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Temperature- and pH-dependence of the oxygen-binding reaction of human fetal haemoglobin

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O_2 binding to human haemoglobin F_0 was studied at high haem concentrations (3 mM) in the temperature range 15–35 °C and in the pH range 6.8–8.7 at 25 °C. Comparison with O_2 binding to human adult haemoglobin A_0 under identical solution conditions reveals striking similarities in the Bohr effect and the enthalpy of oxygenation between the two haemoglobins.

INTRODUCTION

Human fetal haemoglobin (HbF_0) is composed of two α-subunits that are identical with those found in human adult haemoglobin (HbA_0) and two subunits that differ from the adult β-subunits at 39 amino acid locations (Schroeder *et al.*, 1963). Despite this large number of substitutions the gross tertiary structures of deoxy-HbF_0 and deoxy-HbA_0 are nearly the same (Frier & Perutz, 1977), which raises the question of the influence these substitutions may have on the energetics of O_2 binding to these haemoglobins. We have pointed out elsewhere that striking similarities exist between the two haemoglobins in binding O_2 and CO in the presence of 2,3-bisphosphoglycerate at 37 °C and pH 7.35 when high haem concentrations (2mM) are used (Di Cera *et al.*, 1989). This finding has prompted us to examine in detail the O_2-binding properties of HbF_0 at high haem concentrations, under a wide range of temperature and of pH. The results are compared with those obtained with HbA_0 under identical solution conditions.

MATERIALS AND METHODS

HbF_0 was prepared from umbilical-cord blood by separation on a 1.5 m column of Bio-Rex 70 (mesh 200–400) equilibrated with 50 mM-phosphate buffer, pH 6.5, at 4 °C (Castagnola *et al.*, 1983). The purity of the sample was determined to be greater than 98% on the basis of isoelectric focusing of the globin chains (Castagnola *et al.*, 1984). The haemoglobin was reduced overnight at 4 °C with the enzymic reducing system described by Hayashi *et al.* (1973) under CO, followed by concentration to 8 mM-haem with an Amicon concentrator.

Binding curves were obtained by a thin-layer optical technique (Dolman & Gill, 1978) as described in detail elsewhere (Gill *et al.*, 1987; Di Cera *et al.*, 1987). The basic property of this method is the possibility of precisely setting the solution activity of O_2 by equilibrating the sample with a well-defined ligand gas phase. The sample is held vertically between an optical window and a membrane permeable to O_2 in a gas-tight stainless-steel cell connected with a dilution valve. The thickness of the layer is precisely defined by a spacer shim and is typically 50 μm, so that high haem concentrations (up to 12 mM) can be studied. Initially the gas-tight chamber is filled to atmospheric pressure with O_2. The dilution-valve bore is then flushed at atmospheric pressure with N_2 and connected to the chamber. This procedure yields stepwise dilutions of the O_2 partial pressure p in the gas phase according to the equation:

$$p_i = p_0 D^i \quad (1)$$

where D is a dilution factor (typically $D = 0.6955 \pm 0.0005$) depending on the geometry of the cell (Dolman & Gill, 1978), and p_0 is the starting O_2 partial pressure. Changes in O_2 saturation of the haemoglobin sample are typically monitored as changes in absorbance at 576 nm by using a Cary 219 spectrophotometer. The underlying equation is:

$$\delta A_i = \delta A_T (Y_i - Y_{i-1}) \quad (2)$$

where δA_i is the absorbance change after the ith dilution, δA_T is the total absorbance change observed in going from deoxy- to oxy-haemoglobin, and Y is the fractional O_2 saturation equal to (Wyman, 1964):

$$Y = \tfrac{1}{4} \mathrm{d}(\ln Z)/\mathrm{d}(\ln p) \quad (3)$$

The function Z is the partition function:

$$Z = 1 + \beta_1 p + \beta_2 p^2 + \beta_3 p^3 + \beta_4 p^4 \quad (4)$$

where β_i is the overall equilibrium constant for the reaction $Hb + iO_2 = Hb(O_2)_i$.

The equilibrium constants and the optical parameter δA_T were estimated by non-linear least-squares regression as described in detail elsewhere (Gill *et al.*, 1987; Di Cera & Gill, 1988). The median O_2 pressure, p_m, was calculated in each case from the value of β_4 as $p_m = \beta_4^{-\frac{1}{4}}$ (Wyman, 1964).

RESULTS AND DISCUSSION

Fig. 1 summarizes the effect of temperature by depicting a van't Hoff plot of the logarithm of p_m as a function

Abbreviations used: HbF, human fetal haemoglobin; HbA, human adult haemoglobin.

