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THE CO AND NO BOHR EFFECT OF HUMAN HEMOGLOBIN WITH AND WITHOUT INOSITOLHEXAPHOSPHATE

Simon H. de BRUIN, Francis J.C. BOEN,
Harry S. ROLLEMA and Gerard G.M. van BEEK
Department of Biophysical Chemistry, University of Nijmegen, Toernooiveld, Nijmegen, The Netherlands

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Using NO and CO as ligands the Bohr effect of human hemoglobin has been measured with and without inositolhexophosphate. It appears that in the absence and presence of inositolhexaphosphate hemoglobin shows a distinct ligand specificity with respect to the Bohr effect. Ligation with NO is accompanied by release of a larger number of Bohr protons than ligation with CO. However, in the presence of IHP the observed NO Bohr effect is smaller than the CO Bohr effect. It is shown that this latter result is due to the fact that the number of protons taken up upon binding of inositolhexaphosphate to ligated hemoglobin is larger for HbNO than for HbCO. It is suggested that this additional proton uptake is partially due to a restoration of the saltbridge between His 146 β and Asp 94 β upon addition of IHP.

1. Introduction

Up to now it is still unknown to which extent the changes in tertiary and quaternary structure which hemoglobin shows upon ligation, contribute to the Bohr effect (for a review see ref. [1]). This is due to the phenomenon that under normal solvent conditions the ligation induced changes in tertiary structure of the α and β chains are accompanied by the change in quaternary conformation from the deoxy- or T-structure to the oxy- or R-structure. Recently Cassoly [2] and Salhany et al. [3] have reported that addition of IHP to a solution of HbNO induces large changes in the visible absorption spectrum of the protein. After that, evidence has been presented that HbNO with IHP bound possesses the T-structure [4–9]. In other words when deoxyhemoglobin is ligated with NO in the presence of IHP, the protein will not change its quaternary conformation from T or R. In view of this we measured i) the number of Bohr protons released upon ligation of Hb with NO and CO in the presence and absence of IHP and ii) the proton uptake upon binding of IHP to HbNO, HbCO and HbO_2 [‡].

‡ Abbreviations used are: IHP, inositolhexaphosphate; HbNO, HbCO, HbO_2, hemoglobin fully ligated with NO, CO and O_2 respectively; Hb, non-ligated hemoglobin.

2. Materials and methods

Solutions of HbNO were prepared according to Drabkin [10] and freed from organic phosphates by passing them through a mixed bed ion exchange column (Amberlite IRA 400 and IR 120).

HbNO was prepared by adding NO to a solution of deoxyhemoglobin. NO (Matheson) was washed through H_2SO_4 and 8 M KOH. The visible absorption spectrum of HbNO prepared in this way was identical to that observed for HbNO, prepared according to Trittelvitz et al. [11]. All experiments with HbNO were carried out under anaerobic conditions.

The number of Bohr protons and the proton uptake upon IHP binding to ligated hemoglobin were measured with a very accurate pH stat equipment [12]. For the latter experiments IHP was added using an automatic titration apparatus described earlier [13].

Deoxygenation of HbO_2 was performed in a rotating tonometer by a constant flow of argon. From this tonometer each time 4 ml of solution was transferred anaerobically to the titration vessel.

Ligation with CO or NO was achieved by injecting these gasses through a serum cap into the titration vessel using normal syringes. The titration vessel was sealed-off just before injection.

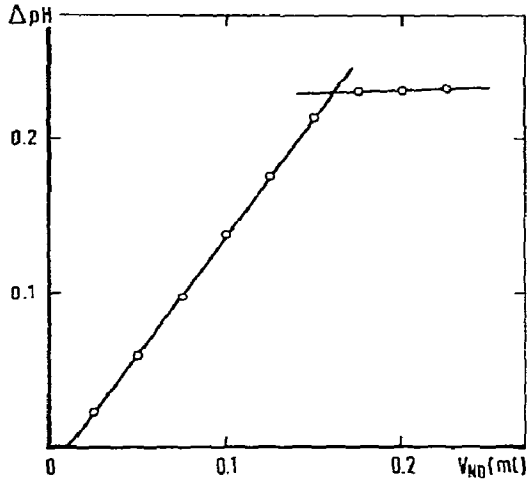

Fig. 1. Change in pH observed upon addition of NO to a solution of Hb. Protein concentration, 0.4 mM; 0.1 M KCl; pH 7.5; 25°C.

pH changes caused by incremental additions of 0.02 ml of gas of atmospheric pressure were recorded. The total change in pH was compensated by addition of 0.05 M NaOH or HCl. The Bohr effect with NO and CO as ligands was measured using the same stock solution. After ligation with NO the absorption spectrum of the product was measured to check for complete saturation. Hemoglobin concentrations are given on tetramer basis.

