Biophys. Struct. Mechanism 4, 201–207 (1978)

Biophysics of Structure and Mechanism

A Mechanism for Indirect Allosteric Action of Charged Effectors*

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Abstract. A mechanism for indirect allosteric action of charged effectors on substrate binding to a macromolecule is proposed. It is accounted for by electrostatic interaction among effectors in the solution, away from their receptors. The possibility of the mechanism proposed is tested in the allosteric action of univalent salt and 2,3-diphosphoglycerate on oxygen binding to hemoglobin. A model for electrostatic interaction between these two effectors in the solution and for their overall effect on oxygen binding is introduced. The 2,3-diphosphoglycerate binding constant to deoxygenated hemoglobin as a function of univalent salt concentration and the median ligand activity as a function of the concentration of univalent salt and 2,3-diphoshoglycerate are calculated and compared with experimental data. The obtained results indicate that electrostatic interaction in the solution may significantly contribute to indirect allosteric action of charged effectors.

Key words: Hemoglobin – Oxygen binding – Charged allosteric effectors – Model.

Introduction

The equilibrium between different conformations of a macromolecule can in general be affected by several kinds of specific action of effectors. This action changes the overall affinity for substrate binding to a macromolecule. Detailed although non-exclusive definitions of such specific effectors have been attained by Herzfeld and Stanley (1974). We shall restrict our discussion only to allosteric action which can be generally exhibited in the direct as well as in the indirect way. Allosteric effectors act directly by binding preferentially to a particular conformational state of a macromolecule. The additional indirect effect is usually ascribed to a specific action of effectors which interact with already bound effectors or with their binding sites.

* Partly presented at the "11th FEBS Meeting" in Copenhagen, August 1977

0340-1057/78/0004/0201/$ 01.40

Thus effectors binding constants of a particular conformation depend on this competitive interaction in the binding sites.

We shall present another mechanism for the indirect allosteric action, which could be important if charged effectors are involved. The main feature remains the same, namely, effectors binding constants depend on the interaction among effectors. It should be pointed out, however, that in case of charged effectors indirect allosteric action can be at least partly accounted for by their mutual non-specific electrostatic interaction which takes place in the solution, away from a macromolecule and their receptors.

To explore the mechanism proposed we shall consider as an example allosteric action of univalent salt and 2,3-diphosphoglycerate (DPG) on oxygen binding to hemoglobin. Both effectors are charged, univalent salt is dissociated and a molecule of DPG carries in the range of physiological pH between three and five negative charges. It is well-known that each of these effectors, by increasing its concentration alone, lowers the binding of oxygen (Chanutin and Curnish, 1967; Benesch and Benesch, 1967; Benesch et al., 1971; Ruckpaul et al., 1971; Benesch et al., 1969; Antonini et al., 1972). The mechanism of the direct effect of univalent salt has not been explained yet because of many ambiguities occuring in the oxygen-linked chloride binding sites in hemoglobin. On the other hand, the mechanism of the direct action of DPG is well accounted for by existing knowledge of hemoglobin structure (Perutz et al., 1968). A hemoglobin molecule exists in two possible quaternary conformations, i.e. the deoxy and the oxy one. DPG binds preferentially with the molar ratio 1 : 1 to the deoxy conformation, the corresponding binding constant being at least 20–50 times greater than the binding constant to the oxy conformation (Kilmartin and Rossi-Bernardi, 1973). According to the Perutz's model (Perutz, 1970), DPG stabilizes the deoxy conformation by binding to its specific binding site in the central cavity between the hemoglobin β-chains in this conformation. This has actually been confirmed by x-ray structural studies (Arnone, 1972). In the oxy conformation the central cavity becomes too small to accommodate a molecule of DPG (Perutz et al., 1968). There are other binding sites for DPG in the oxy conformation, but their number and position are still unknown (Chanutin and Herman, 1969; Garby et al., 1969; Hedlund and Lovrien, 1974).

Experiments (Benesch et al., 1969) show that the system described also performs indirect allosteric action. If both effectors are involved, an increase of the concentration of either of them diminishes the effect on oxygen binding of the other effector. This action may be accounted for either by competitive interaction in the receptors (Arnone, 1972; Chiancone et al., 1972, 1975) or by electrostatic interaction in the solution. It is most probable, however, that it is affected by both of them. The magnitude of the latter mechanism will be estimated.

Model

The free energy of the electrostatic interaction between DPG and univalent ions in the solution will be calculated by the following simple model. It is assumed that the charge of DPG is uniformly distributed on the surface of a hard sphere with a radius R_0. The free energy is approximated by the Linderstrøm-Lang formula

$$G_e(c_1) = [(Ze_0)^2/(8\ \pi\varepsilon_0\varepsilon)]\ [1/R_0 - \kappa(c_1)/(1 + \kappa(c_1)R)]\,, \tag{1a}$$

$$\kappa(c_1) = [2\ N_L e_0^2/(\varepsilon_0\varepsilon kT)]^{1/2}\ c_1^{1/2}\,, \tag{1b}$$

where κ is the well-known Debye-Hückel parameter, c_1 denotes the molar concentration of univalent salt, e_0 is the unit charge in As, ε_0 is the vacuum permittivity, N_L is the Avogadro's number, k is the Boltzmann's constant, T is the absolute temperature, ε is the dielectric constant of the solution, Z is the number of unit charges of a DPG molecule, R_0 is its effective radius, and R is the effective distance of the closest approach between a DPG molecule and a univalent ion, as it is indicated in Figure 1.

