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Simulation of biphasic oxygen equilibrium curves of hemoglobin in the presence of small amounts of 2,3-diphosphoglycerate

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SUMMARY

Oxygen equilibria of hemoglobin in the presence of different concentrations of 2,3-diphosphoglycerate were simulated by using Adair's stepwise oxygenation scheme in which changes of the concentration of free 2,3-diphosphoglycerate during oxygenation were taken into account. The calculated equilibrium curves fitted well to experimental points and also well expressed biphasic features in the presence of less than 1 mole of the phosphate per mole of hemoglobin.

Benesch *et al.*[1] have shown that oxygen equilibrium curves of hemoglobin are biphasic whenever less than 1 mole of 2,3-diphosphoglycerate or inositol hexaphosphate per mole of hemoglobin tetramer is present. They interpreted the biphasic curves as a result of successive oxygenation of two molecular species, *i.e.* stripped (phosphate-free) hemoglobin with a high oxygen affinity, followed by the phosphate-complexed hemoglobin with a much lower oxygen affinity, since the breaking point between the first and second phases in the curves moved downward with the increase in the molar ratio of the phosphates to hemoglobin tetramer. There may be an idea, however, that the interpretation will not be valid, since during the preferential oxygenation of stripped hemoglobin the amount of the phosphate-complexed hemoglobin will decrease gradually by releasing the phosphates. We have now tried to simulate the biphasic oxygenation process on the basis of Adair's scheme[2] which is of general validity.

Fractional oxygen saturation of hemoglobin, Y, in the absence of phosphates is given by the following function of oxygen pressure, p,

$$Y = \frac{a_1 p + 2a_2 p^2 + 3a_3 p^3 + 4a_4 p^4}{4(1 + a_1 p + a_2 p^2 + a_3 p^3 + a_4 p^4)} \tag{1}$$

where $a_1 = 4k_1$, $a_2 = 6k_1k_2$, $a_3 = 4k_1k_2k_3$, and $a_4 = k_1k_2k_3k_4$, and k_i is intrinsic oxygen association constant for $Hb(O_2)_{i-1}$. In the presence of a phosphate, A, Y is also given by Eqn 1 where a_1, a_2, a_3 and a_4 have been replaced by b_1, b_2, b_3 and b_4, respectively, which are related to the a values as follows,

$$b_i = a_i \frac{1 + M_i[A]}{1 + M_0\,[A]}, \quad i = 1, 2, 3, 4 \tag{2}$$

where [A] expresses concentration of free A, and M_i (i = 0, 1, 2, 3, 4) is the binding constant of the phosphate for $Hb(O_2)_i$. In this case, the phosphate is assumed to combine with hemoglobin tetramer in mole-for-mole ratio[1]. [A] is given by the solution of the following equation;

$$Z[A]^2 + \{X + Z([Hb]_{total} - [A]_{total})\}[A] - X[A]_{total} = 0 \tag{3}$$

where $[Hb]_{total}$ and $[A]_{total}$ are the total concentrations of hemoglobin and added phosphate, respectively, $X = 1 + a_1p + a_2p^2 + a_3p^3 + a_4p^4$ and $Z = M_0 + a_1pM_1 + a_2p^2M_2 + a_3p^3M_3 + a_4p^4M_4$. Thus using the positive solution of [A] and the value of M, Y in the presence of any given amount of the added phosphate can be calculated as a function of p from Eqns 1 and 2.

Oxygen equilibrium curves of human adult hemoglobin in the presence of different concentrations of 2,3-diphosphoglycerate from 0 to 0.1 mM were determined by the method of Imai *et al.*[3] and are presented in Fig.1. As Benesch *et al.*[1] previously demonstrated by using inositol hexaphosphate, the curves are biphasic in the presence of 2,3-diphosphoglycerate added to hemoglobin tetramer in the ratio 0.25, 0.50 and 0.75, and the slope of the curves changes clearly around Y = 0.75, 0.50 and 0.25, respectively. However, the curves are apparently monophasic in the presence of more than 1 mole of 2,3-diphosphoglycerate per mole of hemoglobin tetramer.

