spin state. 2,3-DPG, on the other hand, gives little or no heat of binding to methemoglobin at pH 7.4 but shows appreciable binding at pH 6.0. Differences in binding heats between the three hemoglobin forms are interpreted to mean that different functional groups participate in the binding of the same ligand to the various hemoglobin quaternary states.

As a first step toward the understanding of this special anionic effect, measurements of the binding affinities of organic phosphates for the oxygenated and deoxygenated states of the hemoglobin tetramer have been attempted (3-7). There has been considerable variation in the methods used and the conclusions drawn from these studies, the principal discrepancy being the degree of binding of the organic phosphates to oxyhemoglobin under physiological conditions. The results obtained by Benesch et al. (3) and Bunn et al. (6), using the shift of the hemoglobin oxygenation curve in the presence of the organic phosphates as the objective criterion for binding differ sharply, concerning the binding of 2,3-DPG1 to oxyhemoglobin, from those of Chanutin and Hermann (4) and Carby et al. (5), obtained by equilibrium dialysis. This question has important consequences with regard to the mechanism of hemoglobin oxygenation. Benesch et al. (8) concluded that the reported stability constants for the oxyhemoglobin-2,3-DPG complex (4, 5) were inconsistent with the observed shifts in the oxygenation curve. The binding constants obtained from shifts in the oxygenation curves (3), on the other hand, require the assumption of true competitive binding between 2,3-DPG and oxygen for deoxyhemoglobin. Those measurements are, therefore, more indirect than those performed by equilibrium dialysis. As a consequence, the question of binding of 2,3-DPG to oxyhemoglobin is as yet unanswered.

The binding of oxygen to hemoglobin is accompanied by readily discernible spectral changes, but the binding of 2,3-DPG is not. In view of the problems in obtaining direct spectral measurements and of the rather large ΔH values reported (3) for the deoxyhemoglobin-2,3-DPG interaction, we felt that a direct microcalorimetric observation of the binding reaction would help to resolve the discrepancies in the published literature. Calorimetric measurements on horse hemoglobin (9) had indicated that, in the concentrations at which they occur in the red cell, 2,3-DPG does form stable complexes with oxyhemoglobin. Whether this is also true of human hemoglobin and under conditions described (3), we hoped to answer by a flow microcalorimeter titration technique as a direct probe of the binding. This method is capable of providing ΔG, ΔH, and ΔS values for such a binding reaction, provided a heat change occurs and the binding constant is within the necessary limits.

1 The abbreviations used are: 2,3-DPG, 2,3-diphosphoglycerate; IHP, inositol hexaphosphate; bis-tris, 2,2-bis(hydroxymethyl)-2',2'-2''-nitroethanol; Hb, hemoglobin.
**MATERIALS AND METHODS**

**Preparation of Hemoglobin Solutions**—Hemoglobin was prepared from recently outdated (acid citrate dextrose) blood units which were screened electrophoretically to eliminate variant hemoglobins. The cells were washed three times in isotonic saline, suspended in an equal volume of deionized H₂O, and lysed by three times freeze-thawing using a Dry Ice-ethanol bath. The stroma was removed by centrifugation at 44,000 × g for 20 min and the hemolysate (∼100 ml) was dialyzed at 0° in a hollow fiber “dialyzer” beaker (Bio-Rad Labs) for 2 to 3 hours against 2 liters of buffer solution (0.05 M bis-tris (Aldrich Chemical Co., ultrapure) adjusted to pH with HCl and to a final Cl⁻ concentration of 0.1 mM. Inositol hexaphosphate was purchased as the sodium salt and solutions such as those of hemoglobin since there is no vapor space and solutions can be transferred from a reservoir maintained in equilibrium with the desired gas into gas-tight infusion syringes by means of three-way valves. To produce a titration curve for the hemoglobin-2,3-DPG interaction, the hemoglobin solution previously equilibrated with N₂ or air is drawn into the syringe of the constant speed pump (Pump A). The variable speed pump (Pump B) is loaded with gas-equilibrated 2,3-DPG titrant and the subsequent titration proceeds automatically. Step changes are produced at timed intervals by changing the resistance in the control circuit of the Hall effect drive motor of the variable pump. The titration range covers an approximate 10-fold change in reactant ratio from an initial ∼5 to 1 excess of hemoglobin over organic phosphate to a final ∼2 to 1 excess of the titrant. The heat data can then be expressed in terms of total heat production in microlcalories per min versus micromoles per min of 2,3-DPG added. The time dimension can be eliminated giving −Q per μmoles 2,3-DPG under steady state conditions. The quantity of hemoglobin participating in the reaction remains constant.

