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Research Article

Bohr effect and oxygen affinity of carp, eel and human hemoglobin: Quantitative analyses provide rationale for the Root effect

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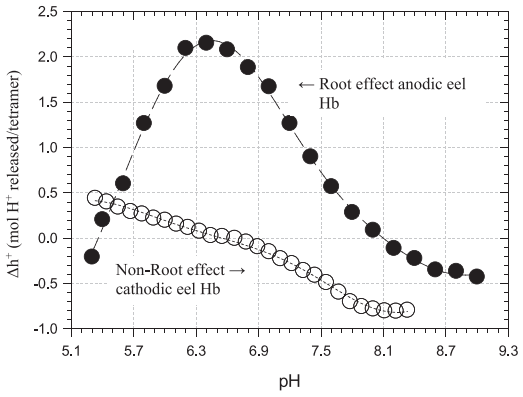
HIGHLIGHTS

- Bohr effect of Root effect Hbs > that of human Hb because they lack Bohr groups with $pK^R > pK^T$.
- Bohr effect of Root effect Hbs accounted for quantitatively on the basis of *three* quaternary structures: R, T, and S.
- pH sensitivity of carp Hb O_2 affinity > human. Carp data simulated from human, given that carp Hb lacks $pK^R > pK^T$ groups.
- Carp Bohr data simulated from human by eliminating human $pK^R > pK^T$and including carp His97β pK^R, pK^T and pK^S contributions.
- Root effect can be switched off by replacing histidines at quaternary structure sensitive locations on the Hb molecule.

GRAPHICAL ABSTRACT



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ABSTRACT

The functional properties of most fish hemoglobins are more complex than those of human hemoglobin. This complexity arises in the form of the Root effect, in which the oxygen affinity of such fish hemoglobins decreases rapidly with pH relative to that of human hemoglobin. Cooperative ligand binding is also diminished below pH ≈ 6.5. The Bohr effect, determined by acid-base titration, has been reported for the Root effect carp and anodic eel hemoglobins. Unlike for mammalian hemoglobins, the Wyman equation for the Bohr effect fails to account quantitatively for these Bohr data. We present a successful quantitative accounting for these data based on evidence for multiple T states in various fish hemoglobins and on their lack of sixhistidine Bohr groups, with $pK^{oxy} > pK^{deoxy}$. On the same bases we also provide a rationale for the higher pH sensitivity of the oxygen affinity of carp compared to human hemoglobin.

1. Introduction

The Root effect of various species of fish and amphibians has been the object of intense study for more than four decades and is yet to be understood at the molecular level. These studies have been the subject of various reviews [1–3]. Like mammalian hemoglobins, Root effect hemoglobins exist in the form of tetramers, each made up of two identical alpha and two identical beta subunits. X-ray crystallographic

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studies indicate that the general structure of Root effect hemoglobins is similar to that of human hemoglobin [4–9]. Nevertheless, although the oxygen binding data of stripped human hemoglobin can be rationalized on the basis of the quaternary two-state allosteric model [10–12] or of the tertiary-two-state (TTS) allosteric model [13–15], it has proved difficult to account for similar data on Root effect hemoglobins with the same models [8]. The major difficulties experienced with Root effect hemoglobins compared to human hemoglobin are that (i) their affinity for oxygen becomes noticeably reduced as the pH of the experimental solution decreases; and (ii) their Hill coefficient, an index of co-operative ligand binding, decreases with decreasing pH and even becomes less than unity at pH lower than *ca* 6.5. These manifestations of the Root effect may be contrasted with the behavior of human hemoglobin for which (i) the oxygen affinity decreases less drastically with decreasing pH (and even increases below *ca* pH 6); and (ii) theHill coefficient remains constant at slightly < 3, irrespective of the solution pH. A major difference between human and Root effect hemoglobins which is not yet widely recognized is that the latter do not possess at least five of the six negatively contributing Bohr effect groups, with $pK^{oxy} > pK^{deoxy}$, that are present in human hemoglobin. These groups are His20α, His45α, His112α, His2β, His77β and His143β (see Fig. 1 of Caruso et al. [16]). The combined contribution of these groups gives rise to the so-called acid Bohr effect in human hemoglobin. Furthermore, there is a residue insertion at the CD corner of fish α-chains. Moreover, the beginning of the α-chain of human hemoglobin contains an NH_3^+ group, which is a positively contributing Bohr group. In fish hemoglobins, on the other hand, this position is occupied by an acetylated residue that cannot contribute to the Bohr effect.

The major focus of our present study is on the Root effect hemoglobins of carp and of the European anodic eel. To the best of our knowledge, no x-ray crystallographic study on either of these hemoglobins has been reported in the literature. Nevertheless, we developed an interest in these two Root effect hemoglobins because the Bohr effect of each (*stripped* and in *de-ionized* water) has been determined directly by acid-base titration [17,18] and their amino acid sequences are known [19,20].The availability of these directly determined Bohr effect data presents us with an opportunity to examine the Root effect from a point of view that is different from those of x-ray crystallographic and oxygen affinity studies.

