TROUT HEMOGLOBIN: OXYGEN BINDING AT SUB-ZERO TEMPERATURES

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1. Introduction

The functional properties of Hb Trout IV, one of the hemoglobin components from trout blood, strongly imply its involvement in the function of the swim-bladder. Thus, Hb Trout IV displays the Root effect, a property common to hemoglobins from teleost fishes, whose characteristic features may be represented by a large pH dependence of the O_2 binding properties, both the O_2 affinity and the apparent cooperativity decreasing as the pH drops from 8.0–6.0 [1].

As to the molecular interpretation, it has been proposed [2,3] that the Root effect is related to a stabilization, induced by protons, of a 'low affinity' conformational state of the hemoglobin tetramer which, at acid pH, would be the dominant state both in the ligand free and ligand bound derivatives. Thus, on the basis of the simple two-states approximation [4], heme–heme interactions at low pH values are necessarily lost, and oxygen binding should correspond to the simple situation of a molecule containing equivalent and non-interacting low affinity sites. On the other hand, at acid pH (≤ 6) the value of the Hill coefficient (n) appears to be even below 1, which indicates a functional difference between the α and β subunits within the hemoglobin molecule. In summary the data now available do not allow to exclude an effect of protons in enhancing such an intrinsic functional difference. If this was the case, intramolecular heterogeneity would become more and more pronounced as pH decreases, leading to an overall decrease in cooperativity even in the presence of homotropic interactions.

2. Materials and methods

A possibility to discriminate between these two hypotheses could be provided by equilibrium experiments performed at sub-zero temperatures. In fact due to the negative ΔH of oxygenation it should be possible, at low enough temperature, to obtain a complete oxygen dissociation curve in a pH region where the protein is largely deoxygenated at room temperature, even at 1 atm of O_2. We have determined the oxygen dissociation curves of Hb Trout IV at low temperatures (down to $-30^{\circ}C$) in mixtures of ethylene-glycol and water.

The choice of the solvent had to take into account the stability of the protein. Thus the results reported in this note were obtained in mixtures of ethylene-glycol and water (50/50, v/v) containing the desired buffer and KCl 0.4 M, since preliminary experiments showed that under these conditions, Hb Trout IV is capable of binding oxygen reversibly. The visible absorption spectra (700–360 nm) of the various derivatives in ethylene-glycol/water (50/50 v/v) are not significantly different from those in water: ligand binding can thus be followed spectrophotometrically in the usual region of wavelengths. All optical

measurements were performed with a Cary 15 spectrophotometer particularly equipped for measurement at sub-zero temperatures [5].

3. Results

Figure 1 reports the fractional saturation ($\overline{Y}$) with oxygen in air of Hb Trout IV as a function of proton activity (pa_H) and at different temperatures in ethylene-glycol and water (50/50v/v. As expected, at constant pa_H and pO_2 (155 mmHg), the fractional saturation with oxygen progressively increases as temperature is lowered (~0.8 at $t = -38°C$).

Complete oxygen dissociation curves were obtained at two different values of temperature and pa_H. At pa_H~5.8 the equilibrium curve reported in fig.2, is clearly heterogeneous, indicating the presence of different oxygen binding sites, each characterized by its ow affinity. Rising pa_H to 6.4 the heterogeneity is less evident and the oxygen equilibrium curve more closely resembles a simple hyperbola.

The O_2 dissociation curves at $-15°C$ and $pa_H = 5.8$ and 6.4 were fitted using the following equation:

$$\overline{Y}_{tot} = \frac{K_1 x}{1 + K_1 x} \varphi_1 + \frac{K_2 x}{1 + K_2 x} \varphi_2$$

where $\overline{Y}_{tot}$ is the observed fractional saturation computed from the spectral changes, and x is the activity of the ligand (pO_2). This equation assumes two sets of independent binding sites (1 and 2), characterized by intrinsic equilibrium constants K_1 and K_2 and fractional abundances φ_1 and φ_2.

