CAT HAEMOGLOBINS A AND B: DIFFERENCES IN THE INTERACTION WITH Cl^-, PHOSPHATE AND CO_2

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SUMMARY

Domestic cat blood contains two haemoglobins called HbA and HbB which occur in variable proportions. The influence of CO_2, Cl^- and inorganic phosphate on the oxygen equilibrium of these haemoglobins has been investigated, as a measure of the oxygen-linked binding of these effector molecules. The effect of Cl^-, CO_2 and inorganic phosphate on HbA was much greater than on HbB which has blocked ß chain N-termini. While increasing concentrations of phosphate finally abolished the CO_2-effect on HbA and HbB, no competition between CO_2 and Cl^- could be observed. While the lower reactivity of HbB toward CO_2 and inorganic phosphate is due to the blockage of the ß N-termini, this does not explain the reduced effect of Cl^-. From the pH dependence of oxygen-linked Cl^- binding to HbA it is concluded that ß chain Histidine (s) which are either missing or not accessible in HbB play a role in the oxygen-linked Cl^- binding of HbA and also seem to contribute to the Bohr effect.

INTRODUCTION

Cat blood contains two haemoglobins A and B. The minor component B constitutes between 10 - 50% of the total haemoglobin (1). The structure of both haemoglobins has been partly elucidated (2,3), and the difference seems to rest entirely on the HbB ß chains which are acetylated at the N-terminal amino group, a site participating in the binding of CO_2 (4) and 2,3 DPG (5). Both haemoglobins lack $His ß_2$ (2,3), a residue involved in the binding of 2,3 DPG (5). Functional studies have consequently shown that 2,3 DPG binds rather weakly to cat HbA (2,3) and not at all to HbB. The oxygen affinity and the Bohr effect of both haemoglobins were re-

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ported to be identical when studied in 0.14M phosphate buffer (2) on the other hand cat HbB has a substantially lower oxygen affinity than HbA when investigated in 0.1 M NaCl (6). We have examined more closely the specific effects of Cl^-, CO_2 and inorganic phosphate on both haemoglobins as organic phosphates play no role in the maintenance of in vivo ligand affinity (7). Secondly as there are no indications of a dependence of the in vivo ligand affinity (3) on the ratio of HbA/HbB, we hoped to find out by which mechanism the two haemoglobins adapt their functional properties in order to guarantee uniformity of gas transport characteristics independent of the prevailing individual haemoglobin pattern.

EXPERIMENTAL

Cat haemoglobins A and B were separated on Bio-Rex 70 as described by Taketa et al. (1). Prior to chromatography elution buffers and haemoglobin samples were equilibrated with CO. The eluted fractions were concentrated and dialysed extensively against several changes of the respective buffers required for oxygen equilibrium studies in an Amicon UF cell equipped with PM 10 Membrane, and stored as carbon monoxy haemoglobin (Hb CO) in liquid nitrogen until used. The purity of the separated haemoglobins was assessed by isoelectric focussing on polyacrylamide gel. For oxygen equilibrium studies Hb CO was converted to deoxyhaemoglobin by intermittent evacuation and exposure to strong light in an ice bath.

Oxygen equilibrium curves and assessment of methaemoglobin formation were carried out according to Benesch et al. (8) using a Hitachi model 124 spectrophotometer. The pH was measured with a Radiometer glass electrode type G 297/G2 connected to Radiometer pH meter 26. Methaemoglobin content at the end of oxygen equilibrium experiments ranged between 6-11%.

RESULTS AND DISCUSSION

The results of this study show that oxygen-linked binding of Cl^-, CO_2 and ortho phosphate as judged by the effect of oxygen affinity is more pronounced for cat HbA than for HbB (Fig. 1 and 2). In agreement with other authors we find that HbB has a much lower O_2 affinity at low concentrations of salt (2,6).

