OXYGEN-DISSOCIATION PROPERTIES AND REGULATION OF OXYGEN AFFINITY OF POLYMERIZED BOVINE HEMOGLOBIN

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It is extremely important to take into account the mechanism of allosteric regulation of the affinity of hemoglobin or oxygen when an artificial oxygen carrier, intended to function in blood plasma, outside the specific erythrocyte environment, is created on its basis. It has been shown that human hemoglobin, like its polymer which circulates for a long time in the blood stream (hemoglobin polymer — HbP) [2, 5], possesses high affinity for oxygen (P_{50} = 16-17 mm Hg), and this makes the giving up of oxygen to the tissues difficult. A lasting reduction in affinity for oxygen has been successfully achieved by irreversible addition of an allosteric regulator, namely pyridoxal-5'-phosphate (PP), to HbP [3, 7]. Nevertheless, there is undoubted interest in the investigation of the possibility of using hemoglobin with specifically and structurally determined low affinity for oxygen, very slightly reduced by 2,3-diphosphoglycerate, such as bovine hemoglobin [4], as the basis of an artificial oxygen carrier.

The object of the present investigation was to study the gas-transport properties of bovine HbP synthesized in the writers' laboratory and the character of its interaction with allosteric effectors such as H^+, Cl^-, and PP.

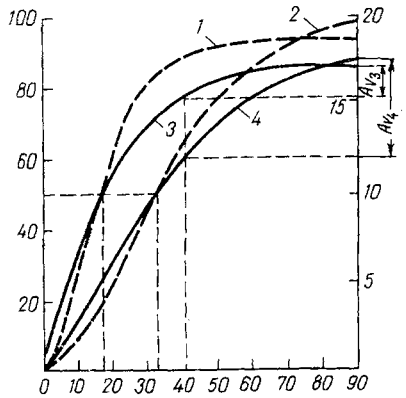

Fig. 1. Oxygen dissociation curves. 1) Human Hb, 2) bovine Hb, 3) human HbP, 4) bovine HbP. pCO_2 = 40 mm Hg, pH 7.4, 37°C, concentration as Hb 10-12%. Phosphates removed by ultrafiltration. Abscissa, pO_2 (in mm Hg); ordinate: right — O_2 concentration (in vols. %), on left — Hb saturation with oxygen (in %).

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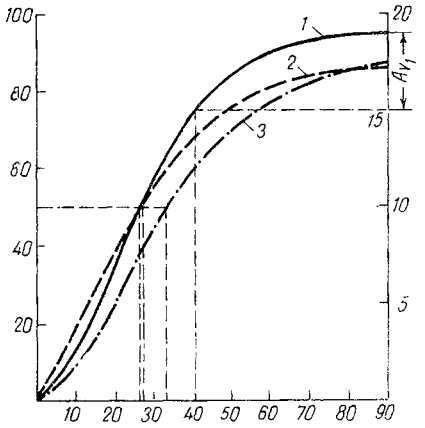

Fig. 2. Comparison of oxygen transport characteristics of human blood (1), of 12% human HbP with covalently bonded PP (2), and of 12% bovine HbP (3). pCO_2 = 40 mm Hg, pH 7.4, 37°C; Cl concentration 0.15 M. Remainder of legend as to Fig. 1.

EXPERIMENTAL METHOD

Oxygen dissociation curves (ODC) of hemoproteins in 0.15 M NaCl or 0.2 M borax-borate buffer were recorded on a "Hem-O-Scan" apparatus (Aminco, USA) under physiological conditions (37°C, pCO_2 40 mm Hg, pH 7.4). The ionic composition was balanced beforehand by ultrafiltration: The pH of the solution was corrected with the aid of Tris (Serva, West Germany) or aspartic acid (Reanal, Hungary). For quantitative evaluation of the ODC the values of P_{50} (the partial pressure of oxygen at 50% hemoglobin saturation), determining the affinity of the hemoprotein for oxygen, and A_V — the quantity of oxygen (in vols. %) given up by the hemoglobin during a partial oxygen pressure drop from 90 to 40 mm Hg, corresponding to that in the body between artery and vein, were calculated.

