# A GENERAL MODEL OF COOPERATIVITY AND ITS APPLICATION TO DPG INHIBITION OF HEMOGLOBIN OXYGENATION

Judith Herzfeld[+] and H. Eugene Stanley[*]

[+]Department of Chemistry, [*]Department of Physics, and
[+*]Center for Materials Science and Engineering
Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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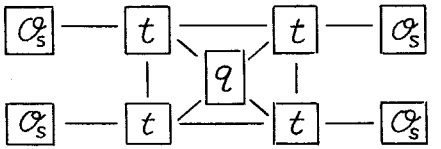
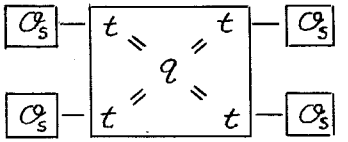
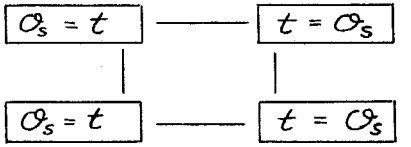
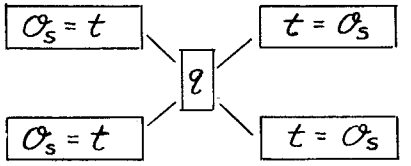
Summary: A general model of cooperativity has been developed that combines the concepts of preferential binding, tertiary subunit interaction, and quaternary constraint. The models of Monod, Wyman and Changeux, and of Koshland et al., are limiting cases of this general theory. Another special case of the general model, approximating the Perutz description of hemoglobin, fits mono- and bi-phasic hemoglobin oxygenation data, over a wide range of 2-3 Diphosphoglycerate (DPG) concentrations, with a total of five adjustable parameters, and predicts that the change from deoxy to oxy quaternary structure occurs just before or just after the third heme is oxygenated, depending on the DPG level.

The allosteric model of Monod, Wyman, and Changeux[1] (MWC) explains cooperativity in terms of preferential binding of substrate to different molecular conformations, and quaternary constraints which maintain molecular symmetry. Cooperativity has also been explained by Koshland, Nemethy and Filmer[2], in terms of induced-fit binding of substrate and interactions between subunits in different tertiary conformations. Both theories explain positive cooperativity. However, only the cooperative mechanism of Koshland, et al. explains negative cooperativity, and only the cooperative mechanism of MWC explains cooperativity when structural change does not coincide with substrate binding.

The cooperative mechanisms of both theories have been combined in a single model which is described in greater detail elsewhere;[3,4] the MWC and Koshland theories are special cases of this general model (see Table I). Another special case is particularly applicable to hemoglobin: Perutz[5] has suggested that cooperativity arises from the combination of induced-fit binding of oxygen, as hypothesized by Koshland, and quaternary constraints, as hypothesized by MWC (see Table I).

The general model also incorporates two mechanisms for effector action: (1) Effector molecules may interact directly ('direct effectors') with bound

TABLE I

| Model | Binding | Allosteric Mechanisms | Schematic Diagram |
|---|---|---|---|
| General | Preferential binding | Tertiary-tertiary interactions<br>Quaternary constraints |  |
| Monod, Wyman, and Changeux[1] | Preferential binding | No tertiray-tertiary interactions<br>Infinite quaternary constraints (symmetry) |  |
| Koshland, Nemethy, and Filmer[2] | Induced-fit binding<br>(Infinitely preferential binding) | Tertiary-tertiary interactions<br>No quaternary constraints |  |
| Perutz[5] | Induced-fit binding<br>(Infinitely preferential binding) | No tertiary-tertiary interactions<br>Quaternary constraints |  |

This table shows how previous models are special cases of the general model. The last column indicates schematically this relation. The notation $\mathcal{O}_S$, t, q corresponds respectively to substrate binding, tertiary conformation, and quaternary conformation. A solid line indicates an interaction, while a double line, together with an enclosing box, indicates an infinitely strong interaction.

substrate molecules or with the substrate receptors, changing the stability of the bound complex and the observed binding affinity. In the limiting case of extreme antagonistic interactions beween substrate and effector, the effector competes (' competitive effectors') with substrate for occupancy of the same binding sites.

(2) Effector molecules may shift the equilibrium between accessible conformations (' allosteric effectors') by binding preferentially to a particular tertiary or quaternary conformation (' t-effectors' and ' q-effectors').

In particular, for the case of hemoglobin oxygenation, the following types of effectors are included in the model:

(A) Carbon monoxide, as an example of a competitive effector.

(B) The protons involved in the alkaline Bohr effect (which bind preferentially to the intact salt bridges of subunits in the deoxy tertiary conformation[5]), as an example of a t-effector.

(C) DPG (which binds preferentially to the deoxy quaternary structure of hemoglobin[6]), as an example of a q-effector.

(D) Since binding of DPG is pH dependent, pH influences the quaternary equilibrium in the presence of DPG, and protons can be considered ' second-order' q-effectors, as well as t-effectors.

