Vol. 80, No. 1, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS

January 13, 1978 Pages 56-62

HIGH pK VALUE OF THE N-TERMINAL AMINO GROUP OF THE γ-CHAIN CAUSES LOW CO_2 BINDING OF HUMAN FETAL HEMOGLOBIN

Gerolf Gros and Christian Bauer

Institut für Physiologie, Universität Regensburg,
8400 Regensburg, W-Germany

Received October 24,1977

SUMMARY: The N-terminal amino acid residue of the γ-chains of human fetal hemoglobin (Hb F_{II}) is glycine rather than valine like in many other hemoglobins including the human adult pigment (Hb A). In the course of an evaluation of functional implications associated with this replacement we have studied the CO_2 binding properties of Hb F_{II} in comparison with Hb F_{Ic} where the N-termini of the γ-chains are blocked. By comparing Hb F_{II} and Hb F_{Ic} it is possible to specifically estimate carbamate formation at the N-termini of the γ-chains in Hb F_{II}. These data were used to calculate the carbamate equilibrium and ionization constant of these groups. At 37 °C, $-\log_{10}$ of the ionization constant (pK_z) was found to be 8.1 and is thus significantly higher than pK_z of the N-terminal valines of the ß-chains of Hb A which has been reported to be 6.6 at 37 °C. The high pK_z value of the γ-chain α-amino group explains the much lower carbamate formation in Hb F_{II} compared to Hb A.

The effect of molecular CO_2 on the oxygen affinity of human fetal hemoglobin (Hb F_{II}) is much weaker than in the adult (Hb A) pigment (1, 2), suggesting that less oxygen-linked carbamate is formed by Hb F_{II} than by Hb A. The reason for the different behaviour of the two hemoglobins is not clear. In Hb A, the amino groups of the N-terminal valines at the ß-chains are responsible for the major portion of the oxylabile carbamate (2, 3, 4). These valine residues are replaced by glycines in the γ-chain of Hb F_{II} so that one might expect that the different CO_2 binding properties of Hb F_{II} and Hb A are related to the structural difference of the γ-chain and ß-chain N-terminus.

In this paper, we report the CO_2 binding properties of the γ-chain N-terminal amino groups. Using a method described previously (5), we have measured the carbamate formation of Hb F_{II} and Hb F_{Ic}. Hb F_{Ic} is a minor hemoglobin fraction of human fetal blood and is identical to Hb F_{II} except for the γ-chain N-termini which are acety-

0006-291X/78/0801-0056$01.00/0

lated in Hb F_{Ic} (6) and thus not available to the binding of CO_2. The difference in the carbamate formation of Hb F_{II} and Hb F_{Ic}, therefore, has been taken to represent the carbamate formed at the α-amino groups of the γ-chains.

MATERIALS and METHODS

Fetal hemoglobins F_{II} and F_{Ic} were prepared from human cord blood as described previously (2). Purity of the hemoglobin fractions was checked by isoelectric focusing electrophoresis. Hemoglobin solutions were dialyzed against 0.15 M NaCl, adjusted to hemoglobin concentrations of 1.5 g/dl, and to various pH values by addition of 0.1 M NaOH or HCl. 0.01 g/dl acetazolamide (Lederle, München) was added to all hemoglobin solutions to ensure inhibition of traces of carbonic anhydrase possibly present. Methemoglobin content was < 7 %.

CO_2-free hemoglobin solutions were mixed with equal volumes of a CO_2 solution (0.15 M NaCl equilibrated with CO_2) in a stopped-flow rapid-reaction apparatus equipped with a pH-sensitive glass electrode. The time course of pH after rapid mixing was recorded, and used to estimate the change of pH due to the formation of hemoglobin carbamate, ΔpH_{carb} (5). The number of protons released in the course of carbamate formation per hemoglobin tetramer, Q_H^+/Hb_4, was obtained from:

$$\frac{Q_H^+}{Hb_4} = \Delta pH_{carb} \cdot \beta \quad , \qquad (1)$$

where β is the buffer capacity of the hemoglobin which was read for the pertinent pH range from an independently determined H^+ titration curve. For further details of the method see ref. (5).