3. Results and discussion

Fig. 1 shows the change in pH generated by the addition of NO to a solution of deoxyhemoglobin. The sharp equivalence point proves that under our experimental conditions the pH changes caused by the reaction of NO with water are small compared to the changes in pH due to heme ligation.

Fig. 2 shows the number of Bohr protons released upon ligation of deoxyhemoglobin with NO or CO in the absence of IHP. It is seen that the alkaline Bohr effect observed upon ligation with NO is about 25 percent larger than when hemoglobin is ligated with CO. This result is surprising since Moore and Gibson have presented evidence that at neutral pH values an ap-

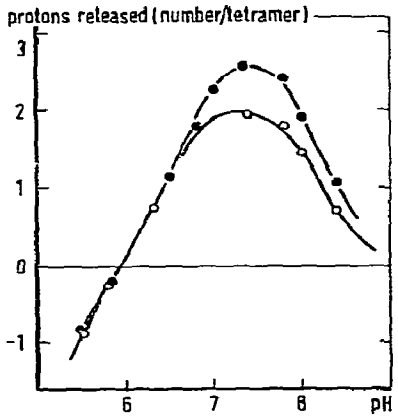

Fig. 2. Number of protons released per tetramer upon ligation of Hb with NO (•) and CO (○). Protein concentration, 0.4 mM; 0.1 M KCl; 25°C.

preciable number of the HbNO molecules possesses a T-like quaternary structure [8]. So, in case the Bohr effect is related to the T or R transition a suppressed Bohr effect is expected when deoxyhemoglobin is ligated with NO. An explanation on the assumption that NO and CO show a difference in interaction with the distal histidine resulting in a pK value for this residue which is lower for HbNO than for HbCO, is ruled out by the observation of McCoy and Caughey [14] and Maxwell and Caughey [7] that the infrared stretching frequencies of several heme ligands show no pH dependence. In other words in both HbNO and HbCO the distal histidine seems to be not titratable over a wide pH range.

It must be noted here that in contrast to the ligand specific influences on the Bohr effect we observed for human hemoglobin, Chien [15] did not observe this ligand specificity for horse hemoglobin.

In going from a solution of deoxyhemoglobin without IHP to a solution of fully ligated hemoglobin in the presence of IHP two different pathways can be followed. The first includes the addition of IHP to a solution of deoxyhemoglobin followed by heme-ligation. Along the second pathway deoxyhemoglobin becomes first heme-ligated followed by addition of IHP.

Indicating the proton uptake accompanying the binding of IHP to deoxyhemoglobin by ΔZ_a, the number of Bohr protons measured in the presence of IHP

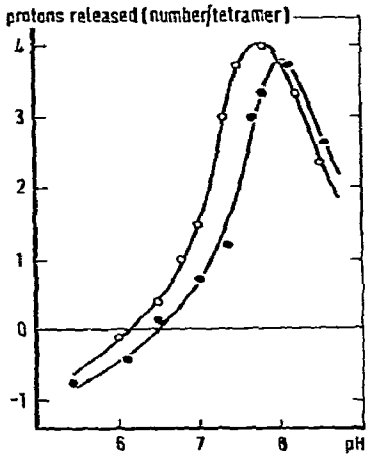

Fig. 3. Number of protons released per tetramer upon ligation of Hb with NO (•) and CO (○) in the presence of IHP. Protein concentration, 0.4 mM; 0.6 mM IHP; 0.1 M KCl; 25°C.

by Δz_b, the number of Bohr protons measured without IHP by Δz_c and the proton uptake accompanying the binding of IHP to ligated hemoglobin by Δz_d, the following relation will hold at any particular pH:

$$\Delta z_a + \Delta z_b = \Delta z_c + \Delta z_d,$$

or $$\Delta z_b - \Delta z_c = \Delta z_d - \Delta z_a.$$

Fig. 3 shows that in the presence of IHP the number of Bohr protons released upon ligation is smaller using NO as ligand than using CO. The difference in IHP induced Bohr effect using CO and NO as ligands is shown in fig. 4 (curve A). This difference can be obtained by subtracting the value for $\Delta z_b - \Delta z_c$ using NO as ligand from the value for this quantity using CO as ligand. The above relation points out that when $\Delta z_b - \Delta z_c$ changes in going from NO to CO the same change will be observed for Δz_d. Therefore we measured at different pH values the number of protons bound to HbNO, HbO_2 and HbCO upon addition of IHP. The curves measured at pH 7.4 (fig. 5) show clearly that the binding of IHP to HbNO is accompanied by a proton uptake which is larger than that observed for the binding of IHP to HbCO or HbO_2. The difference in proton uptake between HbNO and HbCO or HbO_2, observed at a molar ratio of IHP to hemoglobin of 3:1 ($n = 3$) is ca 1.5. This value together