We shall henceforth specialize the model to the treatment of the effect of DPG on hemoglobin oxygenation. At constant levels of other effectors (e.g., constant pH), the fractional saturation of the oxygen and DPG binding sites is given by

$$\bar{y}_{O_2} = \frac{1}{4} Z^{-1} p_{O_2} (\partial Z / \partial p_{O_2}) \tag{1a}$$

$$\bar{y}_{DPG} = Z^{-1} [DPG]_{free} (\partial Z / \partial [DPG]_{free}) \tag{1b}$$

where

$$Z = Z_{deoxy} + Z_{oxy} \ , \tag{2}$$

$$Z_{deoxy} = K_q (K_{DPG, deoxy} [DPG]_{free} + 1)$$

$$x\,(p_{O_2} K^{\alpha}_{O_2,\,deoxy} + 1)^2\,(p_{O_2} K^{\beta}_{O_2,\,deoxy} + 1)^2 \qquad (3a)$$

$$Z_{oxy} = (K_{DPG,\,oxy}\,[DPG]_{free} + 1)$$

$$x\,(p_{O_2} K^{\alpha}_{O_2,\,oxy} + 1)^2\,(p_{O_2} K^{\beta}_{O_2,\,oxy} + 1)^2 \qquad (3b)$$

and

$$[DPG]_{free} = [DPG]_{total} - [Hb]\,\bar{y}_{DPG} \cdot \qquad (4)$$

The parameters appearing in the above equations are defined as follows: $K^{\alpha}_{O_2,\,deoxy}$ and $K^{\beta}_{O_2,\,deoxy}$ are, respectively, the constraints for induced-fit binding to $\alpha$ and $\beta$ subunits constrained by the deoxy quaternary conformation. $K_{DPG,\,deoxy}$ is the constant for DPG binding to the deoxy quaternary conformation. Three more parameters are similarly defined with 'deoxy' quaternary conformation replaced by 'oxy' quaternary conformation. A seventh parameter, $K_q$, is the constant for the equilibrium between the two quaternary conformations in the absence of oxygen and DPG.

A partial test of the present model was obtained in attempting to fit both monophasic and phasic oxygenation data by adjusting the seven parameters of the above equations. A nearly equally good fit to the data was obtained (see Fig. 1) by assuming that

$$K^{\alpha}_{O_2,\,deoxy} = K^{\beta}_{O_2,\,deoxy} \equiv K_{O_2,\,deoxy} \qquad (5a)$$

and

$$K^{\alpha}_{O_2,\,oxy} = K^{\beta}_{O_2,\,oxy} \equiv K_{O_2,\,oxy} \qquad (5b)$$

so that the seven adjustable parameters reduce to five. Thus our model interprets the data to suggest that the $\alpha$ and $\beta$ chains are nearly equivalent with respect to oxygen affinity and quaternary constraints.

The five-parameter model was then used to predict the change of the quaternary structure as successive heme groups are oxygenated. Equations (3a) and (3b) may be rewritten in the form

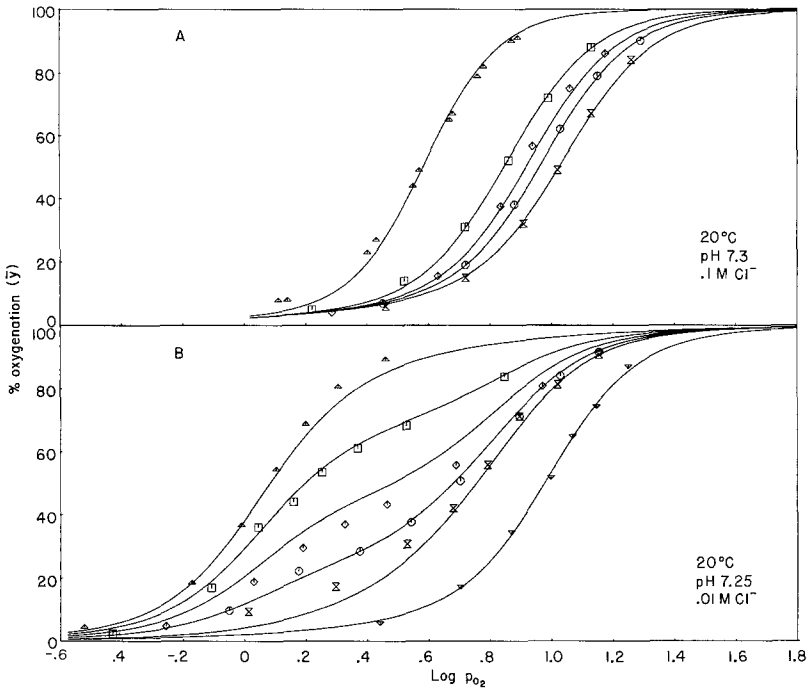



Figure 1. Experimental oxygenation data compared with the theoretical equilibrium curves.