RESULTS

In Fig. 1, Q_H^+/Hb_4, the number of protons released by carbamate formation per hemoglobin tetramer, is plotted as a function of pH. pCO_2 was approximately 60 Torr, temperature 37 °C. In Fig. 1a the carbamate formation of deoxygenated Hb F_{II} is compared to that of deoxygenated Hb F_{Ic}, in Fig. 1b the carbamate formation of the oxygenated derivatives is compared. Each data point in Fig. 1 is the average of 4 single determinations (the standard deviations of the mean values of Q_H^+/Hb_4 average ± 0.21). It can be seen that, under identical conditions, Hb F_{II} binds significantly more CO_2 than Hb F_{Ic}. This holds in the entire pH range studied, and for deoxy- as well as oxyhemoglobin.

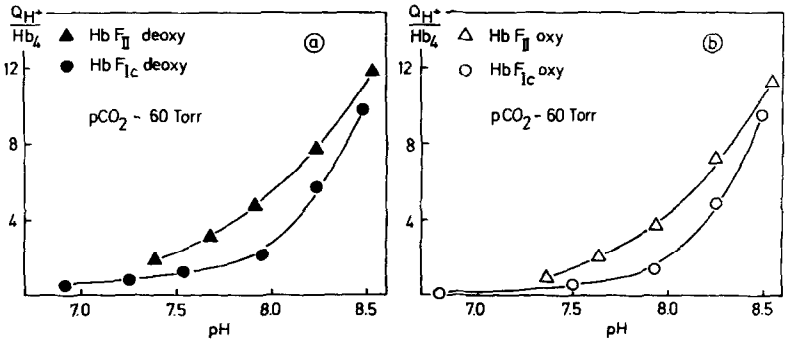

Fig. 1. Q_H^+/Hb_4, the number of protons released by carbamate formation per hemoglobin tetramer, as a function of pH. pCO_2 was approximately constant at 60 Torr, temperature 37 °C, ionic strength 0.15. Fig. 1a: deoxy Hb F_{II} and Hb F_{Ic}. Fig. 1b: oxy Hb F_{II} and Hb F_{Ic}.

DISCUSSION

In the following analysis, the difference in the carbamate formation of Hb F_{II} and Hb F_{Ic} is assumed to represent the carbamate formed by two γ-chain N-terminal amino groups. Stegink et al. (6) have shown that both N-terminal amino groups of the γ-chains are acetylated in Hb F_{Ic}. Thus, they cannot participate in carbamate formation.

Differences between Q_H^+/Hb_4 values of Hb F_{II} and Hb F_{Ic}, $\Delta(Q_H^+/Hb_4)$, were read at various pH values from the curves of Fig. 1, as close to the experimental points as possible. $\Delta(Q_H^+/Hb_4)$ is plotted in Fig. 2 as a function of pH, for deoxy- and oxyhemoglobin. Fig. 2 shows that $\Delta(Q_H^+/Hb_4)$ is consistently greater for deoxy- than for oxyhemoglobin, indicating a larger CO_2 affinity of the γ-chain N-termini in deoxygenated compared to oxygenated Hb F_{II}. At constant pCO_2, $\Delta(Q_H^+/Hb_4)$ increases with increasing pH, and levels off at a value of ~ 2.3 at pH ≈ 8.4. The data points of Fig. 2 have been used to calculate the equilibrium constants governing carbamate formation of the γ-chain α-amino groups as will be shown below.

The fraction of amino groups, f, that at a given CO_2 concentration, $[CO_2]$, and H^+ concentration, $[H^+]$, have reacted with CO_2 is given by (7):

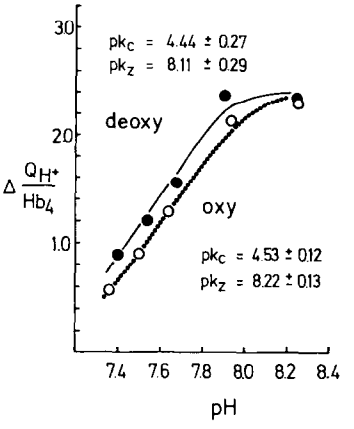

Fig. 2. $\Delta(Q_{H^+}/Hb_4)$, the difference in Q_{H^+}/Hb_4 of Hb F_{II} and Hb F_{Ic}, as a function of pH, for deoxyhemoglobin (●) and oxyhemoglobin (○). The data points were read from the curves of Fig. 1. They represent the protons liberated by carbamate formation at the γ-chain α-amino groups. Also shown are the pK_c and pK_z values of these groups (with standard deviations; top: for deoxy Hb, bottom: for oxy Hb), and the theoretical curves calculated from them for the deoxy (solid line) and the oxy (dotted line) derivative. Conditions: 37 °C, ionic strength 0.15, $pCO_2 \approx 60$ Torr.