From the oxygen equilibrium data for stripped hemoglobin and for hemoglobin in 0.1 mM 2,3-diphosphoglycerate, the intrinsic oxygen association constants, k, were estimated as previously described[4]. The estimates of k are summarized in Table I together

TABLE I

ADAIR'S CONSTANTS (k_i, mm Hg) AND 2,3-DIPHOSPHOGLYCERATE BINDING CONSTANTS (M_i, M^{-1}) AT pH 7.4 AND 25 °C

	i = 0	*i = 1*	*i = 2*	*i = 3*	*i = 4*
k_i (stripped)	–	0.079	0.295	0.75	4.35
k_i (in 0.1 mM 2,3-diphosphoglycerate)	–	0.011	0.031	0.04	4.35
M_i	$1.3 \cdot 10^7$	$1.8 \cdot 10^6$	$1.9 \cdot 10^5$	0	0

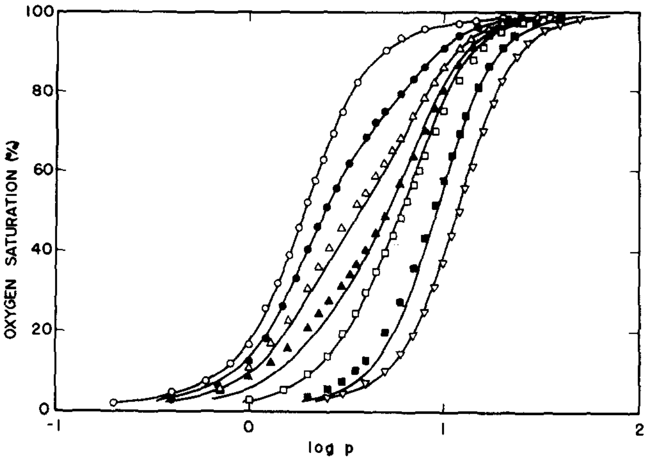

Fig. 1. Oxygen equilibrium curves of hemoglobin in the presence of different amounts of 2,3-diphosphoglycerate. Hemoglobin concentration, $1.5 \cdot 10^{-5}$ M on a tetramer basis; 0.05 M bis-Tris buffer (pH 7.4); 25 °C. Points and lines express experimental data and calculated curves, respectively. ○——○, no 2,3-diphosphoglycerate; ●——●, $3.75 \cdot 10^{-6}$ M 2,3-diphosphoglycerate (0.25 mole/mole); △——△, $7.5 \cdot 10^{-6}$ M 2,3-diphosphoglycerate (0.50 mole/mole); ▲——▲, $1.13 \cdot 10^{-5}$ M 2,3-diphosphoglycerate (0.75 mole/mole); □——□, $1.5 \cdot 10^{-5}$ M 2,3-diphosphoglycerate (1.0 mole/mole); ■——■, $4.5 \cdot 10^{-5}$ M 2,3-diphosphoglycerate (3.0 moles/mole); ▽——▽, $1.0 \cdot 10^{-4}$ M 2,3-diphosphoglycerate (6.7 moles/mole).

with the 2,3-diphosphoglycerate binding constants, M. The binding constants were obtained from a values for stripped hemoglobin and b values for hemoglobin in 0.1 mM 2,3-diphosphoglycerate by using Eqn 2 on the assumption that [A] = [added 2,3-diphosphoglycerate] = constant and 2,3-diphosphoglycerate has no affinity toward fully oxygenated hemoglobin $(M_4 = 0)$[1]. M_3 is zero since k_4 is identical for stripped hemoglobin and hemoglobin in 0.1 mM 2,3-diphosphoglycerate.