A. Data points,[6,7] taken with $6 \times 10^{-5}$ M hemoglobin (by tetramer) and 0.05 M Bis Tris buffer. Total DPG: Δ none; □ $1.3 \times 10^{-4}$ M; ◇ $2.5 \times 10^{-4}$ M; ○ $4.0 \times 10^{-4}$ M; ⧖ $1.0 \times 10^{-3}$ M.

Theoretical equilibrium curves shown for parameter values:

$K_{DPG,\,deoxy} = 1.07 \times 10^{5}\ M^{-1}$; $K_{DPG,\,oxy} = 1.22 \times 10^{3} M^{-1}$;
$K_q = 1.27 \times 10^{10}$; $K_{o_2,\,deoxy} = 0.0234\ mmHg^{-1}$; $K_{o_2,\,oxy} = 91.3 mmHg^{-1}$.

B. Data points[8] taken with $1.17 \times 10^{-4}$ M hemoglobin (by tetramer) and Bis Tris buffer. Total DPG: Δ none; □ $2.87 \times 10^{-5}$ M; ◇ $5.75 \times 10^{-5}$ M; ○ $8.62 \times 10^{-5}$ M; ⧖ $1.15 \times 10^{-4}$ M; ▽ $1.15 \times 10^{-3}$ M.

Theoretical equilibrium curves shown for parameter values:

$K_{DPG,\,deoxy} = 7.14 \times 10^{6}\ M^{-1}$; $K_{DPG,\,oxy} = 2.70 \times 10^{3}\ M^{-1}$;
$K_q = 1.32 \times 10^{2}$; $K_{o_2,\,deoxy} = 0.0220 mmHg^{-1}$; $K_{o_2,\,oxy} = 2.52 mmHg^{-1}$.

Notice that the method of Tyuma et al.[9] would involve 20 parameters to fir the 5 monophasic Benesch curves and at least 24 parameters to fit the 6 Bunn curves.

$$Z_{deoxy} = \sum_{n=0}^{4} K_q (K_{DPG,\,deoxy}[DPG]_{free} + 1) \frac{4!}{n!\,(4-n)!} p_{O_2}^{n} K_{O_2,\,deoxy}^{n}$$

$$= \sum_{n=0}^{4} P(deoxy\,|\,n) \qquad (6a)$$

and

$$Z_{oxy} = \sum_{n=0}^{4} (K_{DPG,\,oxy}[DPG]_{free} + 1) \frac{4!}{n!\,(4-n)!} p_{O_2}^{n} K_{O_2,\,oxy}^{n}$$

$$= \sum_{n=0}^{4} P(oxy\,|\,n) \qquad (6b)$$

Here $P(deoxy\,|\,n)$ and $P(oxy\,|\,n)$ are proportional, respectively, to the probabilities that a single hemoglobin molecule with n hemes oxygenated will be in the deoxy and oxy quaternary conformations. Figure 2 shows the changed balance between the two quaternary conformations as oxygenation proceeds. The change from deoxy to oxy quaternary strucutre is predicted to occur just before or just after the third heme is oxygenated, depending on the DPG level. This prediction is in rough accordance with the observations of Gibson and Parkhurst.[10]

The present model provides a useful means of analyzing abnormal oxygenation. The substituted residues of several variant hemoglobins have been found to shift the allosteric equilibrium between either the tertiary or the quaternary conformations.[11] Such effects should be reflected in the parameters of the monophasic oxygenation curves.

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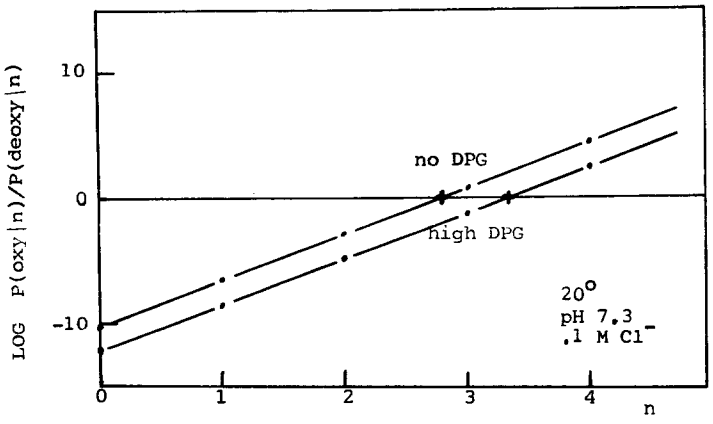


Figure 2. The relative probabilities of the oxy and deoxy quaternary states in a hemoglobin molecule with n hemes oxygenated. (Theoretical parameters as in Figure 1A.)

The "change" from one quaternary conformation to another is marked by a '+'.

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Note added in proof: The equations applied here to DPG inhibition of hemoglobin oxygenation are a special case of our general model. This model is more general than that of Szabo and Karplus,[12] which was very recently applied to the Bohr effect (but not to the DPG effect). In particular, the models differ significantly in that we make no a priori assumptions regarding at what stage of oxygenation the change in quaternary conformation occurs, but rather deduce this information from the oxygenation data as interpreted by our model.

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