EFFECT OF INOSITOL HEXAPHOSPHATE AND OTHER ORGANIC PHOSPHATES ON THE COOPERATIVITY IN OXYGEN BINDING OF HUMAN HEMOGLOBINS

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Summary: On the contrary to 2,3-diphosphoglycerate (DPG) and adenosine triphosphate (ATP), inositol hexaphosphate (IHP) reduces the affinity of human adult hemoglobin (Hb A) to the 4th oxygen molecule as well as the affinity to the 1st, 2nd, and 3rd molecules. Thus, the over-all free energy of interaction (ΔF_I) increases only by about 600 cal per site and the maximum slope of the Hill plot, n, decreases significantly in the presence of 1.7 mM IHP. Therefore, the mechanism of action of IHP in lowering the oxygen affinity of hemoglobin seems to be quite different from that of DPG or ATP. The effect of DPG on the oxygen equilibrium function of human fetal hemoglobin (Hb F) is similar to but less than that on Hb A.

In a previous communication we have reported that DPG markedly reduces the affinity of Hb A to the 1st, 2nd, and 3rd molecules of oxygen without affecting the affinity to the 4th molecule, increasing ΔF_I by about 1400 cal per site (1). The present paper is an extension of the previous investigation and includes the effect of IHP and ATP on the four successive association constants of Hb A for oxygen and the effect of DPG on the oxygen equilibrium function of Hb F.

Preparation of Hb A and oxygen equilibrium measurements were performed as described previously (1). Hb F was isolated from cord blood by the method of Zade-Oppen (2) and freed from phosphates. All the equilibrium measurements were performed at 25° on 1.5×10^{-5} M hemoglobins (tetramer) in 0.01 M Tris-HCl or 0.05 M bis-Tris-HCl buffer (pH 7.40), which gave exactly the same oxygen equilibrium curve. Data analysis was carried out by the Scatchard (3) and Hill plots (4) and four intrinsic microscopic association constants (k's) of hemoglobins for the binding of oxygen based on the Adair's successive oxygenation theory (5) were estimated by fitting the plots with simulated

curves calculated by a digital computer, as reported previously (1).

The values of k's, ΔF_I, the maximum value of Hill's parameter (n_{max}), and oxygen pressure at half saturation (p_{50}) obtained are summarized in Table 1, which also includes the previous data on Hb A with and without DPG for comparison.

As seen in the Table, the effect of ATP on the equilibrium function of Hb A is very similar to that of DPG, although the magnitude of ΔF_I and p_{50} in the presence of ATP is slightly lower than that in the presence of the same concentration of DPG.

Table 1. Summary of k's (mm Hg^{-1}), ΔF_I (cal/site), n_{max}, and p_{50} (mm Hg) for the equilibrium of oxygen with Hb A and Hb F at pH 7.4 and 25°.

	k_1	k_2	k_3	k_4	ΔF_I	n_{max}	p_{50}
Hb A							
stripped	0.079	0.295	0.75	4.35	2300	2.52	1.9
in 2 mM DPG	0.008	0.037	0.02	4.35	3670	3.02	15.3
in 2 mM ATP	0.0126	0.035	0.06	4.35	3460	3.07	11.4
in 1.7 mM IHP	0.0044	0.01	0.0025	0.56	2880	2.35	70.8
Hb F							
stripped	0.063	0.28	0.32	4.0	2480	2.42	2.6
in 2 mM DPG	0.0177	0.07	0.045	4.0	3210	2.77	8.7

On the other hand, as expected from the results of Benesch et al. (6), IHP much more reduces k's than DPG: in 1.7 mM IHP k_1, k_2, and k_3 decrease to about 1/20, 1/30, and 1/300, respectively. Moreover, in contrast to DPG and ATP, IHP also reduces k_4 to 1/8 as evidently seen in the Scatchard plot indicated in Fig. 1. Thus, the asymmetry of the Hill plot is more marked than in DPG and the maximum value of the slope of the plot, n_{max}, is considerably lower than in stripped Hb A. Calculation of ΔF_I by the methods described

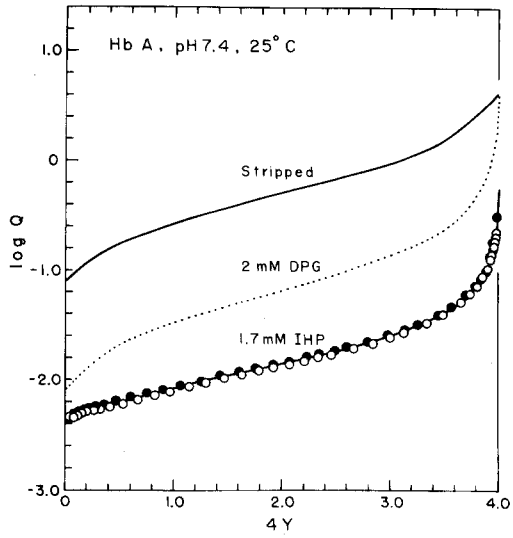

Fig. 1. Scatchard plot of the oxygenation of stripped Hb A in the presence of 1.7 mM IHP. Different symbols refer to separate experiments on different samples. Solid line is the simulated curve constructed by using the values of k's shown in Table 1. The simulated curves for Hb A in the presence and absence of 2 mM DPG are also shown for comparison.