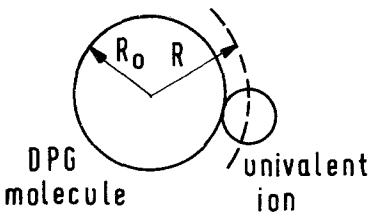

Fig. 1. The model for derivation of the Linderstrøm-Lang formula

(For number values and dimensions of symbols see Table 1.)

By definition, the equilibrium binding constant, K, is given by the relation

$$K = \exp\,(-\,\Delta G/kT)\,, \tag{2}$$

where ΔG is the change in standard Gibbs free energy during the reaction. It is assumed that a DPG molecule, after being bound to hemoglobin, completely loses its electrostatic interaction with univalent ions in the solution. The binding constant for the binding of DPG to hemoglobin can then be represented by using Equations (1) and (2) as

$$K = K^0\ \exp\,[-\,ac_1^{1/2}/(1 + bc_1^{1/2})]\,. \tag{3a}$$

The constant term K^0 includes all contributions to ΔG in Equation (2) which do not depend of c_1, hence it is the binding constant in the absence of univalent salt. a and b are constants and are defined as follows

$$a = [2\ N_L/(\varepsilon_0\varepsilon kT)^3]^{1/2}\ Z^2 e_0^3/(8\ \pi)\,, \tag{3b}$$

$$b = [2\ N_L e_0^2/(\varepsilon_0\varepsilon kT)]^{1/2}\ R\,. \tag{3c}$$

Since a and b are positive, the calculations predict a decrease in the DPG binding constant if the concentration of univalent salt is increased.

The Equation (3) can be applied to the binding of DPG to deoxygenated hemoglobin (see Table 1). Namely, the structural studies (Arnone, 1972) indicate that by the binding to the deoxy conformation a DPG molecule replaces the electrostatic interaction with univalent ions in the solution by the electrostatic interaction with complementary charges in its specific binding site. In fact, a decrease in the DPG binding constant to deoxygenated hemoglobin has actually been observed by direct binding measurements (Hedlund and Lovrien, 1974) as well as from oxygenation equilibrium curves measured at different univalent salt concentrations (Maeda et al., 1972; Tyuma et al., 1973).

As DPG binds much more strongly to the deoxy than to the oxy conformation, it is reasonable to assume that binding sites in the oxy structure are not so specific and that a bound DPG molecule still interacts to some extent with solution ions. Hence in our further calculations, the DPG binding constant to the oxy conformation is considered to be independent of univalent salt concentration. It is also assumed that there is only one DPG binding site in the oxy structure.

The effect of an effector on oxygen binding to hemoglobin can be envisaged from the expression of the median ligand activity of a macromolecule, p_m (Wyman, 1964). This expression is well-known for the direct action of DPG (Baldwin, 1975; Szabo and Karplus, 1976), but for the direct action of univalent salt it still cannot be given explicitely. In case of simultaneous action of the two effectors the expression for p_m becomes much more complex because of additional indirect action. In order to estimate the magnitude of the indirect action due to the electrostatic interaction between the two effectors in the solution, their competitive interaction in the binding sites is not taken into account. This makes it possible to express the direct action of the two effectors as two separate terms in the expression for p_m:

$$\log p_m = \log p_m^0 (c_1) + \log [(1 + K_d(c_1)c_2)/(1 + K_o c_2)]^{1/4}, \tag{4}$$

where c_1 and c_2 are molar concentrations of univalent salt and DPG, respectively, $K_d(c_1)$ and K_o are the DPG binding constants to the deoxy and the oxy conformation, respectively, $K_d(c_1)$ is given in Equation (3). The first term, $p_m^0(c_1)$, describes the direct action of univalent salt, and the second one the direct action of DPG, the latter being modulated by additional action of univalent salt.