**RESULTS AND DISCUSSION**

Calorimetric titration curves are shown for the interaction of 2,3-DPG and IHP with the three forms of hemoglobin in Figs. 1 and 2. Statistical analysis of the curves for the evaluation of both the log K and ΔH values for these reactions was carried out according to published procedures (14, 15) by an iterative curve fitting routine based upon the 1:1 equilibrium expression:

\[
K = \frac{[\text{Hb-2,3-DPG}]}{[\text{Hb}][\text{2,3-DPG}]}
\]

The mass balance equations:

\[
[\text{Hb}_{\text{total}}] = [\text{Hb}] + [\text{Hb-2,3-DPG}]
\]

\[
[2,3\text{-DPG}_{\text{total}}] = [2,3\text{-DPG}] + [\text{Hb-2,3-DPG}]
\]

and the heat equation:

\[
Q = \Delta H \cdot \frac{[\text{Hb-2,3-DPG}]}{\text{vol}}
\]

**Binding of 2,3-DPG to Hemoglobin at pH 7.4**—Fig. 1 shows curves for the titration of three forms of hemoglobin with 2,3-DPG at pH 7.4. With deoxyhemoglobin, a large exothermic heat change is observed. In contrast, hemoglobin in the oxygenated (oxyhemoglobin) or in the oxidized (methemoglobin) form shows little or no heat evolution upon addition of 2,3-DPG. From the position of the equivalence point in the curve at a molar ratio of 1.0, the complex formed between 2,3-DPG and deoxyhemoglobin is seen to be 1:1 in terms of the tetrameric hemoglobin.

**Binding of IHP to Hemoglobin at pH 7.4**—Fig. 2 gives analogous binding curves with IHP replacing 2,3-DPG as the titrating agent. In this case as well, little or no heat change is observed with oxyhemoglobin. However, methemoglobin does produce a measurable heat change (about one-third of that seen with deoxyhemoglobin). Both deoxy- and methemoglobin appear to bind IHP with 1:1 stoichiometry. Since the IHP and 2,3-DPG deoxyhemoglobin complexes are stable, the calorimetric titration curves are relatively independent of the binding constant, and therefore the uncertainty encountered when the constants are evaluated by statistical methods is increased.
Fig. 1 (left). Binding of 2,3-DPG to oxy-, deoxy-, and methemoglobin at pH 7.4. Calorimetric titration curves (determined in 0.05 M bis-tris buffer and 0.1 M total Cl\(^-\) at 25°) are plotted with the exothermic heat change (in millicalories) on the ordinate versus ratio of 2,3-DPG titrating agent to total hemoglobin on the abscissa. Plotted points represent the average of corresponding points from three titrations corrected for heat effects due to dilution and viscous heating. The average standard error per point is ±0.03 mcal. Deoxyhemoglobin curves at pH 7.2 and 7.0 (not shown) had similar error intervals. Hemoglobin concentration is 0.9 mM.

Fig. 2 (right). Binding of IHP to oxy-, deoxy-, and methemoglobin at pH 7.4. Calorimetric titration curves are identical with those in Fig. 1 with IHP replacing 2,3-DPG as the titrating agent. Error intervals are somewhat smaller than those of Fig. 1. Deoxyhemoglobin titrations at pH 7.2 and 7.0 (not shown) had similar error intervals. Hemoglobin concentration is 1.3 mM.

Although binding constants can be determined for the 2,3 DPG deoxyhemoglobin interaction with an acceptable accuracy, in the case of IHP they have only been estimated. AH values, on the other hand, are virtually independent of the binding constant and can be obtained reliably for both interactions.

