THE INTERACTION OF CHLORIDE IONS WITH HUMAN HEMOGLOBIN

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Received March 14, 1974

SUMMARY: Studying the effect of KCl on the Bohr effect of human hemoglobin, it appeared that at low Cl^- concentration the alkaline Bohr effect is considerably smaller than it is at a Cl^- ion concentration near 0.1 M. The data show that at least part of the Bohr effect, that thus far could not be attributed to a particular residue in hemoglobin, is due to interaction of hemoglobin with anions. The effect of KCl on the Bohr effect shows a striking similarity with the effect of 2,3-diphosphoglycerate (DPG) on the Bohr effect. Based on this a mechanism is proposed which satisfactorily explains the observed salt effect.

Recently (1) we have shown that the effect of DPG on the Bohr effect can be attributed to the fact that binding of DPG to both deoxyhemoglobin (Hb) and oxyhemoglobin (HbO_2) is accompanied by an uptake of protons. It was established that the binding of DPG to HbO_2 increases the acid Bohr effect (or decreases the alkaline Bohr effect) whereas the binding to Hb enhances the alkaline Bohr effect. These results were confirmed by Kilmartin (2). Studying the influence of high salt concentrations on the proton dissociation behaviour of Hb and HbO_2, we recently observed (unpublished results) that surprisingly the free energy of saltbridges occurring in Hb and thought to be responsible for the Bohr effect was not influenced by high concentrations of univalent salt. This weakening of the salt bridges at high ionic strength has long been assumed to occur (3-6). In view of these results and the fact that high salt concentration decreases the oxygen affinity of hemoglobin in a way similar to DPG (7), it can be hypothesized that the influence of salt on the Bohr effect as observed by Antonini et al. (3) and ourselves (unpublished results) might equally well be

attributed to a different interaction of univalent anions with Hb and HbO_2, respectively. We present therefore in this paper preliminary results concerning the influence of chloride ions on the Bohr effect at various pH values.

The measurements were carried out following the pH stat procedure described earlier (1). With this method the number of protons released upon oxygenation of Hb are measured. Isoionic solutions of hemoglobin freed from DPG (1) were adjusted to a known Cl^- ion concentration (KCl, Merck, suprapur). Starting from the isoionic point pH values were adjusted with HCl or NaOH. The Cl^- ion concentrations were corrected for the small amounts of HCl added. In all experiments the hemoglobin concentration was 1.6×10^{-4} M on tetramer basis. The measurements were carried out at 25°C.

Fig. 1 shows the dependence on the chloride concentration of

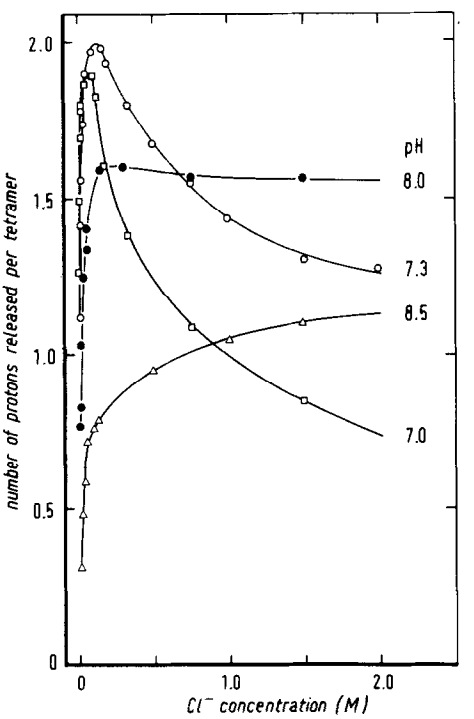

Fig. 1. The dependence on the chloride ion concentration of the number of protons released upon oxygenation of deoxyhemoglobin. The experiments were carried out at pH 7.0 (□), pH 7.3 (o), pH 8.0 (•) and pH 8.5 (Δ).

the number of protons released upon oxygenation of Hb. All curves obtained show a striking similarity with the curves obtained studying the dependence of the Bohr effect on the DPG concentration (see preceding paper). Also in the presence of Cl^- ions the curves resulting from measurements at pH 7.0 and pH 7.3 at first show a sharp increase in the number of protons released followed by a rather gradual decrease. Similarly at pH 8 and 8.5 a strong increase in the number of released protons is at first observed at low Cl^- concentration, but above a certain salt concentration the curves tend to level off at these pH values. Similar behaviour was seen when the influence of DPG on the Bohr effect was examined. In the case of this DPG effect we were able to elucidate the mechanism causing it. The most important feature of this mechanism is that the binding of DPG to both Hb and HbO_2 is accompanied by an uptake of protons. It was possible to prove this since solutions of DPG can be added to solutions of Hb or HbO_2 while keeping the ionic strength constant. However this kind of experiments cannot be carried out with KCl. The model we propose for the influence of Cl^- ions on the Bohr effect will therefore be based on the observed similarity in behaviour of Cl^- ions and DPG as far as the influence on the Bohr effect and oxygen affinity (7) is concerned. The model is identical to that which proved to be valid for the interaction of DPG with hemoglobin. It can be formulated as follows.
a] Chloride ions bind to positively charged groups in both Hb and HbO_2; b] due to this binding the pK of these positively charged groups is increased which means that upon binding of Cl^- ions protons are taken up; c] the groups to which chloride ions are bound in HbO_2 have a lower pK than the groups which are the binding sites in Hb; d] chloride ions are weaker bound to HbO_2 than to Hb.