Given that the general structures of fish hemoglobins are similar to that of human hemoglobin [4–9], we assumed that it would be possible for us to carry out quantitative analyses of the carp and anodic eel Bohr effect data based on the procedures that we previously employed for analyzing mammalian and avian hemoglobin Bohr effect data [21–24]. In the present report we provide detailed quantitative analyses of the carp and anodic eel hemoglobin Bohr effect data based on the Wyman equation for the Bohr effect. Unlike mammalian and avian hemoglobin Bohr effect data, the Bohr data for carp and for anodic eel hemoglobin cannot be fitted with the simple Wyman equation that assumes that a single, T → R, quaternary structure transition results from oxygen binding to deoxyhemoglobin. We demonstrate that the Bohr effect data of the two Root effect hemoglobins can be successfully fitted with a modified Wyman equation which takes into account the existence of multiple T quaternary states, including the liganded T state [8,25–27]. We also provide explanations for the higher pH sensitivity of the oxygen affinity of carp compared to human hemoglobin.

2. Results

In previous reports [21–24] we demonstrated that the magnitude of the Bohr effect of a given hemoglobin is determined solely by the number of Bohr groups it contains and by their Bohr group positions, provided there are no unusual structural effects. For example, human hemoglobin and mouse hemoglobin contain the same number of Bohr groups, at identical Bohr group positions, and the magnitudes of their Bohr effect are exactly the same (see Fig.8 of Okonjo [23]). It is seen from this that the position of a Bohr group determines its pK_as in the R and T quaternary structures. We shall refer to these pK_as as pK^R and pK^T, respectively. Such pK^R and pK^T parameters have been accurately determined with the ^1H NMR technique by Ho and co-workers [28–30] for human hemoglobin histidine residues. Table 1 of Lukin and Ho [30] summarizes the values of these parameters. For convenience, these parameters are also tabulated in Table 1 of this paper. We demonstrated previously [21–24] that these pK^R and pK^T parameters are valid for mammalian and for avian hemoglobins. It would be interesting to determine whether they are also valid for fish hemoglobins, especially for Root effect hemoglobins.

The amino acid sequences of carp and of anodic eel hemoglobin have been determined [19,20]. In Table 1 we present the groups that contribute to the Bohr effect of human hemoglobin, together with their Bohr group positions and their pK^R and pK^T values. For comparison we present in the same table the residues that occupy equivalent Bohr group positions in carp and in anodic eel hemoglobin. It is seen that of the 13 histidine Bohr groups (per αβ dimer) in human hemoglobin only three remain in carp and in anodic eel hemoglobin. This is equally true for other Root effect hemoglobins (see Fig. 3 of Caruso et al. [16]). The 14th Bohr group in human hemoglobin is the terminal NH_3^+ group of the α-chain [31]. In both carp and anodic eel hemoglobin, as well as in

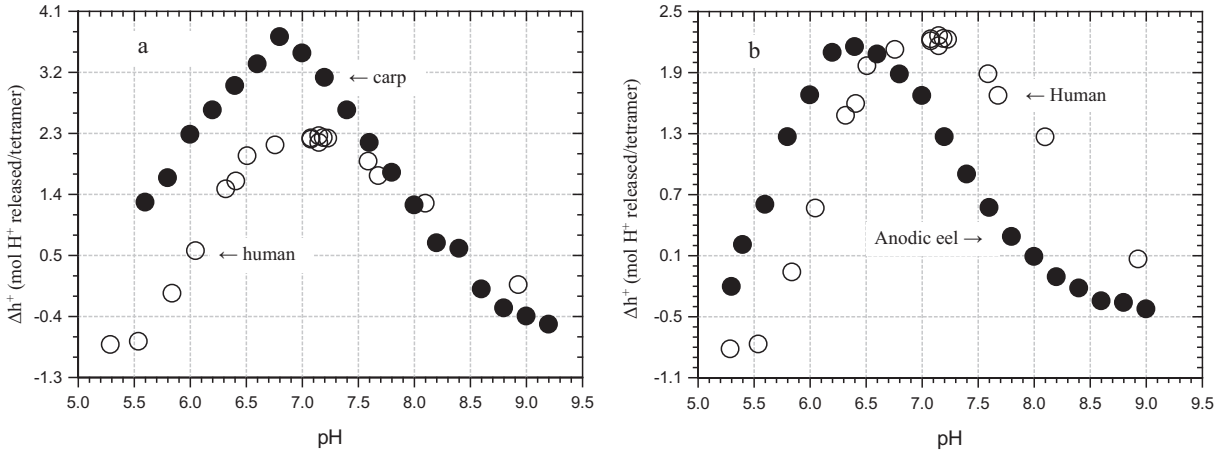

Fig. 1. Comparison of the Bohr effect of (a) stripped carp hemoglobin (filled symbols) and human hemoglobin (open symbols) and (b) stripped anodic eel hemoglobin (filled symbols) and human hemoglobin (open symbols). The carp data were obtained by acid-base titration (see Table 1 of Chien & Mayo [17]); the anodic eel data were obtained from Fig. 3 (dotted curve) of Brauner & Weber [18], using the Snipping Tool of Windows 10, as detailed elsewhere [22]. The human data are from Bailey et al. [32].