Effect of Blood Plasma and Cerebrospinal Fluid on Functional Properties of Human Hemoglobin

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The oxygen affinity of hemoglobin (Hb) obtained by hemolysing erythrocytes directly after blood centrifugation and decanting the plasma (without the usual washing of erythrocytes with physiological saline) is higher than that of Hb prepared from washed erythrocytes [6,7]. It has been hypothesized that this phenomenon may result from the effect of plasma (P) which remains in the microspaces between erythrocytes after centrifugation and comes in contact with Hb. The data presented below are the results of experiments undertaken to verify this hypothesis. Cerebrospinal fluid (CF) and K-phosphate buffer (B) were also used to compare their effect with that of P. Human and animal Hb fulfills two most important functions in the organism: respiratory and buffering (due to the ability of its ionogenic groups to interact with the ligands in the surrounding medium).

In the present work we studied the relationships between these functional properties of Hb.

MATERIALS AND METHODS

Human Hb and blood P of healthy men and women and CF of patients with craniocerebral trauma were used. Hb was prepared from unwashed erythrocytes. The effect of native P (NP) and of P preliminarily boiled with or without alcohol and centrifuged to remove insoluble material (15 min, 8,000 rpm) (PPA and PP, respectively) was studied. To 1 ml of 0.99-1.31 mmol/liter Hb, 0.2-0.3 ml of NP, PPA, PP, CF, and B were added. The oxygen affinity of Hb was measured with the aid of a system of four consecutive desaturators enabling the measurement time to be markedly reduced. The method of spectrophotometry [2] with subsequent pumping of the air out of the desaturators was used (37°C). The correlation between the dynamics of affinity and the pH was calculated by the formula: $\Delta H^+ = \Delta \log p_{50} / \Delta pH$. The buffering properties of Hb were measured by titering with 0.1 M HCl and 0.1 M NaOH.

RESULTS

As reported by Berkovskii *et al.* [1], a 20% Hb solution may contain up to 0.8 g/dl nonHb proteins. In our experiments, 1 ml of erythrocytes after moderate centrifugation entraps some 0.02 ml residual plasma which, when unwashed, may interact with 0.3 g Hb obtained by hemolysing this volume of erythrocytes. As shown in Table 1, human Hb prepared from unwashed erythrocytes exhibits moderate oxygen affinity at 37°C (p_{50} is approximately 24 mm Hg); 0.3 ml of P with mean pH 7.8 increases the pH of the Hb solution by 0.1 unit and the oxygen affinity by almost 40%. The effects of NP and PP are similar, although the pH of the latter constitutes 7.9-8.0. Alkalinization of Hb solutions resulting from the addition of NP or PP is insufficient to provide an explanation of the p_{50} shift as being due to the Bohr effect, because ΔH^+ exceeds the standard values for this effect more than threefold.

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TABLE 1. Oxygen Affinity (mm Hg) and pH of Hb Solutions ($X \pm m_x$)

Experimental conditions	p_{50} (n=7)	pH (n=6)	ΔH^+
Control Hb	23.6±1.5	7.45±0.04	–
Hb + 0.3 ml P	15.7±1.4**	7.55±0.03***	-1.79
Hb + 0.3 ml PP	16.5±1.5**	7.55±0.04*	-1.57
	(n=4)	(n=5)	
Control Hb	22.3±0.5	7.32±0.01	–
Hb + 0.3 ml B	17.6±0.3*	7.58±0.02*	- 0.40
	(n=7)	(n=6)	
Control Hb	27.7±1.1	7.36±0.02	–
	(n=5)	(n=5)	
Hb + 0.2 ml PPA	20.6±2.1*	7.55±0.02*	- 0.67

Note. Here and in Table 2: *) $p<0.01$; **) $p<0.05$; ***) $p<0.01$.

As seen from Table 1, the oxygen affinity of Hb increases by the usual "Bohr" ratio along with the pH.

When boiled in the presence of alcohol, P preserves the capability of markedly increasing the oxygen affinity of Hb; at the same time, treating with alcohol twofold reduces the resulting value, the correlation between p_{50} and pH approaching the Bohr effect.