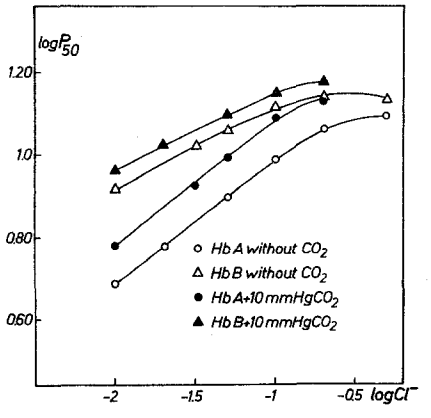

Fig. 1 Influence of CO_2 and Cl^- on the oxygen affinity of cat HbA and HbB
Conditions: In the absence of CO_2 0.05 M bis-Tris + NaCl, pH 7.3 with 10 mm Hg CO_2 $NaHCO_3$/NaCl buffer pH 7.3, t = 20°C, [Hb] = 3 x 10^{-5} M Hb_4

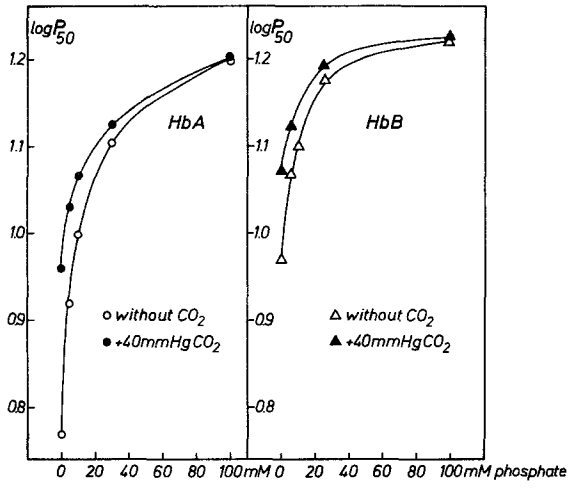

Fig. 2 Influence of phosphate on oxygen affinity and CO_2 binding to HbA and HbB. pH 7.3 t = 20°C, [Hb] = 3×10^{-5} M Hb_4

The lesser effect of CO_2 and phosphate on HbB which has blocked ß chain N-termini is compatible with the experimental results obtained in human Hb, which demonstrate that CO_2 binds to the four N-termini and that oxygen-linked CO_2 binding occurs predominantly at the ß chain N-termini (9) which site also seems to be important for the binding of phosphate ions (10). As both CO_2 and phosphate share sites at the ß chain one should expect reduction of O_2-linked CO_2 binding with increasing phosphate concentrations in the case of HbA as indicated in Fig. 2. An unexpected result however is the finding that oxygen-linked CO_2 binding to HbB , which is restricted to the α chain N-termini is also reduced by inorganic phosphate (Fig. 2). That this cannot be a nonspecific effect i.e. a shift of the carbamate equilibrium due to increased ionic strength is borne out by the fact that Cl^-

does not interfere with CO_2 binding to either haemoglobin (Fig. 1). One has to conclude, that the reduction of CO_2 binding at the α chain N-termini is due to specific binding of inorganic phosphate which interfers with the combination of CO_2 and the free α amino group.

As shown in Fig. 1, Cl^- reduces the oxygen affinity of cat HbA much more effectively than that of HbB so that with increasing concentrations of Cl^- the difference in ligand affinity is reduced though not abolished. If we look at the pH dependence of oxygen-linked Cl^- binding (Fig. 3) it becomes obvious that in this range of Cl^- concentrations (i.e. 0.01 - 0.1 MCl^-) the main difference exists at physiological pH i.e. there is a maximum of oxygen-linked Cl^- binding to HbA between pH 7.0 - 7.3 which then rapidly decreases with increasing pH, so that above pH 8.5 there is a nearly identical effect of Cl^- on both HbB and HbA. As the binding

