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The oxygen-binding properties of erythrocyte suspensions of Salmo gairdneri and of haemolysates in various buffers in the physiological pH range

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Salmo gairdneri erythrocytes were lysed and the oxygen-binding properties of the haemolysates in 0.1 M tris-HCl, veronal-HCl, imidazole-HCl and phosphate buffers were determined over the pH range 7—7.9. The oxygen-binding properties of erythrocyte suspensions were determined over the same pH range (tris-HCl). In this range the haemolysates in tris-HCl, veronal-HCl and phosphate buffers behaved similarly: their oxygen affinites were the highest and their ability to bind oxygen was least affected by the changing pH. In atmospheric oxygen tension the oxygen saturation of these haemolysates was depressed (Root effect) only below pH 7.2. Below this pH the oxygen affinity also decreased rapidly as the pH was lowered (an increase in the Bohr effect). In the erythrocyte suspensions oxygen affinity decreased rapidly with decreasing pH throughout the pH range measured (Bohr constant = —1.0). The oxygen saturation of the suspension was depressed at pH values other than 7.9. The difference between the suspension and the haemolysates is suggested to be due to the lower pH within the red cells. The imidazole buffer caused a marked decrease in the ability of the haemolysate to bind oxygen; up to pH 7.47 the haemolysate was only 35 % oxygen-saturated when equilibrated with air.

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1. Introduction

The oxygen-binding properties of fish haemolysates have been studied in several buffers, e.g. tris-HCl (Iuchi 1973) and phosphate (Morpurgo et al. 1975). At present, however, it is not known whether these properties differ in different buffers within the physiological pH range 7—7.9.

The oxygen-binding properties can be described by the following parameters: P_{50} value, Root effect and Bohr effect. The P_{50} value is the oxygen tension at which the haemoglobin is 50 % saturated with oxygen, the Root effect is the decrease in the oxygen saturation of haemoglobin at high partial pressures of oxygen when the pH is lowered, and the Bohr effect is the decrease in the oxygen affinity of haemoglobin as the pH is lowered. The Bohr effect can be descriped by the Bohr constant, which is the change of $\log P_{50}$ value between two pH values divided by the pH change.

In this study these parameters were determined for haemolysates in 0.1 M imidazole-HCl, tris-HCl, veronal-HCl and phosphate buffers. The same parameters were also determined for erythrocytes, in suspension in physiological saline to see whether the oxygen-binding properties of the intact cells differed from those of the haemolysates.

2. Material and methods

The 0.1 M buffers were made up according to McKenzie (1969). Their pH values are given in Table 1. The physiological saline for the red cell suspension was made up according to Wilson (1972) — physiological saline for freshwater teleosts, but with 0.1 M tris-HCl buffer instead of phosphate to stabilize the pH of the solution.

The fish used were 2-year-old rainbow trout (*Salmo gairdneri*) from the Laukaa Fish Culture Research Station (n = 16, mean weight 385 ± 30 g). They were acclimated for a month to filtered and dechlorinated Helsinki tap water, and fed twice a week with Ewos pelleted trout food. The water temperature was 4 — 5 °C, oxygen saturation 95 — 100 % and pH 7.2 — 7.5. The 2 ml blood samples were taken from stunned fish into heparinized syringes by cardiac puncture.

Table 1. The pH values of the buffers.

Buffer	pH range 7.0—7.1	7.2—7.3	7.4—7.5	7.8—7.9
0.1 M tris(hydroxymethyl)-aminomethane-HCl	7.13	7.33	7.44	7.89
0.1 M phosphate	7.03	7.24	7.41	7.78
0.1 M veronal - HCl	—	7.23	7.49	7.95
0.1 M imidazole - HCl	6.98	—	7.47	—
physiological saline + 0.1 M tris - HCl	—	7.19	7.50	7.91

A 0.1 ml portion of the blood sample was left intact for measurements of oxygen capacity. Immediately after sampling the rest was centrifuged for 2 min at 13 000 g and the plasma removed. The red cells were washed twice with 0.9 % sodium chloride solution, and lysed by freezing and thawing in 4 volumes of the buffer to be used. The haemolysate was then centrifuged for 2 min at 13 000 g to remove the debris and the clear red solution was used for the oxygen equilibrium studies. The red cell suspension was handled similarly, except that instead of lysis the red cells were stirred into 4 volumes of physiological saline and the oxygen-binding properties investigated.

The oxygen capacities of the red cell suspension and haemolysates were measured according to Tucker (1967) and the P_{50} values according to Edwards & Martin (1966) with the modifications described earlier (Nikinmaa & Soivio 1979). The Root effects at different pH values could be determined by measuring the oxygen capacities of the haemolysates and the red cell suspension at different pH values and comparing these with the oxygen capacities at pH 7.9, at which the Root effect is inoperative. The Bohr constant was calculated as described earlier in this paper. The blood samples were equilibrated with a tonometer of rotating type and all measurements with the Radiometer BMS 3 Mk 2 and PHM 71 systems were carried out at 25 °C.

3. Results

The oxygen capacity of the whole blood at pH 7.9 was 4.73 ± 0.14 mmol O_2/l blood ($n = 16$) at 25 °C. The Root effects for the haemolysates in different buffers and for the red cell suspension are given in Fig. 1. For the haemolysate in the tris-HCl, veronal-HCl and phosphate buffers the Root effect started at pH 7.2, and for the red cell suspension between pH 7.5 and 7.9. In the imidazole buffer the haemolysate showed an extreme Root effect, being only 35 % oxygen-saturated at both pH values investigated (i.e. 6.98 and 7.47).

