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Diphosphoglycerate and Inosine Hexaphosphate Control of Oxygen Binding by Hemoglobin: A Theoretical Interpretation of Experimental Data*

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Abstract. A theoretical equation is presented for the control of cooperative adsorption on proteins and other linear macromolecules by hormones, drugs, ATP, and other "cardinal adsorbents." With reasonable accuracy, this equation describes quantitatively the control of oxygen binding to hemoglobin by 2,3-diphosphoglycerate and by inosine hexaphosphate.

To function as a coherent unit, a living cell must possess the means for long-range information and energy transfer. A theory for these fundamental processes was presented in 1962 as part of a general theory of the living state called the association-induction hypothesis.[1–3]

The key mechanism involves three postulates: (*i*) various solutes and the bulk of cell water are adsorbed on cellular proteins; (*ii*) this adsorption is not random but is synchronized as a result of interaction among neighboring adsorption sites (i.e., the adsorption is auto-cooperative); and (*iii*) these auto-cooperative adsorptions and desorptions are controlled by a smaller number of molecular agents collectively referred to as "cardinal adsorbents," but better known as drugs, hormones, ATP, etc. Cardinal adsorbents exercise their control by interacting with certain key sites (cardinal sites) on the same proteins; they serve as king pins in the harmonious functioning of living cells.

A quantitative theory for the cooperative adsorption was derived by Yang & Ling using statistical mechanical methods (ref. 4, see also 5).

In 1963, Monod, Jacob, and Changeux considered the phenomenon of enzyme control by interaction at a distance and introduced the name "allosteric control."[6] In 1965, Monod, Wyman, and Changeux also interpreted "allosteric" control in terms of cooperative interaction; they introduced another cooperative adsorption isotherm.[7] In 1966, still another cooperative adsorption isotherm was presented by Koshland, Nemethy, and Filmer.[8] This isotherm is similar to that of Monod, Wyman, and Changeux in that both are not statistical-mechanical and not general. The differences between our model and that of Monod, Wyman, and Changeux were discussed in a recent review.[3]

The Yang-Ling isotherm for the cooperative adsorption on a linear polymer is represented as

$$[p_i]_{ad} = \frac{[f]}{2}\left(1 + \frac{\xi - 1}{\sqrt{(\xi - 1)^2 + 4\xi \exp(\gamma/RT)}}\right), \quad (1)$$

where $[p_i]_{ad}$ is the concentration of adsorbed ith species, and $[f]$ is the concentration of adsorption sites for the ith (and jth) species. ξ is defined as follows:

$$\xi = \frac{[p_i]_{ex}}{[p_j]_{ex}} \cdot K^{\circ\circ}_{j\to i}, \quad (2)$$

where $[p_i]_{ex}$ and $[p_j]_{ex}$ are the concentration of free ith and jth solute and $K^{\circ\circ}_{j\to i}$ is the intrinsic equilibrium constant for the jth $\rightarrow$ ith solute exchange. $K^{\circ\circ}_{j\to i}$ is related to the intrinsic free energy of exchange $\Delta F^{\circ\circ}_{j\to i}$ by the relation:

$$\Delta F^{\circ\circ}_{j\to i} = -RT \ln K^{\circ\circ}_{j\to i}. \quad (3)$$

It is to be noted that $\Delta F^{\circ\circ}_{j\to i}$ refers to the free energy change in a jth $\rightarrow$ ith exchange of adsorption, which involves no change in the total number of $i - j$ pairs of nearest neighbors within the system. This is the case when the exchange on the middle site occurs in a triad of sites: $ijj \rightarrow iij$. A total of one ij neighboring pair exists before the exchange and afterwards.

On the other hand, in an exchange of $jjj \rightarrow jij$, two ij neighboring pairs are created. The creation of each additional mole of new ij pairs entails another energy term equal to $-(\gamma/2)$. Thus, in this case, the total free energy change is not merely $\Delta F^{\circ\circ}_{j\to i}$ but $\Delta F^{\circ\circ}_{j\to i} + 2[-(\gamma/2)] = \Delta F^{\circ\circ}_{j\to i} - \gamma$.