The values of K_1 and K_2, obtained by trials and

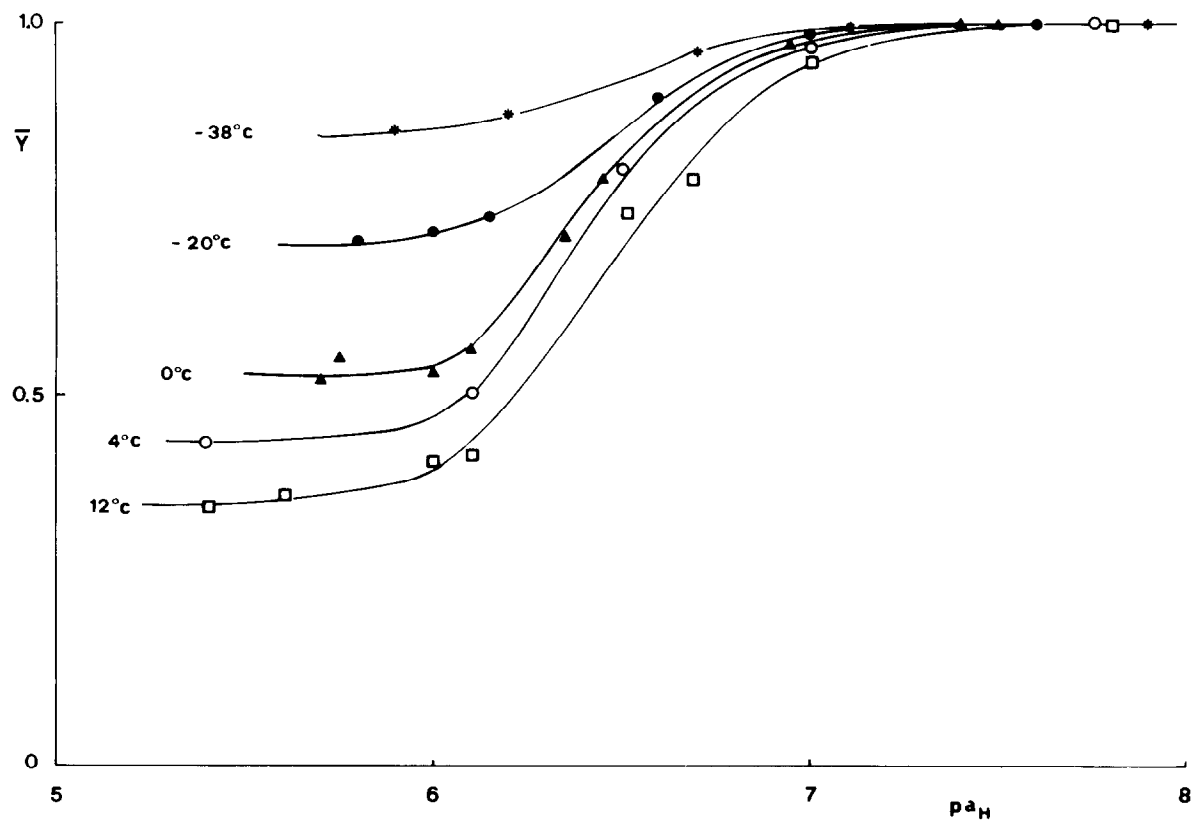

Fig.1. Fractional saturation with oxygen of Hb Trout IV as a function of proton activity and temperature. Phosphate or acetate buffer 0.1 M + KCl 0.4 M in ethylene-glycol/water (50/50, v/v).

