DETERMINATION OF THE EQUILIBRIUM CONSTANTS FOR OXYGEN-LINKED CO_2 BINDING TO HUMAN HEMOGLOBIN

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1. Introduction

The binding of CO_2 to the α-amino groups of hemoglobin as carbamate [1] contributes significantly to CO_2 transport and is partly responsible for lowering the oxygen affinity of hemoglobin to its physiologic value [2]. Direct measurements at pH 7.4 of the CO_2 binding constant (λ) of hemoglobin specifically modified by cyanate at the α-amino groups [3] (i.e., $\alpha_2\beta_2^C$, $\alpha_2^C\beta_2$ and $\alpha_2^C\beta_2^C$) have shown that the β-chains have a higher affinity than the α-chains for CO_2 in Hb. In HbCO, however, both α- and β-chains have been found to have the same CO_2 affinity, although lower than in Hb [4]. The pH-dependent λ is related to $[H^+]$ as follows [2,5]:

$$\lambda = K_c K_z / (K_z [H^+] + [H^+]^2) \quad (1)$$

where K_c is the equilibrium constant of the reaction $\alpha\text{-}NH_2 + CO_2 \rightleftarrows \alpha\text{-}NHCOOH$ and pK_z is the pK of the α-amino group. Below pH 7.6, CO_2 binding to lysines is negligible [2,6]. Here we report the determination of K_c and K_z for the α- and β-chain α-amino groups of Hb and HbCO from CO_2 binding data. The knowledge of these equilibrium constants makes it possible to calculate the amount of carbamino hemoglobin at any physiologic value of P_{CO_2} and pH and to clarify the mechanism of the oxygen-linked CO_2 binding to hemoglobin.

Abbreviations: $\alpha_2\beta_2^C$, the derivative of hemoglobin where cyanate carbamylates the terminal α-amino group of the β-chain; $\alpha_2^C\beta_2$, the derivative of hemoglobin where cyanate carbamylates the terminal α-amino group of the α-chain; $\alpha_2^C\beta_2^C$, the derivative of hemoglobin where cyanate carbamylates the terminal α-amino group of the α- and β-chains; Hb, deoxyhemoglobin; HbCO, carbonmonoxyhemoglobin; HbO_2, oxyhemoglobin; IHP, inositol hexaphosphate.

2. Materials and methods

Human erythrocytes suspended in 0.9% saline were supplied by Centro Trasfusionale Policlinico, Milano, Italy. Hemoglobin was prepared by the method of Adair [7], and organic phosphates were removed according to Berman et al. [8]. The oxyhemoglobin solution, at a concentration of 18–20 mM heme/liter and at an ionic strength of 0.1 M KCl, was used within six days; its methemoglobin content was not greater than 2%. Additional details have been previously published [5].

The IHP concentration in the hemoglobin solution was calculated from the known weight of added solid IHP corrected for hydration and purity grade as stated by the manufacturer. The method of carbamino hemoglobin determination has been previously described [5] and was used without further modification.

3. Results and discussion

Deduction of the values of K_c and K_z for HbCO was possible by use of CO_2 binding data on unmodified human HbCO, since according to Garner et al. [9] the α-amino groups of the α- and β-chains have similar K_z values. Therefore, since λ-values are similar,

K_c must also be the same for both chains. Thus, using eq. (1), a plot of $1/\lambda[H^+]$ against $[H^+]$ for unmodified HbCO was expected to give a straight line with slope $1/K_cK_z$ and intercept $1/K_c$ on the vertical axis. We have carried out CO_2 binding experiments on HbCO to supplement those already reported [5]. The data plotted in this manner gave a satisfactory fit to a straight line and yielded the values of K_c and K_z shown in table 1.

Values of K_c and K_z for the α- and β-chain α-amino groups of Hb could not be obtained directly from CO_2 binding data on unmodified hemoglobin [5]. They could, in principle, be obtained from data on CO_2 binding to deoxy $\alpha_2^C\beta_2$ and $\alpha_2\beta_2^C$, over a range of pH values, but the amount required of these derivatives would be prohibitive. Instead, we have calculated K_c for the α- and β-chains in Hb from eq. (1) by use of the K_z values of Garner et al. [9], corrected for temperature, and the values of λ for deoxy $\alpha_2^C\beta_2$ and $\alpha_2\beta_2^C$ at pH 7.4 [4].