The thermodynamic quantities obtained by calorimetric titration are likely composite values of more than one interaction occurring between 2,3-DPG and the deoxyhemoglobin and, as such, are “apparent” thermodynamic quantities since the calculations involve the assumption of a single 1:1 binding reaction. The AH value would therefore also be a composite of enthalpy changes resulting from the formation of salt bridges as well as from proton association and dissociation reactions involving the hemoglobin, and possibly the organic phosphate and the buffer. The fact that addition of 2,3-DPG to deoxyhemoglobin at pH 7.3 does not produce a measurable pH change (6) does not preclude a proton involvement since hemoglobin itself is zwitter ionic and may function as its own buffer.

pH Dependence of Thermodynamic Values—Thermodynamic values are listed for the 2,3-DPG-deoxyhemoglobin binding reaction in Table I including the fitted values for the logarithmic binding constants at pH 7.0, 7.2, and 7.4. While we were unable to show a pH dependence of log K similar to that reported by Benesch et al. (3), the values are roughly comparable in magnitude. In addition, Bunn et al. (6) report a similar value of log K = 4.64 at pH 7.3. The failure to observe an obvious pH dependence in log K may be due to the inherent uncertainty in its calorimetric determination. The ΔH values do, however, show a marked pH dependence, the exothermic heat change increasing with decreasing pH. This would indicate an involvement of ionizable protons in the binding reaction of organic phosphates to deoxyhemoglobin. Such a conclusion is supported by the pH dependence of the binding constant (3) and by the demonstrated involvement of NH\(_2\)-terminal amino groups in the binding reaction (7, 16–18). A shift in the pK\(_a\) of the α-amino group of the β chain terminal valine or the β 143 histidine to a higher value in the presence of ligand could account for this pH effect. Such a shift might be expected in conjunction with the binding of a polyvalent anion which would facilitate the protonation of hemoglobin groups lying in sterically favorable orientations for the formation of salt bridges with the ligand.

<table>
<thead>
<tr>
<th>pH</th>
<th>Log K</th>
<th>-ΔG°</th>
<th>-ΔH</th>
<th>-ΔS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cal/mole</td>
<td>Cal/mole</td>
<td>cal/deg X mole</td>
<td></td>
</tr>
<tr>
<td>2,3-DPG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>4.5 ± 0.3</td>
<td>6.1 ± 0.4</td>
<td>13.0 ± 0.2</td>
<td>~23</td>
</tr>
<tr>
<td>7.2</td>
<td>4.5 ± 0.3</td>
<td>6.1 ± 0.4</td>
<td>11.4 ± 0.3</td>
<td>~18</td>
</tr>
<tr>
<td>7.4</td>
<td>4.4 ± 0.2</td>
<td>6.0 ± 0.3</td>
<td>10.3 ± 0.3</td>
<td>~14</td>
</tr>
</tbody>
</table>

2,3-DPG +
10-fold excess Mg\(^{2+}\)
7.4  | 3.5 ± 0.1 | 4.8 ± 0.1 | 10.7 ± 0.2 | ~20  |

* ΔG values were obtained from log K by -ΔG = -2.30 RT log K.
* Hemoglobin concentration was 1.6 mM.

ΔH Values—Our ΔH value of -10.3 Cal per mole (43.1 kJ...
per mole) for the 2,3-DPG-deoxyhemoglobin reaction obtained at pH 7.4 and 25° is roughly comparable to the Benesch et al. (3) value of $\Delta H = -13.2$ Cal per mole at pH 7.3 obtained by a van’t Hoff plot over the temperature range 10–30° and the value of Bunn et al. (6) of $\Delta H = -11$ Cal per mole at pH 7.3 obtained by the same method. The buffer and Cl– concentration in our experiments were identical with those used in the above studies (3, 6) although our calorimetric data were obtained using hemoglobin solutions approximately 30-fold more concentrated. The similarities between the enthalpy values observed for the interaction of both 2,3-DPG and IHP with deoxyhemoglobin at pH 7.0 to 7.4 would suggest that both interactions are responsive to pH in a very similar manner.