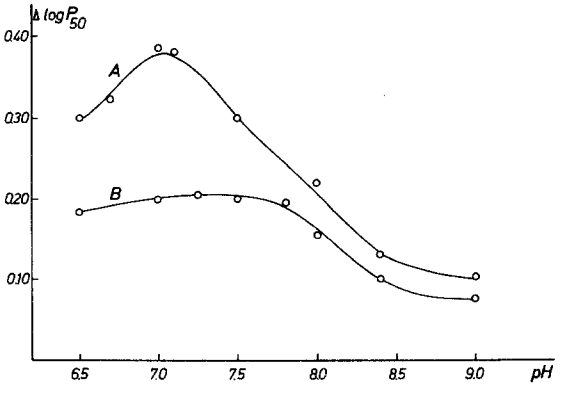

Fig. 3 Dependence of oxygen-linked Cl^- binding to HbA and HbB on pH, as measured by the change in log P_{50} due to an increase of the Cl^- concentration from 0.01 to 0.1 M. [Hb] = 3 x 10^{-5} M Hb_4, t = 20°C, bis-Tris or Tris buffer

of Cl^- involves positively charged groups the rapid decline of Cl^- binding to HbA above pH 7.3 points to participation of groups which have their pK around pH 7. Theoretically these could be Histidines or the free N-terminal α amino groups. In view however of the absence of competition between CO_2 and Cl^- binding (Fig. 1) a participation of N-terminal α groups in Cl^- binding seems unlikely.

Aside from the effect of oxygen affinity it has recently been shown that Cl^- also contributes to the alkaline Bohr effect measured at physiological concentrations of Cl^- i.e. 0.1 M (11). In our experiments we found an increase of the Bohr effect by raising the Cl^- concentration (from 0.01 to 0.1 M Cl^-) only in the case of HbA, where $\Delta \log P_{50}/\Delta pH$ increased substantially (Table 1). One can deduce from these findings that the Cl^- binding sites mis-

TABLE 1

$\Delta \log P_{50}/\Delta pH$ (pH 7.0 - pH 7.5)

	HbA	HbB
0.01 M Cl^-	0.60	0.61
0.1 M Cl^-	0.77	0.64
0.1 M Cl^- + 40 Torr CO_2	0.48	0.51

Effect of Cl^- and CO_2 on the Bohr effect of cat HbA and HbB.

sing in HbB contribute about half of the effect of Cl^- on O_2 affinity at physiological pH and are almost entirely responsible for the enhancement of the alkaline Bohr effect.

Finally we wish to discuss the physiological implications of the different affinities of cat HbA and HbB for Cl^- and CO_2 which under in vivo conditions are the main allosteric effectors. Due to the larger oxygen-linked binding of both Cl^- and CO_2 to HbA the oxygen affinity of both haemoglobins is the same when physiological concentrations of Cl^- and CO_2 are given (Fig. 4). Secondly the enhancement of the Bohr effect by Cl^- in the case of HbA effectively counterbalances the reduction of the Bohr effect due to oxygen-linked CO_2 binding (Table 1). If both HbA and HbB had the same Bohr effect in the absence of CO_2 and at physiological concentrations of Cl^- than the Bohr effect in the presence of CO_2 would depend on the HbA/HbB

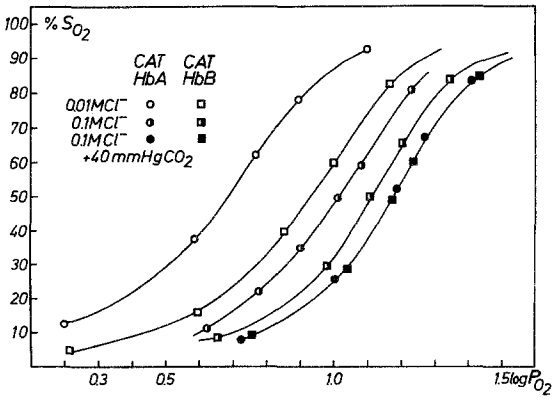

Fig. 4 Comparison of oxygen affinities of HbA and HbB in dependence of CO_2 and Cl^-, pH 7.3, t = 20°C [Hb]= 3 x 10^{-5} M Hb_4

ratio as HbA has a much larger oxygen-linked carbamate formation and hence a greater reduction of the Bohr effect than HbB. Concluding one can say that by virtue of different interaction with Cl^- and CO_2 the two haemoglobins are adapted to each other in such a way that variation of the haemoglobin pattern leaves unaltered the functional properties of the system in vivo.

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