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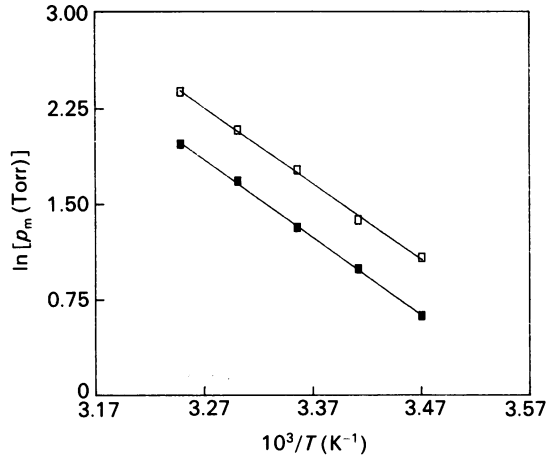

Fig. 1. van't Hoff plot of the logarithm of the median O_2 partial pressure versus the inverse of temperature for HbF_0 (□) and HbA_0 (■)

Measurements were taken in the temperature range 15–35 °C, under solution conditions of 0.1 M-NaCl/0.1 M-Hepes buffer, pH 7.41, 3 mM-haem and the presence of an enzymic reducing system. Enthalpy values are [see eqn. (5) in the text]: $\delta H_T = -38.07 \pm 0.88$ kJ/mol (-9.10 ± 0.21 kcal/mol) for HbF_0 and $\delta H_T = -36.53 \pm 1.26$ kJ/mol (-8.73 ± 0.30 kcal/mol) for HbA_0. Conversion factor: 1 Torr ≈ 133.3 Pa.

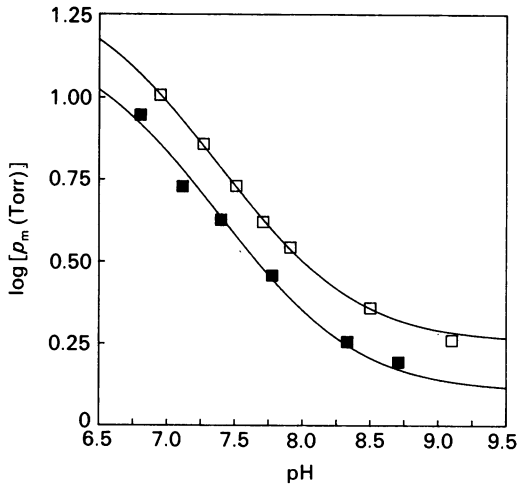

Fig. 2. Comparison of the alkaline Bohr effect of HbF_0 (□) with that of HbA_0 (■) shown as the logarithm of the median O_2 partial pressure plotted versus pH

Curves were calculated by using the pK values reported elsewhere for HbA_0 (Di Cera *et al.*, 1988). The lower curve (for HbA_0) was drawn simply by subtracting a constant factor (0.15 log units) from the upper curve, and is intended for a visual comparison of the Bohr effects of the two haemoglobins. The slope of the curve yields the number of protons released per molecule of O_2 taken up [see eqn. (6) in the text], and is equal to −0.52 at pH 7.4 in both cases. Solution conditions were: 0.1 M-NaCl/0.1 M-Hepes/0.1 M-Bicine buffer, 3 mM-haem and the presence of an enzymic reducing system at 25.0 °C. Conversion factor: 1 Torr ≈ 133.3 Pa.

of $1/T$ for both HbF_0 and HbA_0. The slope of the straight line is related to the total heat of saturation per haem site, δH_T, according to (Wyman, 1964):

$$d(\ln p_m)/d(1/T) = \delta H_T/R \quad (5)$$

where R is the gas constant. The essentially equal enthalpic contributions to the oxygenation process of these two haemoglobins supports the idea that the two macromolecules operate by the same overall co-operative mechanism. Further support to this conclusion comes from analysis of the alkaline Bohr effect of HbF_0, which is shown in Fig. 2 as the change in the logarithm of p_m as a function of pH. The slope in Fig. 2 is equal to (Wyman, 1964):

$$d(\log p_m)/dpH = X_{oxy} - X_{deoxy} = \delta X \quad (6)$$

and yields the difference in protons bound to the oxy and the deoxy forms of Hb per haem site, i.e. the number of mol of protons released per mol of O_2 taken up. This is a quantitative measure of the Bohr effect and strongly depends upon the pH. The maximum value of δX is obtained for pH 7.4 and is equal to −0.52, in agreement with previous studies (Mann & Seymour, 1968). This value is identical with that found in the case of HbA_0 at the same pH (Di Cera *et al.*, 1988).

The results reported in this study complement those obtained on CO and O_2 binding to HbF_0 and HbA_0 under nearly physiological conditions, i.e. in the presence of 2,3-bisphosphoglycerate at 37 °C and pH 7.35 (Di Cera *et al.*, 1989). Altogether they draw attention to the similarities between the energetics of the two major human haemoglobins and suggest a common molecular mechanism for the underlying reactions. The similarity also points out that both haemoglobins function as O_2 carriers with the same efficiency, but have different overall O_2 affinities, which has obvious physiological reasons (Di Cera *et al.*, 1989). Interestingly, this difference is observed in the present study even in the absence of 2,3-bisphosphoglycerate, contrary to conclusions drawn from previous measurements done at low haem concentrations (Tyuma *et al.*, 1973).

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