In order to obtain reliable estimates of K_c by this method, it was necessary to recheck the accuracy of the λ values, since the CO_2 binding data on the carbamylated hemoglobins had been corrected approx for some contaminating normal hemoglobin [4]. For the present study, we blocked the α-amino groups of the β-chains of Hb with IHP and then measured the CO_2 binding to the α-chains. The amount of CO_2 bound by the β-chains was obtained by subtraction of the data on Hb plus IHP from the data on stripped Hb. The data on CO_2 binding to α and β-chains obtained by this method are shown in fig.1 as reciprocal plots of ϕ (moles CO_2 bound/hemoglobin tetramer) against CO_2 concentration at pH 7.4. Data previously obtained on carbamylated derivatives [4] are also shown for comparison.

The data from Hb plus IHP shown in fig.1 were

Table 1
Values of pK_c and pK_z for the α-amino groups of α- and β-chains of Hb and HbCO at 37°C

	Hb	HbCO
α-Chain		
pK_c	4.76 ± 0.10 (4.78 ± 0.05)	5.30 ± 0.05
pK_z	7.46 ± 0.20 (7.43 ± 0.10)	6.93 ± 0.10 (6.70 ± 0.13)
β-Chain		
pK_c	4.54 ± 0.05 (4.55 ± 0.02)	5.30 ± 0.05
pK_z	6.63 ± 0.20 (6.58 ± 0.12)	6.93 ± 0.10 (6.80 ± 0.05)

Values of pK_c and pK_z not in parentheses were obtained from our binding data as described in the text. Values of pK_z in parentheses are adapted from Garner et al. [9], corrected for temperature, assuming as values of the heat of ionization of the α-amino group ΔH = 14.0 kcal/mol for the α-chains of Hb and ΔH = 10.0 kcal/mol for the β-chains of Hb and for both chains of HbCO [14,15]. pK_c Values in parentheses were calculated from the pK_z values of Garner et al. and our binding data.

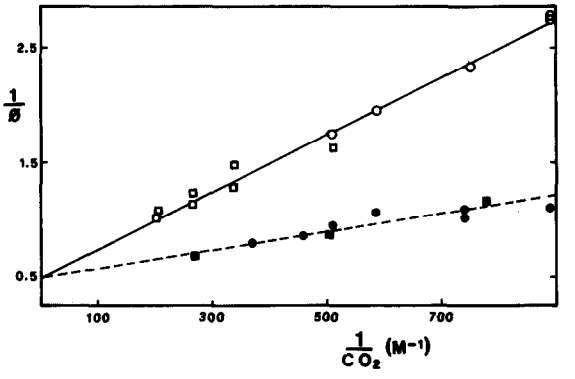

Fig.1. Reciprocal plots of CO_2 bound to 3.5 mM (in tetramer) Hb against CO_2 concentration at 37°C, pH 7.4, I = 0.15. The method of carbamino hemoglobin determination and hemoglobin preparation has been previously described [5]. ϕ = mol CO_2 bound/tetramer. (□) $1/\phi$ for α-chains (β-chains carbamylated, $\alpha_2\beta_2^C$) [4]. (■) $1/\phi$ for β-chains (α-chains carbamylated, $\alpha_2^C\beta_2$) [4]. (○) $1/\phi$ for α chains (β-chains of Hb blocked with IHP, 3 mol/tetramer). (●) $1/\phi$ for β-chains (IHP method, see below for details). The $1/\phi$ data for β-chains by the IHP method were obtained by subtracting the amount of CO_2 bound to Hb plus IHP from that bound to stripped Hb under the same conditions. In order to reduce data scatter, the experimental value of the amount of CO_2 bound by Hb plus IHP in the above calculation was replaced by the value calculated for the same conditions by use of the binding constant given below. The solid and broken lines were drawn by a least square procedure and include the intercept point $1/\phi$ = 0.5. The CO_2 binding constants calculated from the slope reciprocals of the lines were: solid line, λ_α = 201 ± 5.0 M^{-1} compared with 190 M^{-1} measured from $\alpha_2\beta_2^C$; broken line, λ_β = 620 ± 20.0 M^{-1} compared with 579 M^{-1} measured from $\alpha_2^C\beta_2$ [4]. At pH 7.0, λ_α = 44.4 ± 4.3 M^{-1} and λ_β = 201 ± 10.7 M^{-1} were obtained from similar plots. No displacement of IHP by CO_2 was observed at pH 7.0 at any P_{CO_2} value.

unchanged when the IHP concentration was increased from a 2- to an 8-fold molar excess. Effects of ionic strength contribution by IHP were negligible, since IHP in the same concentration range had no effect on CO_2 binding to HbCO at pH 7.4. Furthermore, the best fitting straight lines through the data in fig.1 obtained by the IHP method gave intercepts on the vertical axis very close to 0.5, which is the expected value for the case of two CO_2 binding sites in the hemoglobin tetramer. These are good indications that IHP addition to Hb completely excluded CO_2 binding to the β-chains. Only at P_{CO_2} values higher than 90 mm Hg did the data on Hb plus IHP deviate from the solid line in fig.1 because of a slight displacement of IHP by CO_2. These data were discarded.

