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Bohr effect of native and chemically modified hemoglobins: Quantitative analyses based on the Wyman equation

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ABSTRACT

Thirteen histidines and the α-chain terminal amino group (ACTA) make all of the contributions to the Bohr effect of human hemoglobin. The pK_as of the 13 histidines in carbonmonoxy- and deoxyhemoglobin are known from ^{1}H NMR studies. Those of ACTA are not so precisely known. We employed the Wyman equation and the 13 histidine pairs of pK_as to determine the pK_as of ACTA by curve-fitting to hemoglobin Bohr effect data. Using all 14 pairs of pK_as as preliminary data, we employed the Wyman equation to fit the Bohr data for hemoglobin chemically modified at Cys93β with cystamine, cystine and iodoacetamide. We demonstrate quantitatively that the reduction of the Bohr effect upon chemical modification is due to three negatively contributing Bohr groups: His2β, His77β and His143β. These make twice their normal contributions to the Bohr effect in unmodified hemoglobin. We also find that the ACTA pK_as increase with increasing ionic strength.

1. Introduction

The Bohr effect is an important physiological phenomenon that is linked to the uptake of oxygen from the lungs and its release to the tissues, with the concomitant uptake of carbon dioxide from the tissues and its release through the lungs. These processes are mediated by the hemoglobin molecules present in the red blood cell. Depending on the pH of the solution, the *in vitro* binding of oxygen by deoxyhemoglobin may give rise to the uptake of protons from solution or to their release into solution. These are referred to as the Bohr effect. Experimentally the Bohr effect is observed below pH ≈ 6 as an uptake of protons from solution upon oxygen binding to deoxyhemoglobin; above this pH it is observed as a release of protons into solution.

As a preliminary to our analyses of the Bohr effect of human hemoglobin chemically modified at Cys93β, we have carried out detailed analyses of the Bohr effect data for four native (that is, unmodified) mammalian hemoglobins: human, mouse, guinea pig and dog.

There are 14 ionizable groups (per αβ dimer) that make all of the contributions to the Bohr effect of human hemoglobin. Six of these take up protons upon oxygen (or carbon monoxide) binding to deoxyhemoglobin. We shall refer to them as negative contributors to the Bohr effect. Their pK_as of ionization increase on oxygen (or carbon monoxide) binding to deoxyhemoglobin. The remaining eight Bohr groups release protons into solution upon oxygen (or carbon monoxide) binding to deoxyhemoglobin, and their pK_as of ionization decrease. We shall refer to these as positive contributors to the Bohr effect. All the negative contributors are histidine residues and their pK_as in carbonmonoxy- and deoxyhemoglobin have been accurately determined with the ^{1}H NMR technique by Ho and co-workers [1–3]. We shall refer to these pK_as as pK^R and pK^T, respectively. Of the eight positive contributors, seven are histidine residues and their pK^R and pK^T have also been accurately determined by Ho and co-workers [1–3]. The eighth positively contributing Bohr group is the α-chain terminal amino group. Estimates of its pK^R and pK^T in carbonmonoxy- and in deoxyhemoglobin have been provided from studies of the kinetics of its reaction with chemical reagents [4,5]. Another set of estimates has been provided theoretically by Zheng et al. [6]. Unfortunately, these two sets of estimates differ considerably from each other.

It is reasonable to assume that if accurately determined Bohr effect data and accurately measured pK^R and pK^T of all the Bohr effect groups in carbonmonoxyhemoglobin and in deoxyhemoglobin are available, one should be able to fit the Bohr data with the Wyman equation for the Bohr effect [7,8]. For this purpose we had at our disposal (i) Bohr effect data accurately determined with the pH-stat technique for four mammalian hemoglobins [9] and (ii) pK^R and pK^T values accurately determined for each of the 13 histidine Bohr groups (Table 1 of Lukin and Ho [2]). Unfortunately, the pK^R and pK^T of the α-chain terminal amino group are not as precisely known. Estimates from kinetic studies of the reaction of this group with chemical modifying reagents have been provided by two groups. Garner et al. [4] obtained pK^R and pK^T values of 6.95 and 7.79 for carbonmonoxy- and deoxyhemoglobin, respectively. The corresponding pK^R and pK^T reported by van Beek and de Bruin [5] are 7.25 and 8.00. The mean pK^R and pK^T obtained from