The above mechanism can explain satisfactorily the shape of the curves in Fig. 1. The sharp increase in the number of protons released observed at all pH values is due to a stronger binding of Cl^- to Hb as compared to the binding of Cl^- to HbO_2. The decrease at high ionic strength observed in the curves measured at pH 7.0 and 7.3 is due to the fact that at high Cl^- concentration the effect of the binding of Cl^- to HbO_2 is counteracting the contribution of the binding of Cl^- by Hb to the Bohr effect. The two curves at pH 8 and 8.5 tend to reach a constant level and show no decrease at high salt concentrations, because at high pH the

groups in HbO_2 which bind Cl^- ions are then no longer charged and consequently incapable of binding.

The proposed mechanism is supported by the NMR results of Chiancone et al. (8) and Bull et al. (9) who found that Cl^- ions are bound by Hb and HbO_2 and that the ligand affinity of the binding site in Hb was larger than that of the site in HbO_2. From a NMR study on the chloride binding to hemoglobin Abruzzo, in which His (143)β has been replaced by Arg, Chiancone et al. (10) concluded that this histidine may be involved in binding of Cl^- ions.

In Fig. 2 we enlarged part of Fig. 1 up to a Cl^- concentration

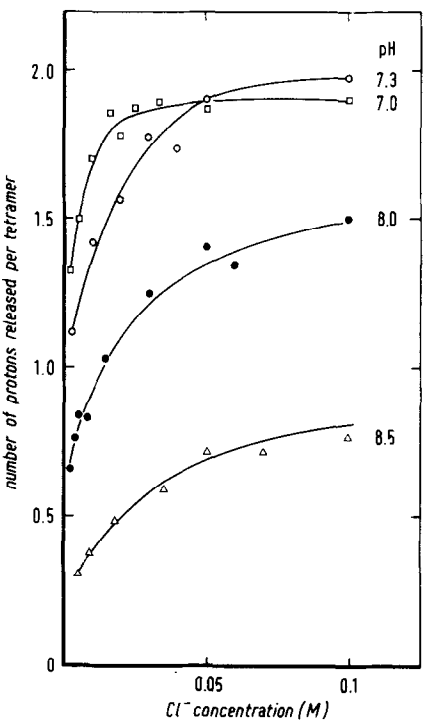

Fig. 2. Enlarged part of Fig. 1 up to a chloride concentration of 0.1 M. For the meaning of the symbols we refer to the legend of Fig. 1.

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of 0.1 M. The curves drawn for the data obtained at pH 7.3 and 7.0 show the usual value of about two protons released at a KCl concentration near 0.1 M. On going down to KCl concentration of 3×10^{-3} M the number of Bohr protons released decreases strongly

and reaches a value of 60 to 70 percent of the effect measured at $[Cl^-]$ = 0.1 M. The decrease observed at pH 8.0 and 8.5 is comparatively even larger than observed at the other pH values. The difference in slope of the curves shown in Fig. 2 support the proposed mechanism for the interaction of chloride ions with hemoglobin as outlined above. In going to high pH the slope of the curves becomes smaller which indicates a decrease in affinity of Cl^- ions to deoxyhemoglobin upon an increase in pH. This decrease in affinity has also been observed with DPG. It is caused by the fact that at high pH groups involved in the binding become ionized and loose their positive charge so that anion will not be bound in that pH range.

From the data reported we are led to important conclusion that part of the Bohr effect measured at (Cl^-) = 0.1 M is due to an interaction of Cl^- ions with deoxyhemoglobin. The effect measured at (Cl^-) = 0.1 M cannot totally be attributed to the so called Bohr groups, which are positively charged groups forming saltbridges with negatively charged partners in Hb. In other words a great part of the Bohr effect is not merely a property of hemoglobin itself being more or less independent from solvent conditions, but on the contrary a great part of the effect is strongly related to interaction of hemoglobin with the solute. It might be noted that our results are consistent with crystallographic data in so far as up till now a part of the Bohr effect could not be attributed to any particular saltbridge (11). Perutz has proposed His H5(122)α which forms a saltbridge with Asp H9(126)α both in Hb and in HbO_2, as a possible Bohr group although they emphasized that they could not find clear crystallographic evidence for a change in free energy of the saltbridge upon oxygenation of deoxyhemoglobin (11).

To conclude we think it should be realized that our conclusion about the part played by Cl^- and other anions will stand even if the model proposed would appear not to be correct.

ACKNOWLEDGEMENT

The authors are indebted to Mrs. H.M.M.L. Rövekamp for her technical assistance.

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