$$f = \frac{[CO_2]}{[CO_2] + \frac{[H^+]}{K_c} + \frac{[H^+]^2}{K_c K_z}} \qquad (2)$$

where K_c is the carbamate equilibrium constant, and K_z is the ionization constant of the amino group. The carbamate fraction f is related to the number of protons released by carbamate formation per hemoglobin tetramer in the following fashion (5):

$$f = \Delta \frac{Q_{H^+}}{Hb_4} \cdot \frac{K_z + [H^+]}{n\,(K_z + 2[H^+])} \quad , \qquad (3)$$

where n represents the number of CO_2 binding sites per hemoglobin tetramer (n = 2 in the present case). When values of f have been measured at various CO_2 and H^+ concentrations, K_c and K_z can be obtained from a plot according to the following form of eq. 2:

$$\frac{1-f}{f}\,\frac{[CO_2]}{[H^+]} = \frac{1}{K_c} + \frac{1}{K_c K_z}\,[H^+] \qquad (2a)$$

If the expression on the left-hand side of eq. 2a is plotted versus $[H^+]$ a straight line should be obtained, the intercept on the y-axis being $1/K_c$, the slope $1/K_c K_z$. Such plots were made from the data of Fig. 2. Since K_z has to be known to calculate f according to eq. 3 from $\Delta(Q_H{}^+/Hb_4)$, a trial and error procedure was employed to generate these plots. They gave straight lines with high correlation coefficients for the deoxy ($r = 0.95$) as well as for the oxy ($r = 0.998$) derivative. pK_c values were computed from the intercepts, pK_z values from the slopes of the first order regression equations. pK_c and pK_z were found to be 4.44 and 8.11, respectively, for deoxyhemoglobin, and 4.53 and 8.22, respectively, for oxyhemoglobin. Curves calculated from these constants are shown in Fig. 2, and may be seen to fit the experimental data well. The standard deviations of pK_c and pK_z are also given in Fig. 2. They were calculated from the standard deviations of the intercepts and slopes using an approximation given by Magar (8).

Perrella et al. (9) have determined the pK_c and pK_z values of the N-terminal amino groups of the ß-chains of Hb A at 37 °C. In the deoxy state, they found a pK_c value of 4.54, which agrees rather well with our value of 4.44 for the γ-chain N-terminal amino groups of Hb F_{II}. The pK_z value reported by these authors for the ß-chain α-amino groups of deoxy Hb, on the other hand, is 6.63 and thus about 1.5 pK-units lower than our value for the γ-chain α-amino groups. Two structural characteristics of Hb F_{II} may contribute to the much higher pK value of the γ-chain α-amino group. Firstly, the amino group of glycine has a higher pK value than the amino group of valine (10). It seems likely, therefore, that the γ-chain α-amino group has an intrinsically higher pK value than the ß-chain α-amino group. Secondly, the γ-chain α-amino group presumably is less affected than the ß-chain α-amino group by the positively charged region at the entrance to the central cavity of hemoglobin because His(143)ß with its positively charged imidazole residue is replaced by Ser in the γ-chain, and because the N-terminus of the γ-chain is farther apart from this region than the N-terminus of the ß-chain (11). Note that in the case of the N-terminal glycine of horse myoglobin,