EXPERIMENTAL RESULTS

It follows from the ODC illustrated in Figs. 1 and 2 that in the absence of organic phosphates, bovine hemoglobin and HbP have a P_{50} (30-31 mm Hg) almost twice as high as P_{50} for human hemoglobin and HbP (16-17 mm Hg), and a little higher than P_{50} for human blood (26-27 mm Hg). For human hemoglobin (Hb) and HbP this value can be reached only in the presence of organic phosphates and, in particular, on the addition of PP [3]. It has been shown that during polymerization of bovine Hb there is no change in the value of P_{50}, although the shape of the ODC is changed (Fig. 1) on account of unavoidable changes in the quaternary protein structure.

The position and shape of the ODC determine the value of A_V of solutions of the test compound, which reflects their ability to give up oxygen under physiological conditions. Calculation shows that this value for bovine HbP is 5.5 ± 0.8 vol. % O_2, which is 4.12 vol. % higher than A_V for human HbP and 1.74 vol. % higher than A_V of blood taken from donors (Figs. 1 and 2).

The effectiveness of oxygen transport by bovine HbP, calculated on the basis of *in vitro* experiments, is thus higher than that of donors' blood, so that there is a possibility of its being used to compensate states of hypoxia [1].

To determine the nature of the reduced regulatory effect of organic phosphates on bovine Hb described in the literature [4, 6] and its manifestation toward bovine HbP, interaction of bovine HbP with Cl^- ions and PP was studied.

Titration of the test compounds with 1% PP solution showed that they bind with the regulator in molar proportions of 3 moles PP to 1 mole Hb.

As Table 1 shows, Cl^- ions and PP reduce affinity of bovine HbP for oxygen equally, just as they do with human HbP, provided that they act independently. Each of these regulators exhibits a stronger effect on bovine HbP (shifting P_{50} on average by 15 mm Hg) than on human HbP (P_{50} shifted by 6-7 mm Hg).

TABLE 1. Effect of Cl^- Ions and PP on P_{50} for Human HbP and Bovine HbP (M ± m)

Test compound	P_{50}, mm Hg			
	in absence of Cl^- and PP	Cl^-	PP	Cl^- + PP
Human HbP	11,07 ± 1,05	17,92 ± 0,90	17,03 ± 0,87	27,77 ± 0,36
Bovine HbP	17,38 ± 0,98	32,59 ± 1,50	32,50 ± 1,21	32,70 ± 1,45

However, the effect of simultaneous exposure to Cl^- ions and PP on the value of P_{50} is realized only for solutions of human HbP, and no corresponding reduction in the affinity of bovine HbP for oxygen is observed. The reduced regulatory effect of organic phosphates observed in the presence of 0.15 M NaCl is thus evidently due to competition between phosphates and Cl^- ions. This fact suggests that Cl^- ions and PP bind with bovine HbP, unlike with human HbP, through the same functional groups of the protein molecule, a conclusion confirmed by data [6] showing differences in the structure of functionally active regions of the bovine and human Hb molecules.

The study of dependence of P_{50} for bovine HbP on pH shows that the ratio $\Delta \log P_{50}/\Delta pH$ (the Bohr effect) is -0.19 ± 0.02 which, in absolute terms, is less than the Bohr effect of human blood (-0.42 ± 0.03) and human HbP (-0.26 ± 0.01), i.e., H^+ ions are weaker regulators for bovine HbP.

To study the effect of the covalently attached regulator on the ODC of bovine HbP, hydrogenation of the HbP = PP complex was carried out with sodium borhydride. The value of P_{50} of bovine HbP solutions with covalently bonded PP, in molar proportions of hemoglobin to PP of 1 : 3 was shown to be 35-38 mm Hg (in borax-borate buffer), but it rises sharply in the presence of Cl^- ions, to reach 53-54 mm Hg. This manifestation of additiveness on covalent bonding of the regulator may be associated with the ability of secondary amines, formed as the result of hydrogenation of azomethine bonds between the PP molecules and protein amino groups, to interact with Cl^- ions, which also probably leads to manifestation of the regulatory effect of Cl^- in the presence of PP. The practical importance of the effect, now discovered for the first time, is that it is possible to obtain HbP with an assigned value of P_{50} between 30 and 50 mm Hg by varying the quantity of covalently bonded PP; this may be useful, in particular, when functional properties of chemically modified hemoglobin are simulated.

The investigations described above showed some particular features of interaction between bovine HbP and the principal allosteric effectors and demonstrated that modification of bovine Hb can yield substances with a broad spectrum of oxygen-transport characteristics; the results are of definite importance for research into the creation of a functional replacement for erythrocytes.

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