By using these Adair constants and 2,3-diphosphoglycerate binding constants, Y in the presence of the same concentrations of 2,3-diphosphoglycerate as shown in Fig.1 were calculated for different values of p as outlined above. The simulated oxygen equilibrium curves are compared with the experimental points in Fig.1. The simulated curves agree well with the experimental data with respect to not only positions but also shapes of all the curves. Hill's coefficient, n, which is defined as $n = \mathrm{d}\log[Y/(1-Y)]/\mathrm{d}\log p$, was calculated on the simulated curves and is plotted against Y in Fig.2. The biphasic feature of the equilibrium curves in the presence of less than 1 mole of 2,3-diphosphoglycerate per mole of hemoglobin is clearly demonstrated by those respective double-peak plots. In the presence of more than 1 mole of 2,3-diphosphoglycerate per mole of hemoglobin, the plots exhibit only a single peak.

Recently, Herzfeld and Stanley[5] have simulated the biphasic oxygen equilibrium curve with a general cooperativity model which involves only five fitting parameters. However, the fitness of their simulated curves to the experimental points is less satisfactory than that indicated in Fig.1, in which the simulated curves were calculated by using seven parameters.

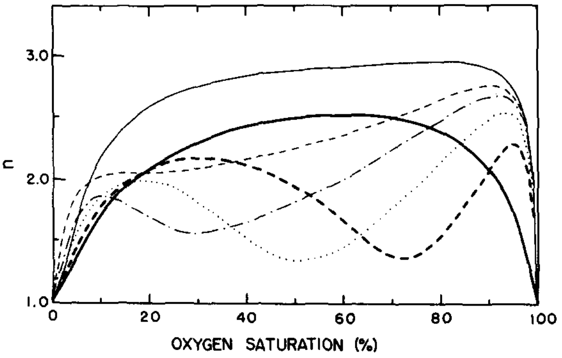

Fig.2. Dependence of Hill's coefficient, *n*, on oxygen saturation. *n* was calculated from the simulated curves in Fig.1. ——, no 2,3-diphosphoglycerate; – – –, 0.25 mole 2,3-diphosphoglycerate/mole; ....., 0.50 mole 2,3-diphosphoglycerate/mole; – . –, 0.75 mole 2,3-diphosphoglycerate/mole; – – –, 1.0 mole 2,3-diphosphoglycerate/mole; ——, 6.7 moles 2,3-diphosphoglycerate/mole. Note that troughs of the plots in the presence of 0.25, 0.50 and 0.75 mole 2,3-diphosphoglycerate/mole are located around 75, 50 and 25% oxygen saturations, respectively.

The saturation of hemoglobin with 2,3-diphosphoglycerate was calculated from the constants in Table I and is plotted against *Y* in Fig.3. The slope of the plots in the presence of 2,3-diphosphoglycerate added in the ratios 0.25, 0.50 and 0.75 sharply increases around *Y* = 0.75, 0.50 and 0.25, respectively, indicating that the 2,3-diphosphoglycerate molecule is substantially released beyond those *Y* values.

The results of the present analysis, which includes the binding of 2,3-diphosphoglycerate to hemoglobin in intermediate stages of oxygenation, quantitatively support the interpretation of Benesch *et al.*[1] for the biphasic oxygen equilibrium curves.

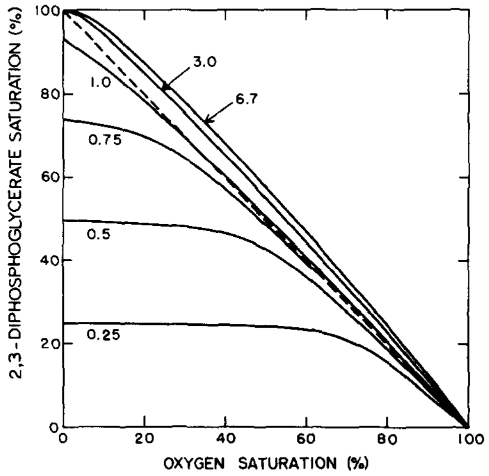

Fig.3. Dependence of the saturation of hemoglobin with 2,3-diphosphoglycerate on the saturation of hemoglobin with oxygen. Solid lines were calculated by using the constants in Table I. Figures attached to the lines indicate their respective molar ratios of 2,3-diphosphoglycerate to hemoglobin tetramer. The broken line indicates proportional dependence.

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