The effect of P on Hb depends upon the concentration of the latter: the effect augments along with the dilution (Table 2). The solution becomes more alkaline, but the essence of the phenomenon is least of all explained by the Bohr effect.

CF also strongly influences Hb, the oxygen affinity of the latter increasing in the same degree as in the presence of NP in the diluted solutions (Table 2).

In this case, the changes of pH are virtually the same, ΔH^+ fivefold exceeding standard values of the Bohr coefficient. Thus, at least, there is no mismatch between the results of experiments with P and the hypothesis that residual P is probably the cause of the increased oxygen affinity of Hb obtained from unwashed erythrocytes. Moreover, Hb exhibited the same strong response to the addition of CF. In contrast to the effect of B, the nature of the effects of P and CF is obviously least of all associated with H^+.

The data in Tables 1 and 2 show that along with the altered oxygen affinity, the buffering properties of Hb also change. As previously reported [6,7], Hb is capable of changing the pH of the aqueous medium by means of ion exchange. In our experiments, the distilled water in which Hb was dissolved had an initial pH of about 6.5, and under the influence of Hb its alkalinization occurred (pH 7.28-7.35). P and CF (pH 7.82 and 7.80, respectively) raised the pH of the Hb solution to 7.44-7.46. This additional effect cannot be attributed to buffering properties of the proteins, because P and CF differ hundreds of times with respect to this parameter [4]. The results of titering Hb solutions with acid and alkali are evidence that after the addition of P, the buffering capacity of Hb in terms of acid and alkaline equivalents increases by a factor of 4 and 2, respectively (Table 3). Hence, under the influence of blood P, other ionogenic groups of the protein become accessible to titration, this indicating its conformational changes. Under the influence of PP the effect is somewhat reduced: the binding of acid equivalents is 35% and of basic equivalents 25% higher than in the control.

Thus, under the influence of P, both the buffering capacity of Hb and its ability to interact with O_2 change. For example, a more variable responsiveness of the titered groups is observed. In fact, the NP and PP responses of oxygen affinity of Hb are similar, whereas those of its ability to bind acid and basic equivalents are markedly different.

The above effects suggest a comprehensive explanation. They are probably determined by the low-molecular-weight ligands contained in P and

TABLE 2. Effect of P and CF on Hb Solutions of Different Concentration (n=6)

Hb concentration, mM/liter and experimental conditions	p_{50}, mm Hg	pH	ΔH^+
0.99–1.31 control Hb	28.7±2.1	7.31±0.03	–
b) Hb + 0.3 ml NP, pH 7.82	20.0±1.9*	7.39±0.03***	-1.96
c) Hb + 0.3 ml CF, pH 7.80	16.7±0.8*	7.44±0.05**	-2.70
0.50–0.65 control Hb	28.4±2.3	7.35±0.02	–
b) Hb + 0.15 ml NP, pH 7.82	16.1±1.3**	7.45±0.04*	-1.79

TABLE 3. Buffering Capacity of Hb (ml/ΔpH) before and after Adding 0.2 ml NP

Experimental conditions	*n*	Solution used for titration	
		NaOH	HCl
Control	9	0.024±0.001	0.025±0.004
Experiment		0.045±0.001	0.116±0.001
Control	6	0.040±0.020	0.029±0.003
Experiment		0.050±0.002	0.039±0.001

CF and, in addition, by the effect of water molecules on the ionogenic groups of Hb. Biologically active compounds contained in the body fluids examined are thermostable but are partially destroyed when boiled with alcohol. Although P and CF vary greatly in terms of their chemical composition [4], the degree of their influence on the oxygen affinity of Hb is almost equal. Inorganic ions, which are known to reduce the oxygen affinity of Hb [5], cannot be such ligands. Judging by the fact that the correlation between p_{50} and pH outruns the range of the Bohr effect, H^+ also has little effect. On the other hand, water may markedly alter the properties of Hb (including its dissociation into dimers and monomers) by hydrating the ions in the Hb molecule, this increasing the oxygen affinity [3,5,9]. Hence, the present data draw attention to unknown features of the interaction between Hb and factors in body fluids and their possible participation in regulating functions of vital importance.

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