EFFECT OF 2,3-DIPHOSPHOGLYCERATE ON THE BOHR EFFECT
OF HUMAN ADULT HEMOGLOBIN
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SUMMARY: The effect of 2,3-diphosphoglycerate (DPG) on the Bohr effect of human hemoglobin has been studied by means of hydrogen ion titration techniques. The results indicate a) that both the acid and the alkaline Bohr effect are equally affected, b) that the DPG binding to deoxyhemoglobin (Hb) is much stronger than to carboxyhemoglobin (HbCO) and c) that Hb binds effectively one DPG molecule. The effect on the Bohr effect can roughly be described by assuming that upon binding two groups per tetramer change their pK from 6.8 to 7.8 and two others from 6.8 to 5.8. These groups very probably are the imidazole groups of the two histidines H21 (143)§ and the two phosphate groups of DPG (second dissociation). From the experiments a value for the dissociation constant K of the Hb-DPG complex of about 10⁻⁵ M⁻¹ could be estimated at pH 6.2 and pH 7.5.

The remarkable effect of DPG in lowering the oxygen affinity of Hb A has been well established (1-5). There is however some disagreement in the recent literature whether DPG also affects the Bohr effect, which is the mutual dependence of the oxygen and proton affinity of hemoglobin. Some authors (6,7) have reported a definite influence of DPG on this effect, while others (4,8,9,10) mention that such an influence is absent. This report tries to give an answer to this problem in a quantitative way.

MATERIALS AND METHODS
Hb A was prepared by the toluene method (11); after dialysis against water, the number of DPG molecules present per hemoglobin molecule, indicated by n, was 0.6 ± 0.02, measured according to the method of Bartlett (13). This value has also been
reported by Benesch et al (5). The DPG was removed by passing the
dialysed solution through a mixed-bed ion-exchange column (Amber-
lite IRA 400 and IR 120) using a recycling system (12). After
passage through the column n was 0.04. The way we used for re-
moving DPG has the advantage that the solution is deionized si-
multaneously. The Bohr effect with and without DPG was studied by
measuring the hydrogen ion titration curve of Hb and HbCO using
the advanced titration equipment as described previously (14).
To obtain the titration curve for Hb and HbCO the same sample was
used; the titrations were carried out at 25°. The protein concen-
trations were 16 mg per ml. DPG was obtained as salt from Cal-
biochem; it was converted into the acid form by repeated passing

![Fig. 1. Difference in protons bound \( \Delta Z_n^B \) between deoxy and car-
boxyhemoglobin vs. pH (Bohr curve) at a molar ratio \( n = (DPG)/(Hb) \) of 0.0 (Δ), 0.5 (○), 1.0 (*) and 2.0 (+), re-
spectively; hemoglobin concentration 16 mg/ml.](image-url)
through Amberlite IR 120. The concentration of the DPG solutions was measured titrimetrically. All results are given per tetramer.

RESULTS

Figure 1 shows the Bohr effect, defined as the difference in protons bound \( \Delta Z_n^B \) by Hb and HbCO at each pH, at several values of n. The results given are those of one set of experiments using the same stock solutions. The figure shows clearly that DPG influences both the alkaline and the acid Bohr effect. The curve with n=1.5 is not shown to avoid overcrowding of the figure, but coincides nearly with the curve for n=2.

The difference titration curve (not shown) of HbCO with and without DPG was similar to the titration curve of DPG alone; the pH value "half way" was in both cases about 7.1. So the presence of HbCO did not strongly affect the titration behavior of DPG.

The difference titration curve of Hb with and without DPG was on the contrary very different from the titration curve of DPG alone. This indicates that the DPG influence on the Bohr effect is mainly caused by an interaction between Hb and DPG. This observation is consistent with the fact that Hb binds DPG much stronger than HbCO does (1,15,16).

Figure 2 shows the difference in Bohr effect \( \Delta Z_n^B - \Delta Z_0^B \) between hemoglobin in the presence and absence of DPG. The data presented are the mean values of two sets of experiments. The accuracy of the data is ± 0.05 charge units. The figure shows clearly that both the alkaline and the acid Bohr effect are equally affected and that the major part of the increase is found up to n=1.