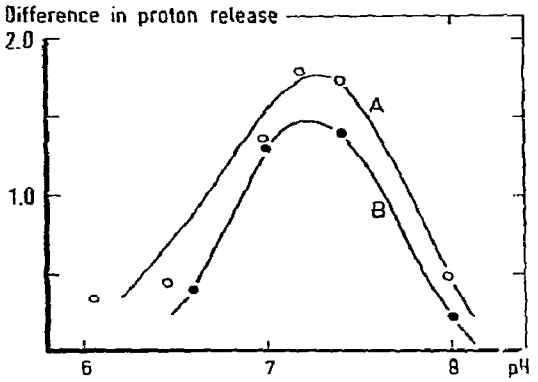

Fig. 4. Difference in IHP induced Bohr effect for the ligation of Hb with CO and NO (curve A). Curve B represents the difference in proton uptake upon binding of IHP to HbNO and HbCO at $n = 3$. n is the molar ratio of IHP to hemoglobin. For further details see text.

with those measured at other pH values is shown in fig. 4 (curve B). The correspondence between the curves A and B in fig. 4 is satisfactory.

There are two alternative explanations for the additional proton uptake occurring upon binding of IHP to HbNO. The first is that the value for the association constant for the HbNO-IHP complex is much larger than for the HbCO or HbO_2–IHP complex. As a result the number of IHP bound by HbNO (and so the proton uptake) will be larger than the amount bound by HbCO (or HbO_2) except for very large values

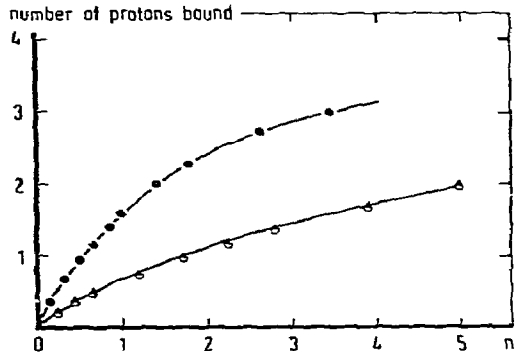

Fig. 5. Number of protons bound per tetramer upon IHP binding to HbCO (○) HbO_2 (△) and HbNO (•) as a function of n, the molar ratio of IHP to hemoglobin. Protein concentration, 0.2 mM; pH 7.4; 0.1 M KCl; 25°C.

of n. However, fig. 5 shows that at any n value the number of protons bound by HbNO is twice that bound by HbCO (or HbO_2). This indicates that the association constants for the binding of IHP to these three ligated states of hemoglobin do not differ substantially. This rules out this explanation.

The second and most likely explanation is that the additional proton uptake observed for HbNO is due to the R to T transition occurring in HbNO upon addition of IHP. Which residues are responsible for this additional proton uptake? First it can be expected that after this conformational change the configuration of the binding site for IHP will be different from the configuration of this site in HbCO. This difference in configuration might affect the pK values of the basic groups, forming the binding site for IHP in both Hb [16] and ligated hemoglobin [17]. As a result a small difference in proton uptake upon IHP binding can be expected. On the other hand Cassoly [5] has found that in the presence of IHP the –SH group of Cys 93β in HbNO shows a strongly decreased reactivity towards p-chloromercuribenzoate; this suppressed reactivity is quite similar to that observed for deoxyhemoglobin. These results have been confirmed by us (unpublished results). Perutz et al. [6] and Taketa et al. [18] have observed a decrease in reactivity of the –SH group of Cys 93β towards 4,4′-dithiobispyrimidine upon addition of IHP to HbNO. Strong evidence has been presented by Perutz [19] that the low reactivity of the –SH group in deoxyhemoglobin is due to sterical hindrance caused by the saltbridge between His 146β and Asp 94β. In view of this it is reasonable to assume that the low SH reactivity in HbNO having IHP bound is also caused by sterical hindrance of that particular saltbridge. As his 146β is also a Bohr group [19] part of the effect shown in fig. 4 can be attributed to this group. In other words upon addition of IHP to HbNO the saltbridge between His 146β and Asp 94β becomes restored. As a result an additional proton uptake is observed.

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