THE BOHR EFFECT OF THE ISOLATED α AND β CHAINS OF HUMAN HEMOGLOBIN

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1. Introduction

Although the Bohr effect (i.e. the linkage between oxygen and proton binding sites) has been discovered already in 1904 [1], still the molecular mechanism of this effect is not completely understood. A number of groups, responsible for a part of the Bohr effect, has been identified [2]. Recently we have shown that part of the Bohr effect is not an intrinsic property of the hemoglobin tetramer, but due to a difference in interaction of chloride ions with oxy and deoxyhemoglobin [3]. A question which is still unanswered is whether the Bohr effect is related to the change in quaternary structure only or also to the change in tertiary structure of the subunits.

Experiments carried out in our laboratory on the Bohr effect of valency hybrids (submitted for publication) suggest that the Bohr effect is related to the state of ligation rather than to the change in quaternary structure. This observation is in accordance with the results of kinetic studies on the rate of proton release upon ligand binding [4–6] which suggest a relation between the Bohr effect and changes in tertiary structure of the subunits. If so, then it becomes difficult to understand why the isolated α and β chains do not show any Bohr effect at all as has been suggested in oxygenation studies [7,8].

We present therefore a study of the Bohr effect of the α and β chains of hemoglobin using the very sensitive pHstat equipment we constructed [9]. This method is more suited to observe small effects than measuring oxygen binding curves.

Horse heart myoglobin was studies for comparison. It appeared that the α and β chains have a small but significant Bohr effect while myoglobin shows no Bohr effect. In the case of β chains a marked influence of Ins-P_6^* on the Bohr effect is found.

2. Experimental

Human hemoglobin was isolated according to the toluene procedure of Drabkin [10]. Organic phosphates were removed by passage over a mixed-bed ion exchange column (Amberlite IRA 400 and IR 120).
Preparation of α^{PMB} and β^{PMB} chains was achieved by incubating carbon monoxide hemoglobin with β-chloromercuribenzoate [11] followed by a chromatographic separation on DEAE-Sephadex.

The α^{PMB} and β^{PMB} chains were demercurated by a β-mercaptoethanol treatment [8], resulting in a complete regeneration of the SH-group of the α-chain. To regain the theoretical number of free SH-groups, the β-chains had to be incubated with a five-fold excess of dithiothreitol for 12 h.

Horse heart myoglobin (obtained from Sigma) was converted to the ferrous form by dithionite in the presence of CO.

The removal of CO from the α and β chains and from myoglobin was achieved by light. The concentration of the Ins-P_6 (Sigma) solutions was determined by weight. The Bohr curves were measured with a pH stat equipment described elsewhere [9].

3. Results

Fig.1 shows the Bohr effect of the α chains in the presence and absence of Ins-P_6. It is seen that the

**Abbreviation:* Ins-P_6, inositolhexaphosphate.

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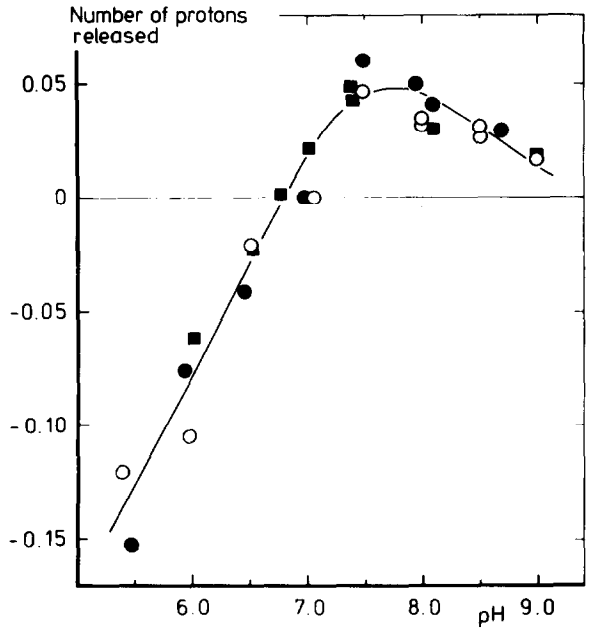

Fig.1. The Bohr effect (measured as the number of protons released per heme upon ligation with O_2) of α chains in the presence (○) and absence (● and ■) of Ins-P_6. Circles and squares refer to different preparations. Protein concentration, 8.0×10^{-4} M on heme basis; Ins-P_6 concentration, 2.5×10^{-3} M; 0.1 M KCl; 25°C.