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in the previous paper (1) gives 2880 cal per site in 1.7 mM IHP, which is about 800 cal lower than in 2 mM DPG but still about 600 cal higher than in stripped Hb A.

These observations are consistent with the results of recent kinetic study of Gibson and Gray (7), who have found that IHP significantly reduces the rate of oxygen dissociation from the fully saturated oxy Hb A. It is strongly suggested, therefore, that the mechanism of action of IHP in lowering the oxygen affinity of hemoglobin is quite different from that of DPG and that IHP combines to oxygen-saturated Hb A as well as to the deoxy form imposing some structural constraint upon both the protein molecules.

The Hill's parameter, n, as defined by

$$n = d \log [Y/(1 - Y)]/d \log p,$$

where Y is the fractional saturation of hemoglobin and p is the oxygen pressure, has been usually determined from the slope of the Hill plot in the middle range and taken as a good index of the interactions among oxygen

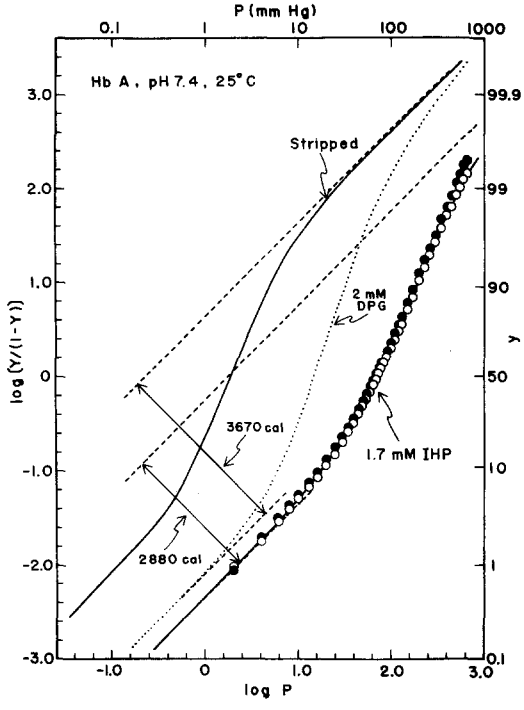

Fig. 2. Hill plots of the data shown in Fig. 1.

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binding sites. As shown in Table 1, however, n_{max} is not proportional to ΔF_I. The slope, n, calculated on the simulated Hill plots in Fig. 2 at different log p is indicated in Fig. 3. The inconsistency between ΔF_I and n_{max} is not irrational since the magnitude of ΔF_I is proportional to the area under the n vs. log p plot (8), not to n_{max} itself, and the shape of the plot markedly depends upon the presence of the organic phosphates, especially IHP, as clearly seen in Fig. 3. The non-proportionality of n to ΔF_I has already been shown by Wyman (8) in Spirographis hemoglobin containing about 80 hemes per molecule. The above results, however, evidently indicate that even in hemoglobin molecules having the same number of hemes the Hill's parameter is not always a good measure of the interactions among oxygen combining sites.

DPG also reduces k_1, k_2, and k_3 of Hb F as shown in Table 1. In accord with our previous observation (9), however, the magnitude of the reduction is smaller than in Hb A: k_1 and k_2 decrease to about a quarter and k_3 to

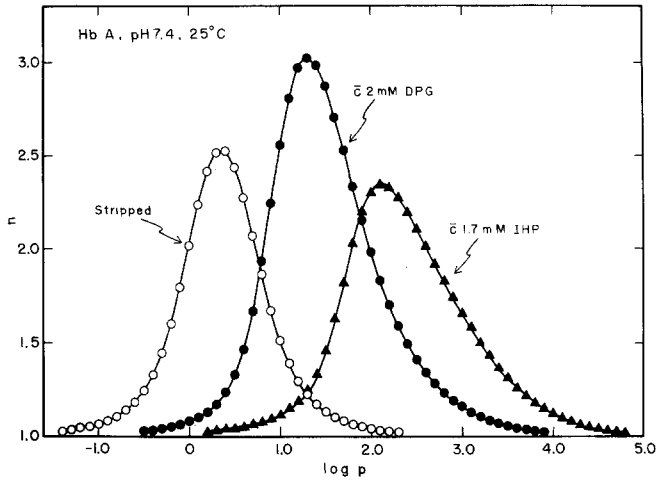

Fig. 3. Plots of the slope, n, of the simulated Hill plots shown in Fig. 2 against log p.

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one-seventh in 2 mM DPG. As in Hb A, k_4 shows no or little, if any, change in the presence of functionally saturating concentration of the phosphate. Thus, the extra ΔF_I due to DPG decreases to 2920 cal per mole in Hb F, which is approximately 2600 cal per mole lower than in Hb A.

In view of the proposed binding mechanism of DPG to hemoglobin mentioned in the previous paper (1), the difference may reasonably ascribed to the loss of two of the four salt linkages with the phosphate in Hb F, since histidine H21(143)β assumed to be involved in the linkage is replaced by uncharged serine in the γ chains.

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