The newly calculated binding constants, λ_α and λ_β, agree well with those calculated from the data on $\alpha_2^C\beta_2$ and $\alpha_2\beta_2^C$ (see legend to fig.1 for details). This supports the validity of the IHP method and also shows that blocking the β-chain α-amino groups with IHP does not significantly alter the CO_2 reactivity of the α-chains. The K_c values calculated by use of λ_α and λ_β determined from the data of fig.1 and the K_z values of Garner et al. [9] are shown in table 1.

The ratio of the binding constants at two different pH values is a function only of K_z of the given site and of the two $[H^+]$ values. Thus, by determination (with the IHP method) of the CO_2 binding constants of α- and β-chain α-amino groups of Hb at one more pH value (pH 7.0), we were able to obtain an estimate of K_z and the corresponding K_c, independent of the K_z values of Garner et al. [9]. It was not possible to follow the more rigorous procedure used for HbCO, i.e., to determine the binding constants over a range of pH values, because at pH 7.4 the CO_2 competes significantly with IHP, and at pH 7.0 the amount of CO_2 bound by the α-chains is too small to be measured with sufficient accuracy. This left a narrow range of pH values suitable for investigation, but, as shown in table 1, our estimate of K_z is in good agreement with that of Garner et al. [9]. However, the experimental error does not allow an accurate assessment of the contribution of the α-amino group of the α-chain to the alkaline Bohr effect [10].

The values of K_c reported in this paper, together with our value of K_z, allow a determination of the carbamino content for Hb and HbCO (and approx. for HbO_2). The accuracy of such a calculation will be comparable with the accuracy in the determination of ϕ (± 0.06 mol. CO_2 bound/Hb tetramer) [5]. The values of K_c and K_z reported here also show that the difference in pK_c between Hb and HbCO is the main reason for the decrease in CO_2 affinity of Hb upon the binding of CO (or oxygen). The difference in pK_z contributes less to this effect. As an example, at pH 7.4 and P_{CO_2} = 80 mm Hg, the two α-amino groups of the α-chains of Hb bind almost the same amount of CO_2 (ϕ = 0.67) as do all the four groups in HbCO (ϕ = 0.74) under the same conditions, in spite of the fact that the former groups have a higher pK_z value.

This interpretation of the oxygen-linked change in CO_2 affinity of hemoglobin is restricted to the physiologic pH range where our data were obtained. Another description of the interaction of CO_2 with Hb and HbCO may be necessary outside this pH range if the pK_c values are pH-dependent, as Garner et al. have suggested may be the case for the pK_z values. pH-dependence of pK_c is to be expected, since the difference in pK_c values between Hb and HbCO probably results from different interactions of the carbamate anions with neighboring charged groups, whose state of ionization may vary with pH. The existence of such interactions between charged residues in hemoglobin and the carbamate anion is suggested by crystallographic studies of human Hb and HbCO [11,12].

Our data are in substantial agreement with the pK_c values of Hb and HbCO that Morrow et al. [13] determined by means of ^{13}C NMR spectroscopy. The data obtained by these authors on Hb are quantitatively similar to ours. Their data on HbCO agree qualitatively in that HbCO was found to have higher pK_c values than Hb. However, they found a difference in pK_c between the α- and β-chain α-amino groups of HbCO. This is in contrast with the evidence for the equivalence of the four α-amino groups of HbCO described in this paper.

This discrepancy cannot be explained on the basis of poor resolution of carbamino hemoglobin determination in our method compared with the NMR method. The CO_2 binding constants of the two classes of α-amino groups measured by Morrow et al. at 25°C by NMR are such that their difference in value could have been easily detected by Perrella et al. [4] at 37°C by our present experimental approach. It is also

unlikely that the nonhomogeneity of the cyanate derivatives prepared by Perrella et al. [4] was responsible for the discrepancy, since it would have affected data on both modified HbCO and Hb. A possible explanation might be found in a different temperature-dependence of the pK_c values of the two classes of α-amino groups.

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