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kinetics are therefore 7.10 ± 0.2 and 7.90 ± 0.1 for carbonmonoxy- and deoxyhemoglobin, respectively. More recently, Zheng et al. [6] obtained 7.80 and 8.60 by theoretical calculations. We found that, used in conjunction with the pK^R and pK^T determined by ^1H NMR for the thirteen histidine Bohr groups [1–3], none of the pK^R and pK^T determined for the α-chain terminal amino group [4–6] gave a good fit to the Bohr data with the Wyman equation for the Bohr effect [7,8]. Consequently, following the method we previously employed to fit the Bohr effect data of avian hemoglobins [10], we obtained good fits to the Bohr data for human, mouse, and guinea pig hemoglobin. When we included the fitting parameters previously reported for dog hemoglobin Bohr data [10], we obtained for the α-chain terminal amino group of the mammalian hemoglobins mean pK^R and pK^T values of 6.127 ± 0.08 and 6.983 ± 0.19 for carbonmonoxy- and deoxyhemoglobin, respectively. These values are comparable with the corresponding values, 6.25 and 7.30, previously reported for fits to avian hemoglobin Bohr data [10]. Using the fits to the native hemoglobin Bohr data as preliminary data, we provide evidence leading to the conclusion that the observed decrease in the Bohr effect of human hemoglobin upon chemical modification of the Cys93β sulfhydryl group arises from increased negative contributions from His2β, His77β and His143β. These increases are associated with changes in the tertiary structure of hemoglobin. We found that the pK^R and pK^T of the α-chain terminal amino group is sensitive to ionic strength; the higher the ionic strength the higher the pK^R and pK^T.

2. Computational procedures

The Bohr effect data employed for the following analyses were collected from the stripped hemoglobins with the pH-stat technique at 25 °C and ionic strength 50 mmol/dm^3 [9,11]. All curve-fittings were undertaken with the Levenberg–Marquardt method, as previously reported [10]. We employed programs that we wrote on a MicroMath Scientist software (Salt Lake City, Utah, USA). Prior to their use for fits, we corrected all Bohr group pK^R and pK^T values determined at 29 °C with the ^1H NMR technique [2] to their appropriate values at 25 °C, the temperature at which the Bohr data were collected [9,11]. For this purpose we assumed a heat of ionization of 27.18 kJ mol^{-1} for the imidazole group of histidine, as determined by Rossi-Bernardi and Roughton [12]. The corrected pK^R and pK^T were employed for all the fits. The goodness of fit to each set of Bohr data was judged by the value of R^2, the square of the correlation coefficient. The value of R^2 was obtained by activating the statistics sub-program of MicroMath Scientist, following each fit. Standard deviations of parameter fits were similarly obtained after each fit.

In carrying out fits to the Bohr data of the various native and chemically modified hemoglobins, we employed without change the (temperature corrected) pK^R and pK^T values determined for the 13 histidine Bohr groups of human hemoglobin by Ho and co-workers [1–3]. We have already justified this by demonstrating that the magnitude of the Bohr effect of a given hemoglobin depends only on the Bohr groups it possesses and their Bohr group positions, not on the species of the animal [10,13]. Consequently, we allowed only the non-histidine pK^R and pK^T values to vary in the various fits, as can be seen in Tables 1–3. Therefore only a maximum of two parameters were allowed to vary in each fit. These are the parameters in the tables that have errors indicating standard deviations in their determination. They are also typed in bold face in Tables 1–3.

3. Results

Using the ^1H NMR technique, Ho and co-workers [1–3] accurately determined the pK^R and pK^T values of the 13 histidine Bohr groups in human carbonmonoxy- and deoxyhemoglobin. A full list of these histidine Bohr groups, together with their respective pK^R and pK^T, appears in Table 1 of Lukin and Ho [2]. The corresponding pK^R and pK^T of the fourteenth Bohr group, the α-chain terminal amino group, were estimated kinetically from its reaction with chemical modifying agents [4,5] and by theoretical calculations [6].