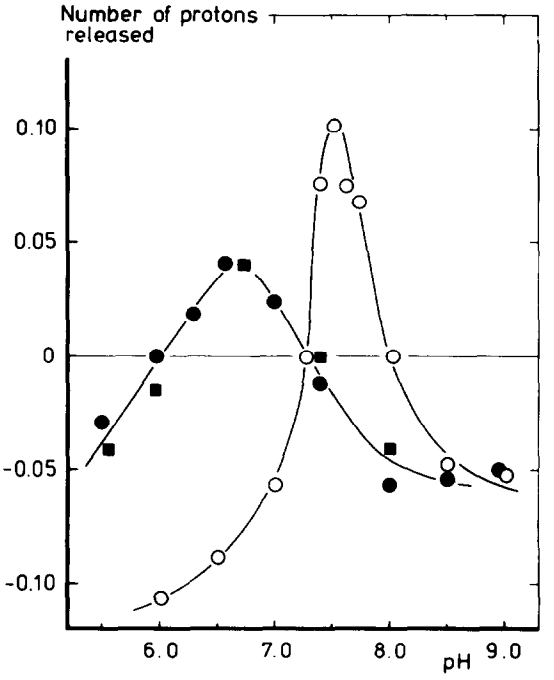

Fig.2. The Bohr effect (measured as the number of protons released per heme upon ligation with O_2) of β chains in the absence (● and ■) and presence (○) of Ins-P_6. Protein concentration, 1.2×10^{-3} M on heme basis; Ins-P_6 concentration, 2.5×10^{-3} M; 0.1 M KCl 25°C. Circles and squares refer to different preparations.

α chain has a small alkaline Bohr effect amounting to about 10% of the effect of hemoglobin. The acid Bohr effect of the α chain, however, is about 30% of that found for hemoglobin. Fig.1 shows that within the experimental accuracy Ins-P_6 has no influence on the Bohr effect of the α chain.

Fig.2 shows the Bohr effect of the β chain with and without Ins-P_6. The β chain has a small Bohr effect with a quite different shape as compared to hemoglobin. For this chain we found a marked influence of Ins-P_6 on the Bohr effect.

Experiments carried out with horse heart myoglobin in the pH range 5.5–9.0 showed that this protein has no Bohr effect. Upon ligation the changes in the number of protons bound by myoglobin in the presence and the absence of Ins-P_6 did not exceed the value of 0.01 and can be regarded as not significant.

4. Discussion

The observed Bohr effect for the α and β chains of human hemoglobin shows that in the case of human hemoglobin the occurrence of heterotropic allosteric interactions is not restricted to the tetramer formed by the two different chains. There is, however, a difference in magnitude of these effects in the hemoglobin tetramer and the α and β chains. The relative large acid Bohr effect observed with the α chains suggests that the tertiary structural change of the α chain in hemoglobin could be responsible for a significant part of the acid Bohr effect of the whole tetramer. The fact that myoglobin does not show any Bohr effect at all indicates that the effects observed with the isolated chains cannot be considered as non specific.

Since the experiments presented in this paper are performed in the presence of 0.1 M KCl there is a possibility that part of the observed Bohr effect of the chains can be explained by a difference in interaction of chloride ions with the oxy and deoxy form of the chains.

Finally the observation of an Ins-P_6 induced change in Bohr effect in the case of the β chain leads to the conclusion, that there is a difference in interaction of oxy and deoxy β_4 with Ins-P_6. This can be explained by assuming that the oxy and deoxy form of the β_4 tetramer have a different structure, which is in accordance with recent oxygenation studies of Bonaventura et al. [12].

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