In Fig. 2 the logarithm of the P_{50} value is plotted against pH. The haemolysates in the tris-HCl, veronal-HCl and phosphate buffers again gave similar responses, their oxygen affinities decreasing rapidly below pH 7.2 and at a much slower rate between pH 7.2 and 7.9 (an increase in the log P_{50} value indicates a decrease in the oxygen affinity of haemoglobin). The oxygen affinity of the red cell suspension was considerably lower at pH 7.5 than at 7.9. The values for the Bohr constant (Table 2) show that the Bohr effect was about the same for the haemolysates below pH 7.2 as for the red cell suspension over the pH range 7.9 — 7.5.

4. Discussion

The Root effect was evident for the red cell suspension at a much higher pH than for the haemolysates in the phosphate, veronal-HCl and tris-HCl buffers. In the haemolysates the pH

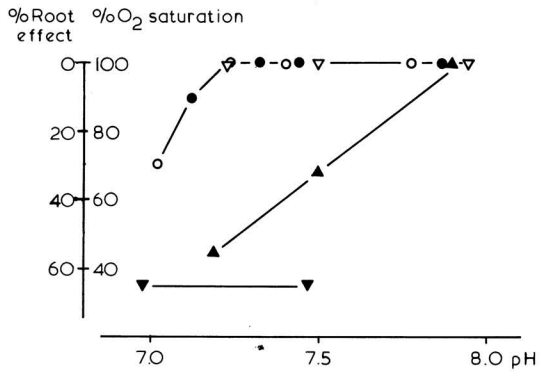

Fig. 1. The percentage Root effects of *Salmo gairdneri* haemolysates in different buffers over the pH range 7—8. = 0.1 M tris-HCl, = 0.1 M phosphate, = 0.1 M veronal-HCl, = 0.1 M imidazole-HCl, = erythrocyte suspension.

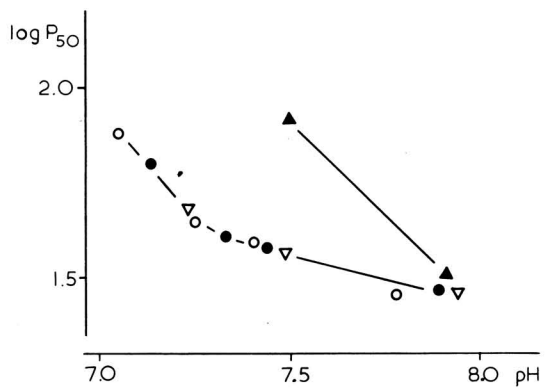

Fig. 2. The logarithmic P_{50} values at different pH values for *Salmo gairdneri* haemolysates in different buffers. Symbols as in Fig. 1.

Table 2. The Bohr constant for *Salmo gairdneri* haemolysates in different buffers.

Buffer	pH range	Bohr constant
0.1 M tris-HCl	7.13—7.33	—0.97
	7.33—7.44	—0.26
	7.44—7.89	—0.25
0.1 M phosphate	7.03—7.24	—1.13
	7.24—7.41	—0.23
	7.41—7.78	—0.40
0.1 M veronal-HCl	7.23—7.49	—0.43
	7.49—7.95	—0.24
Erythrocyte suspension	7.50—7.91	—1.00

measured was that experienced by the haemoglobins, whereas in the red cell suspension the pH inside the cells was probably much lower than the measured pH. At least, the intraerythrocytic pH of eel blood is 0.7 — 0.8 units lower than the plasma pH (Steen & Turitzin 1968, Wood & Johansen 1973). To obtain the Donnan equilibrium the concentration of protons must be much higher inside than outside the cell, as there are negative ions, notably the oragnic phosphates, which cannot cross the cell membrane. When the cells are haemolysed, all the ions can move freely in the solution, and the protons which lower the intraerythrocytic pH on whole blood and in red cell suspensions can be buffered. The difference between the starting points of the Root effect gives an indication of the difference between the intra- and extraerythrocytic pH, which in this case would be about 0.7 units. The differences in the Bohr effects between the red cell suspension and the haemolysates in veronal-HCl, tris-HCl and phosphate buffers is probably also caused by the difference in the pH values experienced by the haemoglobins.

The extreme Root effect observed for the haemolysates in the imidazole buffer cannot be caused by a similar mechanism. However, the imidazole ring of the histidine (143) in the β-chain of the rainbow trout haemoglobins has been found to be of prime importance in mediating the Bohr and Root effects. Neither effect is found in those trout haemoglobins which lack this histidine molecule (Brunori 1975). The deoxygenated form of haemoglobin is stabilized by the formation of salt bridges between the imidazole group of His (143) and certain other groups in the molecule (Kilmartin & Rossi-Bernardi 1973). The introduction of imidazole groups into the solution may cause extreme stabilization of the salt bridges and thus also of the deoxygenated form of haemoglobin, and so in imidazole buffers haemoglobin may fail to become oxygenated even at high oxygen tensions.

The increase in the Bohr constants as the pH is lowered has been described earlier for several fish species, amongst them rainbow trout (Gillen & Riggs 1971, 1977, Weber et al. 1976b). The Bohr constants obtained by Weber et al. were somewhat lower than those obtained in this study. The reason for this difference is apparently that Weber et al. stripped haemoglobins of organophosphates, and in this study that was not done. The presence of organophosphates has been shown to increase the Bohr constant (Gillen & Riggs 1977).

The observation that the haemolysates in phosphate, tris-HCl and veronal-HCl buffers had similar Bohr and Root effects was surprising, since inorganic phosphates have been shown to increase the Root effect in the rainbow trout (Brunori 1975) and the Bohr effect in the eel (Weber et al. 1976a). In the present study, however, since the organophosphates were not stripped from the haemoglobins the effect of the inorganic phosphates was probably masked and so the haemolysates gave similar results in the phosphate buffer to those obtained in the other two buffers.

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