It has been shown that Eq. 1 describes the adsorption of a variety of materials on native and denatured proteins *in vitro* (refs. 1, 3, 9, including oxygen-binding on hemoglobin *in vitro* and *in vivo*). Equation 1 also describes the adsorption of K^+ and Na^+ ion in frog muscles,[3,10] in canine carotid arteries,[11] and in rabbit uterine myometrium.[12]

It was also found that the drugs, lanoxin and ouabain (cardiac glycosides), control the cooperative adsorption of K^+ ion and of Na^+ ion in frog muscle cells by altering $K^{\circ\circ}_{Na\to K}$ in a manner as predicted by the association-induction hypothesis.[3] Similarly, Jones found that the sex hormone, progesterone, controls the cooperative adsorption of K^+ ion in rabbit myometrium by increasing the value of $K^{\circ\circ}_{Na\to K}$.[12]

Although the control of cooperative adsorption by cardinal adsorbents is an integral part of the association-induction hypothesis, so far no quantitative statement of the control has been given. Such a quantitative statement can be readily written out in a straight forward manner.

Let us consider a system of protein chains, each containing one cardinal site and g cooperatively linked adsorption sites, together referred to as a "gang." In Fig. 1, for example, each gang is shown to include three sites; in real cases, there can be more or less sites in a gang. If the total concentration of gangs, and hence cardinal adsorbents, is [F], and the concentration of cardinal adsorbents in the medium is $[C]_{ex}$, the general equation for the concentration of adsorbed cardinal adsorbent is entirely analogous to Eq. 1.

$$[C]_{ad} = \frac{[F]}{2}\left\{1 + \frac{\bar{\Xi} - 1}{\sqrt{(\bar{\Xi} - 1)^2 + 4\bar{\Xi} \exp(\Gamma/RT).}}\right\} \quad (4)$$

where $\bar{\Xi}$ is defined in an analogous manner as shown in Eq. 2:

$$\bar{\Xi} = [C]_{ex} \cdot \mathcal{K}_c^{\circ\circ} \tag{5}$$

Here the alternative adsorbent on the cardinal site is unspecified (e.g., H_2O), but is a constant and thus included in the apparent association constant $\mathcal{K}_c^{\circ\circ}$.

Fig. 1 shows that the adsorption of a cardinal adsorbent potentially can change both ξ and γ. Thus, the total concentration of the ith adsorbed solute is described by the following equation:

$$[p_i]_{ad} = \frac{g[C]_{ad}}{2}\left\{1 + \frac{\xi_c - 1}{\sqrt{(\xi_c - 1)^2 + 4\xi_c \exp(\gamma_c/RT)}}\right\} + \frac{g\{[F] - [C]_{ad}\}}{2}\left\{1 + \frac{\xi_0 - 1}{\sqrt{(\xi_0 - 1)^2 + 4\xi_0 \exp(\gamma_0/RT)}}\right\}. \tag{6}$$

ξ_c and ξ_0 are defined as follows:

$$\xi_C = \frac{[p_i]_{ex}}{[p_j]_{ex}} \cdot K_{(j\to i)C}^{\circ\circ}, \tag{7}$$

and

$$\xi_0 = \frac{[p_i]_{ex}}{[p_j]_{ex}} \cdot K_{(j\to i)0}^{\circ\circ}. \tag{8}$$

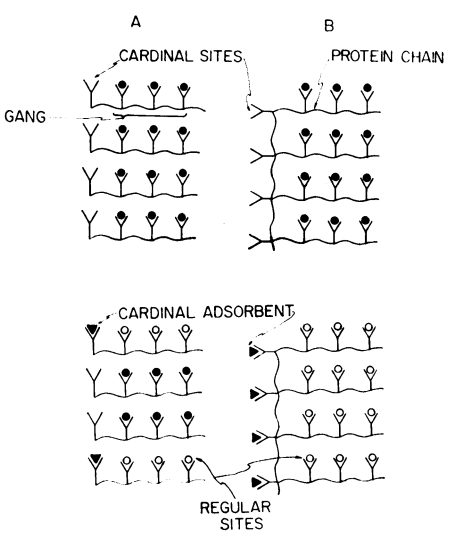

FIG. 1. Diagrammatic illustration of cooperative adsorption controlled by cardinal sites. In *A*, the adsorption at the cardinal sites is independent. In *B*, the adsorption at cardinal sites is cooperative. Solid and empty circles represent alternative adsorbents. Large filled-triangles represent cardinal adsorbents.