The broken line was calculated assuming that upon binding two groups per tetramer change their pK from 6.8 to 7.8 and two other groups from 6.8 to 5.8.

In figure 3 the amplitudes of the maximum influence at pH 7.5 and
Fig. 2. Difference in Bohr effect \((\Delta Z^B_n - \Delta Z^B_o)\) between hemoglobin with and without DPG; the molar ratio \(n\) varies from 0.5 (△), 1.0 (○) and 2.0 (●), respectively. Broken line has been calculated as outlined in the text.

Fig. 3. Difference in Bohr effect between hemoglobin with and without DPG at pH 6.2 (○) and 7.5 (●). Drawn lines are calculated for values of \(K\), the dissociation constant of the Hb-DPG complex, of \(10^{-6}\) (1), \(10^{-5}\) (2), \(2 \times 10^{-5}\) (3) and \(10^{-4}\) M\(^{-1}\) (4), respectively.

pH 6.2 (from the curves in fig. 2) are plotted vs. \(n\). That the increase in \(\Delta Z^B_n - \Delta Z^B_o\) is greatest below \(n=1\) is consistent with the binding of only one DPG molecule (1) per tetramer. The calculated curves are based on the assumption that at constant pH \(\Delta Z^B_n - \Delta Z^B_o\) is proportional to the amount of DPG bound; in addition a maximum binding of one molecule of DPG per tetramer was assumed. A proper
fit for the amplitude of both the acid and the alkaline Bohr ef-
fect was obtained with a value for the dissociation constant $K$ of
the Hb-DPG complex of $10^{-5}$ M$^{-1}$, while the $K$ value reported by
Benesch et al. (5) amounts to $2 \times 10^{-5}$ at pH 7.3. The difference be-
tween the two values lies within the experimental accuracy of our
method.

DISCUSSION

The effect of DPG on the Bohr effect can easily be understood in
view of the model proposed by Perutz (8). Upon binding the 5 nega-
tively charged groups of DPG form saltbridges with positively
charged partners lying inside the central cavity. Both DPG and Hb
now can offer for the formation of saltbridges two groups with a
$pK$ around 7. From the side of hemoglobin these groups are proba-
bly the two histidine residues H21(143)$\beta$; in the DPG molecule
they are made up by the second proton dissociation of the two
phosphate groups. This would explain the $pK$ shifts observed. In
calculating the dotted line in figure 2 we tacitly assumed pH
independence of the DPG binding, which is not quite correct; above
pH 7 the binding of DPG decreases (5,15), probably because of the
deprotonation of the two histidines. This might explain that
around pH 7.5 the calculated curve lies below the experimental
curve (for $n=2$) and above this curve at higher pH. So, actually
the $pK$ shifts might be somewhat larger than we assumed. The fact
that upon binding the increase of the acid Bohr effect is as
great as that of the alkaline Bohr effect suggests that below pH
6.8 the binding is also pH dependent and in such a way that the
binding decreases with decreasing pH. This decrease in binding
would correspond with the protonation of the phosphate groups;
this will loosen the saltbridges between them and their positive
partners.
That the induced extra alkaline and acid Bohr effect are of about the same magnitude implies, further, that the total change in log $P_{50}$ over the pH range 5–9, which can be obtained by integrating the curves in figure 2 (17, 18), is not affected by DPG. Finally we want to say something about the proposal of Tomita and Riggs (7) that the α-amino group of the β-chain, which has a pK of 7.8 in Hb, would be primarily involved in the binding of DPG. If instead of the histidines H21(143)β the two α-amino groups of the β-chains are involved in the binding of DPG, then our results would imply that these two N-termini should have a pK near 6.8 in Hb. From our data we cannot decide between these two alternatives, although we think the involvement of the two histidines H21 is more likely because the binding of DPG to foetal hemoglobin, in which these two histidines are missing, is much lower than to HbA (19). Moreover it would be inconsistent with the pH dependence of $K$ (5, 15).

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REFERENCES.