3.1. Fit of native hemoglobin Bohr data with the Wyman equation

Before embarking on a consideration of the Bohr effect of the chemically modified human hemoglobins, it is important to know the correct pK^R and pK^T of the α-chain terminal amino group in the unmodified (that is, native) hemoglobin. To achieve this we employed the Wyman equation for the Bohr effect, that is, Eq. (1) below. In Eq. (1) $\Delta h_{total}{}^+$ is the sum of the contributions of n Bohr groups to the Bohr effect; $pK_j{}^R$ and $pK_j{}^T$ are the pK^R and pK^T of Bohr group j in carbonmonoxyhemoglobin (or oxyhemoglobin) and in deoxyhemoglobin, respectively. For human hemoglobin the value of n in Eq. (1) is 14, because it contains 14 Bohr groups per αβ dimer (see Table 1).

$$\Delta h^+_{total} = \frac{1}{2}\sum_{j=1}^{n}\left\{\frac{10^{-pK_j^R}}{10^{-pH}+10^{-pK_j^R}} - \frac{10^{-pK_j^T}}{10^{-pH}+10^{-pK_j^T}}\right\} \quad (1)$$

Using Eq. (1), we had expected to be able to reproduce, by simulation, the Bohr effect data for human hemoglobin by using the pK^R and pK^T parameters of the 14 Bohr groups. However, this expectation was not borne out on account of the pK^R and pK^T estimated for the α-chain terminal amino group. The mean pK^R and pK^T of the α-chain terminal amino group estimated kinetically [4,5] are 7.10 and 7.90 for carbonmonoxy- and deoxyhemoglobin, respectively. When we used these, in conjunction with the pK^R and pK^T of the 13 histidine Bohr groups in Table 1 (columns 2 and 3), to simulate the human hemoglobin Bohr data [9] we found that the simulated curve did not follow the trend of the experimental data (Fig. 1, dashed line).

We then carried out the same simulation, but this time we replaced the kinetically derived pK^R and pK^T with the theoretically derived pair [6]: 7.80 and 8.60 for carbonmonoxy- and deoxyhemoglobin, respectively. Fig. 1 (dotted line) shows that, once again, the simulated curve did not follow the trend of the experimental data. We obtained similar results for the mouse and guinea pig hemoglobin Bohr data (not shown). This was what led us to conclude previously [13], and erroneously, that the Bohr effect of mammalian hemoglobins could not be fitted with the Wyman equation, Eq. (1). Since neither the theoretically nor the kinetically derived pK^R and pK^T values for the α-chain terminal amino group allowed for a proper fit to the human hemoglobin Bohr data (Fig. 1), we decided to obtain values for these parameters by curve-fitting to the Bohr effect data, as we previously did for avian hemoglobins [10]. Keeping the pK^R and pK^T of each of the 13 histidine Bohr groups fixed, we allowed only those of the α-chain terminal amino group to vary while fitting the human Bohr data with Eq. (1). Fig. 2 shows the very good fit obtained. The fitting parameters are reported in columns 2 and 3 of Table 1. The mouse hemoglobin Bohr data were similarly fitted. Fig. 3 reports the very good fit to the mouse data. The fitting parameters are shown in columns 4 and 5 of Table 1.

In human and mouse hemoglobin, Bohr group position 50α is occupied by a histidine. However, in guinea pig hemoglobin it is occupied by a non-ionizable proline residue (see Table 1 of [13]). This leaves 12 histidine Bohr groups (per αβ dimer) in guinea pig hemoglobin. However, a new Bohr group arises in guinea pig hemoglobin because in its R2 quaternary state a salt-bridge is formed between the β-chain terminal amino group of one β-chain and the terminal COO^- group of His146β of the partner β-chain in the same molecule [14]. We previously determined the pK^R and pK^T of this β-chain terminal amino group as 6.327 and 4.590 [13]. During the fit of the guinea pig hemoglobin Bohr data with Eq. (1), we fixed these values, as well as the pK^R and pK^T of the 12 histidine Bohr groups. Only the pK^R and pK^T of the α-chain terminal amino group were allowed to vary. As shown in Fig. 4, we obtained a very good fit to the guinea pig data. The fitting