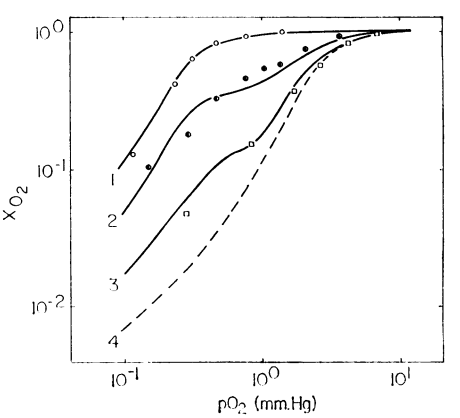

FIG. 2. Oxygen uptake by hemoglobin (stripped) in the presence and absence of 2,3-DPG. Hemoglobin solution was 0.3%, pH 7.0, 10°C. (1) No DPG; (2) 1.2×10^{-5}M DPG; (3) 2.4×10^{-5}M DPG. Points are experimental data of Benesch and Benesch.[13] Lines are theoretical, calculated according to Eq. 9, with the following values for the different parameters: $K_0^{\circ\circ} = 4.0$, $K_c^{\circ\circ} = 0.43$ $(\text{mm Hg})^{-1}$; $-(\gamma_0/2) = 0.64$, $-\gamma_c/2 = 0.47$ kcal/mol, $\mathcal{K}_c^{\circ\circ} = 5 \times 10^{-4}(\text{M})^{-1}$, $-(\Gamma/2) = 0.52$ kcal/mol. The dotted line is the theoretical curve at $[DPG]_{ex} = \infty$.

$K^{\circ\circ}_{(j\to i)C}$ is the intrinsic equilibrium constant of the ith to jth exchange in a gang with its cardinal site occupied by the cardinal adsorbent C; $K^{\circ\circ}_{(j\to i)0}$ is that in which the cardinal site is not so occupied. Similarly, $-(\gamma_c/2)$ and $-(\gamma_0/2)$ refer, respectively, to the nearest-neighbor interaction energy for the gang controlled by cardinal absorbent and the gang not under the control of the cardinal adsorbent. $[C]_{ad}$ is as described by Eq. 4. In the case where there is no interaction among the cardinal sites themselves (as in Fig. 1*A*), $-(\Gamma/2)$ is zero; in the case when there is cooperative interaction among the cardinal sites (as in Fig. 1*B*), $-(\Gamma/2)$ is greater or smaller than zero (auto-cooperative or heterocooperative, respectively).

The recent experimental[13,14] demonstration of the control of the cooperative binding of oxygen by ATP and other phosphates has great significance. Since hemoglobin has no ATPase activity, the data show unequivocally that ATP and other phosphates *per se* (and not their hydrolysis) can control the cooperative adsorption. Such a control of adsorption of K^+ ion in muscle cells by ATP as a cardinal adsorbent has been suggested since 1951[15] and has remained one of the main themes of the association-induction hypothesis.[1-3,16]

The data of Benesch & Benesch on oxygen binding by hemoglobin at 10°C has been replotted on a log-log scale for comparison with predictions of the association-induction hypothesis. Fig. 2 shows the effect of various concentrations of 2,3-diphosphoglycerate (2,3-DPG) on the oxygen binding; Fig. 3 shows the effect of inosine hexaphosphate (IHP). The points are those experimen-