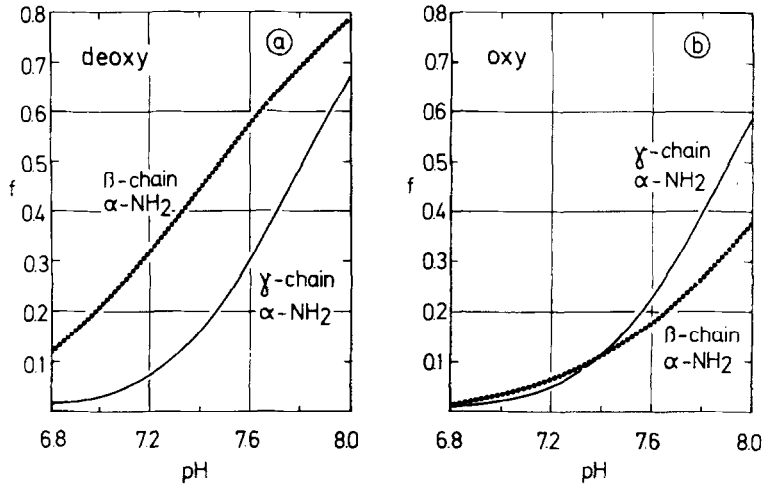

Fig. 3. Fraction of carbamate (i.e. the fraction of amino groups that has reacted with CO_2) as a function of pH. Calculated for the ß-chain α-amino groups of Hb A and the γ-chain α-amino groups of Hb F_{II}, at a constant pCO_2 of 40 Torr, with the constants given in Fig. 2 and in ref. (9). For deoxyhemoglobin (Fig. 3a) and oxyhemoglobin (Fig. 3b).

where no inter-chain interactions are possible, a similarly high pK value has been reported (12).

The substantial difference in the pK_z values of ß- and γ-chain α-amino groups implies that, with physiological pH, at the ß-chains considerably more α-amino groups are free to bind CO_2 than are at the γ-chains. This is illustrated in Fig. 3, where the carbamate fraction, f, is plotted as a function of pH for ß-chain and γ-chain α-amino groups, at a constant pCO_2 of 40 Torr. At physiological pH in the deoxy state, the γ-chain α-amino groups bind only 1/3 to 1/4 of the CO_2 bound by the ß-chain α-amino groups (Fig. 3a). In the oxy state, on the other hand, the CO_2 affinities exhibited by ß- and γ-chains are similar (Fig. 3b). It follows that the formation of oxylabile carbamate by the γ-chains is considerably reduced compared to the ß-chains. Since the ß-chains contribute most of the oxylabile carbamate formed by Hb A (2, 3, 4), the total amount of oxygen-linked carbamate should be markedly smaller in Hb F_{II} than in Hb A. This agrees with the previous finding of a diminished effect of CO_2 on the oxygen affinity of fetal hemoglobin (1, 2).

ACKNOWLEDGEMENTS: We are indebted to Mr. Joseph Pili, Philadelphia, for the construction of the stopped-flow apparatus. We thank Dr. U. Prasser, Krankenhaus St. Josef, and Sr. K. Giordano-Niedermayer, Klinik Dr. Opitz, Regensburg, for collecting human cord blood.

REFERENCES

(1) Bursaux, E., Freminet, A., and Poyart, C. (1974) Resp. Physiol. 20, 181 - 189.

(2) Bauer, C., Baumann, R., Engels, U., and Pacyna, B. (1975) J. Biol. Chem. 250, 2173 - 2176.

(3) Kilmartin, J.V., Fogg, J., Luzzana, M., and Rossi-Bernardi, L. (1973) J. Biol. Chem. 248, 7039 - 7043.

(4) Perrella, M., Kilmartin, J.V., Fogg, J., and Rossi-Bernardi, L. (1975) Nature 256, 759 - 761.

(5) Gros, G., Forster, R.E., and Lin, L. (1976) J. Biol. Chem. 251, 4398 - 4407.

(6) Stegink, L.D., Meyer, P.D., and Brummel, M.C. (1971) J. Biol. Chem. 246, 3001 - 3007.

(7) Rossi-Bernardi, L., and Roughton, F.J.W. (1967) J. Physiol. (London) 189, 1 - 29.

(8) Magar, M.E. (1972) Data Analysis in Biochemistry and Biophysics, p. 175, Academic Press, New York and London.

(9) Perrella, M., Guglielmo, G., and Mosca, A. (1977) FEBS Letters 78, 287 - 290.

(10) Handbook of Biochemistry (1973), Sober, H.A., ed., pp. B-5, B-6, CRC Press, Cleveland.

(11) Frier, J.A., and Perutz, M.F. (1977) J. Mol. Biol. 112, 97 - 112.

(12) Wilbur, D.J., and Allerhand, A. (1977) FEBS Letters 79, 144 - 146.