Results

In Figure 2 $\log p_m$ from Equation (4) is plotted as a function of $\log c_2$ at four different values of c_1 and compared with the experimental data obtained by Tyuma et al. (1973). The $\log p_m^0(c_1)$ versus $\log c_1$ curve was chosen to fit the data measured

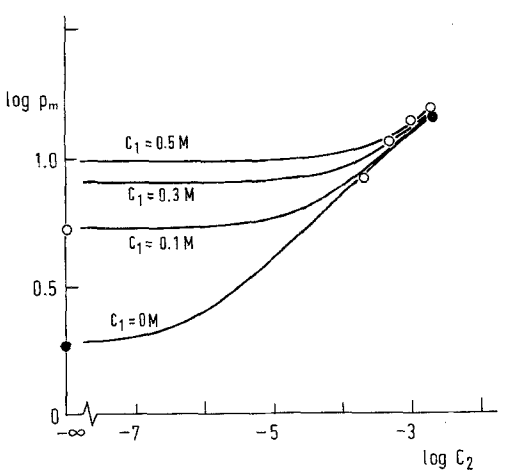

Fig. 2. The calculated dependence of the median ligand activity, p_m, on the molar concentration of univalent salt, c_1, and DPG, c_2. Points are experimental (Tyuma et al., 1973, experimental conditions are the same as mentioned in Table 1): (●) $c_1 = 0$ M; (○) $c_1 = 0.1$ M. (Further explanations in the text)

Table 1. The calculated and experimentally obtained values of the DPG binding constant to deoxygenated hemoglobin, K_d

$K_d[M^{-1}]$ (model)[d]	$K_d[M^{-1}]$ (experiment)[e]	
$c_1 = 0.1$ M	$c_1 = 0$ M	$c_1 = 0.1$ M
2.9×10^4	2.2×10^6	2.6×10^4 [a]
	2.0×10^6	3.1×10^4 [b]
	–	3.6×10^4 [c]

[a] Calculated by Deal (1973) from data of Maeda et al. (1972)
[b] Tyuma et al. (1973)
[c] Benesch et al. (1969)
[d] The values of constants and parameters used in the calculation: $e_0 = 1.6 \times 10^{-19}$ As; $N_L = 6.03 \times 10^{26}$ kmol^{-1}; $\varepsilon_0 = 8.85 \times 10^{-12}$ As (Vm)$^{-1}$; $k = 1.38 \times 10^{-23}$ J K^{-1}; $\varepsilon = 78.54$; $T = 298$ K; $K_d^0 = 2.1 \times 10^6$ M^{-1}; $Z = -4.2$; $R = 5 \times 10^{-10}$ m
[e] Experimental conditions: pH = 7.4; $T = 25°$ C; $[Hb] = 1.5 \times 10^{-5}$ M per tetramer. The datum (c) has been corrected for the same conditions by Tyuma et al. (1973)

by Antonini et al. (1972). However, in the present analysis the curve was shifted in such a way that it corresponds with the data of Tyuma et al. (1973). The values of the parameters for the DPG binding constant to the deoxy conformation from Equation (3) were as follows: $K_d^0 = 2.1 \times 10^6$ M^{-1}, $a = 20.7$ M$^{-1/2}$, $b = 1.6$ M$^{-1/2}$. With these values of the parameters a good agreement can be achieved between calculated and experimentally obtained values of the binding constant (Table 1). Furthermore, parameter a corresponds to the number of unit charges of a DPG molecule $Z = -4.2$, and b corresponds to the closest approach between a DPG molecule and a univalent ion $R = 5$ Å. This seems to be very reasonable for a known ionisation state and known dimensions of a DPG molecule. $K_0 = 1 \times 10^2$ M^{-1} was chosen as the DPG binding constant to the oxy conformation.

Discussion and Conclusions

The shape of the curves indicates that the effect of DPG on oxygen binding is reduced with increasing concentration of univalent salt. On the other hand, the curves for different concentrations of univalent salt join if DPG concentration is increased which indicates a decrease in the action of univalent salt. At a DPG concentration of 0.25×10^{-3} M, oxygen binding is nearly independent of univalent salt concentration, and at a concentration of univalent salt of 0.5 M, oxygen binding becomes independent of DPG concentration. This is also in agreement with the experimentally observed mutual behaviour of these two effectors by Benesch et al. (1969).

Therefore, it is concluded that the mutual effect of univalent salt and DPG on oxygen binding to hemoglobin could be accounted for by the electrostatic interaction in the solution between these two effectors. This does not exclude other possibilities.

The recent observations of chloride binding to hemoglobin (Chiancone et al., 1972, 1975) support the idea of the competitive action of the two effectors in the receptors but show, however, that only a part of oxygen-linked chloride binding sites may be overlapping with the phosphate binding site. The crystallographic evidence of an inorganic salt binding site within the DPG binding site in the deoxy conformation (Arnone, 1972), which is commonly cited in the literature as a structural explanation for the competitive nature of univalent salt and DPG binding, may not necessarily be considered as an oxygen-linked chloride binding site (Arnone, 1972). It seems that the present experimental data cannot give a definitive answer to the problem, therefore more information about chloride binding to hemoglobin and additional systematical measurements of the effectors action are required.

The present analysis shows that the electrostatic interaction between univalent ions and DPG in the solution may essentially contribute to allosteric regulation of oxygen binding to hemoglobin. The obtained estimate also points out that the electrostatic interaction in the solution has to be considered and carefully examined whenever indirect allosteric action is interpreted if charged effectors are involved.

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Received October 4, 1977/Accepted December 5, 1977