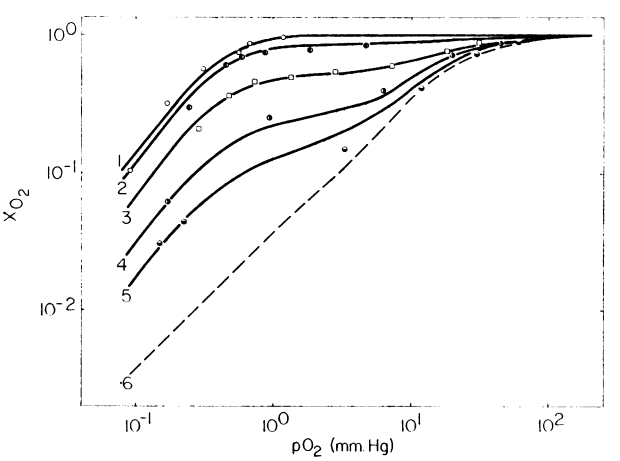

FIG. 3. Oxygen uptake by hemoglobin (stripped) in the presence and absence of IHP. Hemoglobin solution was 0.3%, pH = 7.0, 10°C. (1) No IHP; (2) 1.2×10^{-5} M IHP; (3) 2.4×10^{-5} M IHP; (4) 3.6×10^{-5} M IHP; (5) 4.8×10^{-5} M IHP. Points are experimental data of Benesch and Benesch.[13] Lines are theoretical, calculated according to an equivalent version of Eq. 9, with the following values for the different parameters: $K_0^{\circ\circ} = 3.33$, $K_c^{\circ\circ} = 0.67$ (mm Hg)$^{-1}$; $-(\gamma_0/2) = 0.39$, $-(\gamma_c/2) = 0.21$ kcal/mol; $\mathfrak{K}_c^{\circ\circ} = 4.2 \times 10^{-4}(M)^{-1}$, $-\Gamma/2 = 0.68$ kcal/mol. Dotted line is theoretical curve at $[DPG]_{ex} = \infty$.

tally determined by Benesch and Benesch. The lines are theoretical, calculated according to Eq. 6. In the case of Figure 2, for example, Eq. 6 takes on the following more specific form:

$$X_0 = \frac{[O]_{ad}}{[f]} = \frac{[DPG]_{ad}}{2(F)}\left(1 + \frac{pO_2 \cdot K_C^{\circ\circ} - 1}{\sqrt{(pO_2 \cdot K_C^{\circ\circ} - 1)^2 + 4pO_2K_C^{\circ\circ} \exp(\gamma_c/RT)}}\right)$$
$$+ \frac{[F] - [DPG]_{ad}}{2[F]}\left(1 + \frac{pO_2 \cdot K_O^{\circ\circ} - 1}{\sqrt{(pO_2 \cdot K_O^{\circ\circ} - 1)^2 + 4pO_2K_O^{\circ\circ} \exp(\gamma_0/RT)}}\right), \quad (9)$$

Where X_0 is the mole fraction of heme groups bound to oxygen, $[O]_{ad}$ is the concentration of adsorbed oxygen, and $[DPG]_{ad}$ is the concentration of adsorbed 2,3-DPG. pO_2 is the equilibrium partial pressure of oxygen in the environment. $K_C^{\circ\circ}$, $K_O^{\circ\circ}$, $-\gamma_c/2$, and $-\gamma_0/2$ refer to the oxygen association constant and nearest-neighbor interaction energy under the control of DPG ($K_C^{\circ\circ}$, $-\gamma_c/2$) and not under its control ($K_O^{\circ\circ}$, $-\gamma_0/2$). $[DPG]_{ad}$ is calculated from Eqs. 4 and 5.

In both Figs. 2 and 3, the dashed line represents the theoretical curves for the case where all the cardinal sites are occupied by the cardinal adsorbents. Comparing this with the top curve in each figure (no cardinal adsorption), one finds that the effect of 2,3-DPG on oxygen binding was to shift the intrinsic equilibrium constant $K_{j\to i}^{\circ\circ}$ from 4.0 to 0.43 $(\text{mm Hg})^{-1}$ and the nearest neighbor interaction energy from 0.64 to 0.47 kcal/mol. Similarly the effect of IHP is to change the intrinsic equilibrium constant from 3.3 to 0.67 $(\text{mm Hg})^{-1}$ and the nearest neighbor interaction energy from 0.39 to 0.21 kcal/mol.

The best-fitting curves were calculated on the basis of $\mathcal{K}_c^{\circ\circ}$ equal to $5.0 \times 10^{-4}(\text{M})^{-1}$ (which is equivalent to a dissociation constant of 2.0×10^{-5} M, the value that Benesch & Benesch actually found) and $-(\Gamma/2)$ for 2,3-DPG equal to 0.52 kcal/mol. Similarly, the $\mathcal{K}_c^{\circ\circ}$ value used in the theoretical curves of Figure 3 for IHP is $4.2 \times 10^{-4}(\text{M})^{-1}$ and $-(\Gamma/2)$, 0.68 Kcal/mole. In both cases, somewhat poorer-fitting theoretical curves are obtained with $-(\Gamma/2)$ equal to zero.

In conclusion, we find that within the limits of reasonable accuracy the data for auto-cooperative oxygen-binding by hemoglobin, as well as its control by the adsorption of the cardinal adsorbents, 2,3-DPG and IHP, can be described by Eqs. 1 and 6, respectively.

Abbreviations: DPG, 2,3-diphosphoglyceric acid; IHP, inosine hexaphosphate.

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