The IHP–deoxyhemoglobin binding data are summarized in Table II. $\Delta H$ values are determined with good accuracy by asymptotic extrapolation of the small ratio points on the curve (see Fig. 2). From the shape of the curve at the equivalence point, the logarithmic binding constants were estimated to be greater than 5.5 but less than 6.5. Entropy values were not calculated since the exact log $K$ values are not known. The $\Delta H$ of IHP binding to methemoglobin was determined to be $-3$ Cal per mole (13 kJ per mole). Since the heat change was small, the log $K$ value determined for this reaction is only approximate. From the shape of the curve, however, we can conclude that this binding is also 1:1 in terms of total methemoglobin.

**Table II**

Thermodynamic constants for binding of inositol hexaphosphate to deoxyhemoglobin

Association constants and $\Delta H$ values are given for the interaction of IHP with deoxyhemoglobin and methemoglobin at 25°. Conditions are identical with those of Table I except the hemoglobin concentration is $\sim 1.3$ mM. Binding constants were estimated from the shape of the calorimetric titration curve (see Fig. 2). $\Delta H$ values were determined from the slope of a linear regression of the small ratio points.

<table>
<thead>
<tr>
<th>pH</th>
<th>Log $K$</th>
<th>$-\Delta G$</th>
<th>$-\Delta H$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cal/mole</td>
<td>Cal/mole</td>
</tr>
<tr>
<td>IHP-deoxyhemoglobin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>6.0 ± 0.5</td>
<td>8.2 ± 0.7</td>
<td>12.8 ± 0.2</td>
</tr>
<tr>
<td>7.2</td>
<td>6.0 ± 0.5</td>
<td>8.2 ± 0.7</td>
<td>11.6 ± 0.2</td>
</tr>
<tr>
<td>7.4</td>
<td>6.0 ± 0.5</td>
<td>8.2 ± 0.7</td>
<td>10.1 ± 0.3</td>
</tr>
<tr>
<td>IHP-methemoglobin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.4</td>
<td>~4.8</td>
<td>~6.5</td>
<td>3.1 ± 0.3</td>
</tr>
</tbody>
</table>

at near red cell hemoglobin concentrations, it is likely that dilution effects are minimal. In this context, the effect of various buffer ions, and their net charge, on the hemoglobin molecule is presently under investigation.

**Binding of 2,3-DPG to Hemoglobin at pH 6.0—**Previous reports (5, 19) indicate a strong pH dependence in the binding of 2,3-DPG to oxyhemoglobin, and also equivalent stabilities of the complexes with oxy- and deoxyhemoglobin at pH 6.0. Kinetic studies (20) at pH 7.0 have also been interpreted as evidence for binding of IHP to oxyhemoglobin. As seen in Fig. 3 and Table III, calorimetric results at pH 6.0 present a binding picture quite different from that observed at pH 7.4. At pH 6.0, both oxy- and deoxyhemoglobin show considerable heat evolution upon addition of 2,3-DPG. Deoxyhemoglobin appears to have very similar binding characteristics at pH 6.0 to those observed at pH 7.4 with a somewhat higher log $K$ value (4.9 versus 4.4) and a nearly identical $\Delta H$ value. The oxyhemoglobin complex appears to be about an order of magnitude less stable than that with deoxyhemoglobin and the $\Delta H$ value for the oxyhemoglobin complex appears to be about three-fourths that for deoxyhemoglobin.

**Binding of IHP and 2,3-DPG to Methemoglobin—**Titration of methemoglobin with IHP at pH 7.4 with a somewhat higher log $K$ value (4.9 versus 4.4) and a nearly identical $\Delta H$ value. The oxyhemoglobin complex appears to be about an order of magnitude less stable than that with deoxyhemoglobin and the $\Delta H$ value for the oxyhemoglobin complex appears to be about three-fourths that for deoxyhemoglobin.

**Table III**

Thermodynamic constants for the binding of 2,3-DPG to three forms of hemoglobin at pH 6.0

Association constants, $\Delta H$ and $\Delta S$ values are given for the 2,3-DPG interaction with deoxy-, oxy-, and methemoglobin at pH 6.0 and 25°. Conditions are the same as those in Table I. Calorimetric titration curves (Fig. 3) were analyzed by the curve fitting method to yield the thermodynamic constants. Hemoglobin concentration was $\sim 1.3$ mM and the buffer system was the same as that in Table I.