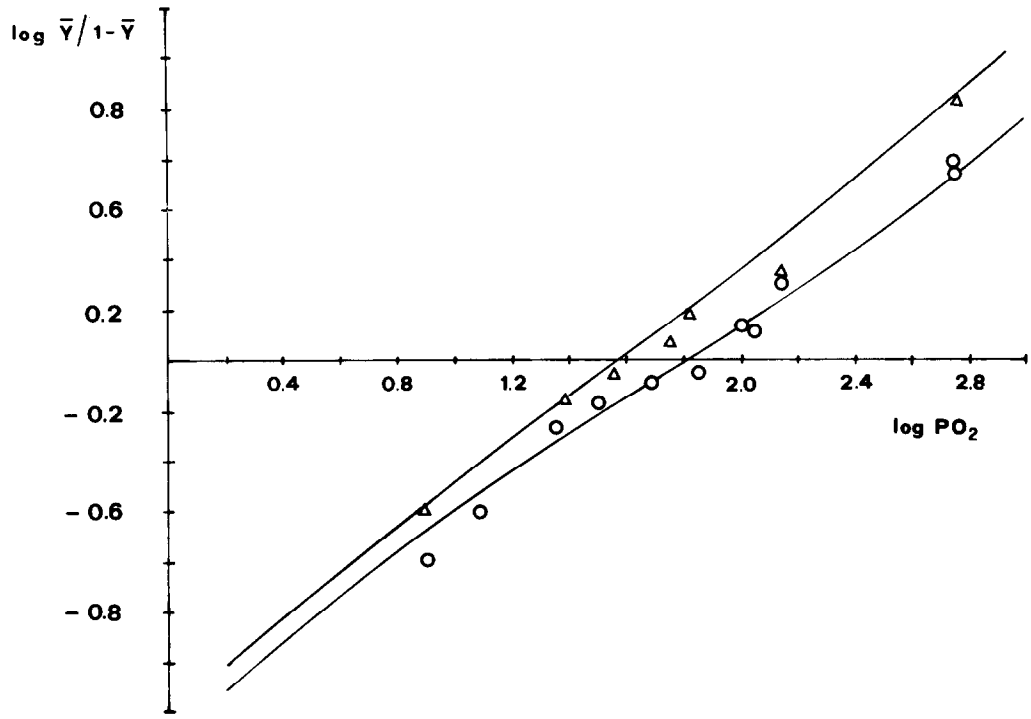

Fig.2. Oxygen dissociation curves at $p\alpha_H$ = 5.8 (○) and $p\alpha_H$ = 6.4 (△). Temperature = −15°C, acetate buffer 0.1 M + KCl 0.4 M in ethylene-glycol/water (50/50, v/v).

errors using $\varphi_1 = 0.45$ and $\varphi_2 = 1 - \varphi_1$, are reported in table 1. It may be noticed that each one of the two equilibrium constants is $p\alpha_H$ dependent, but the $p\alpha_H$ dependence of K_2 is considerably more marked than that of K_1. This implies a differential Bohr effect for the two types of sites. Although it is not clear why φ_1 and φ_2 should be different from 0.5, there are strong arguments to infer that K_1 and K_2 may refer to ligand binding to the two types of chain in the tetramer.

Table1

$p\alpha_H$	K_1 (mm Hg^{-1})	K_2 (mm Hg^{-1})
5.8	0.065	0.005
6.4	0.075	0.012

Values of K_1 and K_2 intrinsic equilibrium constants for the assumed two sets of independent binding sites, were obtained using fractional abundances $\varphi_1 = 0.45$ and $\varphi_2 = 1 - \varphi_1$.

4. Discussion

From the data and the analysis a clear heterogeneity of binding sites in the tetramer is evident at acid $p\alpha_H$. Although possible effects of the mixed solvent cannot be competely excluded, it is beyond any doubt that proton activity enhances the degree of functional heterogeneity between the two types of chains. An unequivocal interpretation however, should consider possible effects on intramolecular heterogeneity induced by intrinsic differences in the heat of oxygenation of each chain. In fact, if this was the case, large changes in temperature could enhance (or depress, depending on the relative sign) the magnitude of the difference in the ligand affinities of the α and β chains.

In summary the results reported here strongly support the existence of functional differences between the two types of oxygen binding sites in Hb Trout IV at acid pH values. This finding is in accord with the results of recent ^{13}C NMR observations

which showed unequivocally that at pH 6.0 the α and β chains in Hb Trout IV have different CO affinities [6]. The functional differences between α and β chains, documented in this paper, could be attributed to local, pH-dependent, structural changes, without necessarily excluding the existence of H^+-dependent conformational equilibria between high and low affinity states of Hb Trout IV.

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