<table>
<thead>
<tr>
<th>Hb Form</th>
<th>Log $K$</th>
<th>$-\Delta G$</th>
<th>$-\Delta H$</th>
<th>$-\Delta S$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cal/mole</td>
<td>Cal/mole</td>
<td>cal/dag x mole</td>
<td></td>
</tr>
<tr>
<td>Deoxy-</td>
<td>4.9 ± 0.3</td>
<td>6.7 ± 0.4</td>
<td>10.0 ± 0.2</td>
<td>~11</td>
</tr>
<tr>
<td>Oxy-</td>
<td>3.9 ± 0.1</td>
<td>5.3 ± 0.1</td>
<td>7.5 ± 0.3</td>
<td>~7</td>
</tr>
<tr>
<td>Met-</td>
<td>4.0 ± 0.1</td>
<td>5.5 ± 0.1</td>
<td>8.6 ± 0.3</td>
<td>~10</td>
</tr>
</tbody>
</table>

**Fig. 3.** Binding of 2,3-DPG to oxy-, deoxy-, and methemoglobin at pH 6.0. Conditions of the calorimetric titration curves are identical with those in Fig. 1 except for a pH of 6.0 and a hemoglobin concentration of 1.3 mM. Error intervals are the same as in Fig. 1.
methemoglobin with IHP produces a curve somewhat more exothermic than that obtained with oxyhemoglobin at pH 7.4 but much less than that observed with deoxyhemoglobin. This larger heat of reaction of methemoglobin compared to oxyhemoglobin might be expected from the suggestion of Kilmartin (21) that at physiological pH values, methemoglobin can assume both the R and T quaternary structures, with the R state predominant and, furthermore, that IHP causes the equilibrium between the quaternary states to shift from the R to the T form. According to this interpretation, since IHP binds specifically to deoxyhemoglobin, i.e. high spin or T state hemoglobin, one would also expect a larger reaction heat at lower pH values due to the increased stability of the T state hemoglobin. Other reports of the binding of organic phosphates (22) suggest multiple binding sites and pH dependent low and high affinity sites for the ATP methemoglobin interaction. Our data indicate that at pH 7.4 methemoglobin possesses a single binding site for IHP but that the IHP binds to all tetramers regardless of the quaternary state, or at least succeeds in transforming all tetramers into an active binding state. Interactions of 2,3-DPG at pH 6.0 and IHP at pH 7.4 with methemoglobin both appear to be quite stable and to involve the total methemoglobin population, although the relative proportions of R and T state methemoglobin at the two pH values would be quite different (23).

The lower heat of reaction compared to deoxyhemoglobin suggests, however, that different functional groups are involved in the binding of IHP to methemoglobin than those responsible for its binding to deoxyhemoglobin. The pH coefficient of this binding is therefore likely to be different from that of deoxyhemoglobin since the pKₐ values of the binding groups are not likely to be identical in the two cases. From a comparison of the methemoglobin curves in Figs. 1 and 3, it is obvious that methemoglobin does increase its binding characteristics for 2,3-DPG at pH 6.0 over pH 7.4. This change is much larger than the small change observed in the case of deoxyhemoglobin and is similar to the change observed in the case of oxyhemoglobin. Both oxyhemoglobin and methemoglobin therefore appear to possess large pH coefficients in the region pH = 6.0 to 7.4.

Effect of Mg²⁺ on the Binding of 2,3-DPG to Deoxyhemoglobin—Magnesium ion has been shown to be an important competitor in the binding of 2,3-DPG to deoxyhemoglobin (5, 6). Calorimetric measurements of the binding of 2,3-DPG to deoxyhemoglobin in the presence of a 10-fold excess of Mg²⁺ at pH 7.4 are shown in Table 1. These demonstrate a marked effect of Mg²⁺ in lowering the apparent binding constant of 2,3-DPG to deoxyhemoglobin. In this case, the binding constant can be determined quite accurately since this log K value is in the optimum range of the calorimetric titration procedure. Using a competitive binding equation, it is then possible, within the accuracy of the log K values determined in the presence and absence of Mg²⁺, to calculate the binding constant of Mg²⁺ to 2,3-DPG at pH 7.4. The calculation gives a log K value of 2.6 for the Mg²⁺:2,3-DPG binding reaction in the presence of 0.1 M Na⁺. This value is in good agreement with two published values (6, 24) obtained by entirely different methods. Since the ΔH value for the deoxyhemoglobin:2,3-DPG interaction remains unchanged in the presence of Mg²⁺, it would appear that the ΔH value for the formation of the Mg²⁺:2,3-DPG complex including proton dissociation, is zero. This agrees well with the ΔH value given by Bunn et al. (6) for this reaction but disagrees with that of Dietzsch and Siegmund (24) obtained by direct calorimetry. It is not clear, however, whether their ΔH value has been corrected for the proton dissociation reaction associated with the Mg²⁺ binding. Nevertheless, the agreement between log K values is very good considering the completely unrelated methods used in their determination.

Agreement of results obtained by different investigators through the use of competitive binding equations points out (a) that Mg²⁺ and deoxyhemoglobin are truly competitive ligands for the available 2,3-DPG stores of the red cell, and (b) that 2,3-DPG and oxygen compete allosterically for the available deoxyhemoglobin. Since the Mg²⁺ complex of 2,3-DPG binds to deoxyhemoglobin either not at all or much more weakly than the free 2,3-DPG, the binding of 2,3-DPG to deoxyhemoglobin is thus a function of the intracellular Mg²⁺ concentration. Conversely, increases in the dioxygen concentration of the red cell due to deoxygenation result in the binding of free 2,3-DPG, with a concurrent dissociation of some Mg²⁺:2,3-DPG complex, and an increase in free Mg²⁺ concentration. This could account for the observed increase in glycolytic rates (25) when red blood cells are transferred from an aerobic to an anaerobic environment. This mechanism would depend on the concentration of free Mg²⁺ which would in turn determine the concentration of the Mg²⁺:ATP complex (6, 20). The concentration of this complex determines the activities of hexokinase and phosphofructokinase, both rate controlling enzymes of the glycolytic sequence in red blood cells.

If oxygen and 2,3-DPG bind competitively to deoxyhemoglobin at pH 7.4, as has been suggested (3, 6, 7), one might expect to see a considerable endothermic heat change upon addition of IHP or 2,3-DPG to oxyhemoglobin, because the stabilization of the deoxy, quaternary structure of hemoglobin would shift the oxygen equilibrium, resulting in the dissociation of 4 oxygen molecules per mole of 2,3-DPG bound. The lack of such a heat change under the conditions of our experiments is, however, readily understandable from a comparison of hemoglobin oxygenation curves in the presence and absence of 2,3-DPG (27). Under the high oxygen partial pressures at which our experiments were conducted, a vertical shift between oxygenation curves determined in the presence and absence of 2,3-DPG results in very little change in oxygen saturation, and consequently very little heat effect associated with heme-oxygen dissociation. At intermediate O₂ saturation, where appreciable dissociation of heme bound oxygen might be expected, this is prevented in a closed system consisting of a single aqueous phase due to an increase in the O₂ partial pressure.

Our calorimetric results, reported here, obtained by direct titration of oxy-, deoxy-, and methemoglobin with 2,3-DPG and IHP, help to resolve differences in results obtained by sedimentation and dialysis equilibrium, gel filtration, and spectrophotometric and potentiometric titration. Our demonstration of a large difference in the heat of binding of 2,3-DPG to oxyhemoglobin observed at pH 7.4 compared to that observed at pH 6.0 may account for some of the apparent inconsistencies in previous reports, since these differences reflect a considerable pH dependence in the binding properties of oxyhemoglobin. Whether or not a degree of multiple binding occurs in any of the hemoglobin-organic phosphate interactions is not completely clear. In some cases, it was necessary to lower the organic phosphate concentration by a small percentage to arrive at the best fit of the experimental data suggesting that some organic phosphate is bound at a second low heat site. It is clear, however, that the predominant heat-producing reaction is 1:1.

REFERENCES
Calorimetric Studies of Hemoglobin Function, the Binding of 2,3-Diphosphoglycerate and Inositol Hexaphosphate to Human Hemoglobin A
Dennis P. Nelson, W. Daniel Miller and Lutz A. Kiesow


Access the most updated version of this article at http://www.jbc.org/content/249/15/4770

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/249/15/4770.full.html#ref-list-1