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MODEL FOR COOPERATIVITY OF BIOLOGICAL MEMBRANES

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We present a mathematical model for the complex cooperativity observed in biological membranes. In our model, it is assumed that the proteins bound on the membrane are noncooperative and possess a Bohr proton. It is further assumed that the net charge of the unliganded state of the protein is different from that of the liganded state owing to the structural change upon binding the ligand. With this model, we show how an all-or-none response, a graded response, and a noncooperative response arise in the binding curve of such biological membranes. In addition, we show how an effector, which can alter the pK_a involved in the binding site, induces a complex cooperativity.

1. Introduction

Many key enzymes in metabolic pathways are believed to be allosteric and hence to exhibit cooperativity upon binding of substrates. There exist two theories to account for the cooperativity: the quatenary structure theory of Monod et al. [1] and the sequential theory of Koshland et al. [2]. The cooperativity observed in biological membranes has been explained by Changeux et al. [3,4] in terms of the relationship between a conformational transition of the repeating units of proteins and the binding of ligands, i.e., in terms of an allosteric nature of the proteins bound to the biological membrane and a highly ordered structure of the proteins. It is nowadays well known that the proteins bound on the membrane are not highly ordered and are sufficiently separated that they do not 'interact' with each other. The objective of this paper is to show that the cooperativity can arise in nonallosteric biological membranes as a consequence of a microenvironmental effect.

If a ligand has a preferential binding to either a protonated or an unprotonated state of the protein (i.e., if a compartmentalized protein is a Bohr protein) and if the net charge of the liganded state is different from that of the unliganded state, the existence of the two environments may cause an anomaly in the ligand-binding property of the protein. That is, if the binding of ligand induces a structural change in the protein resulting in an alteration of its net charge, then a cooperativity may be observed in the Hill plot owing to the dependence of pH on the liganded state of the protein. In the following, we will show quantitatively how the cooperativity may arise as a result of this microenvironmental Bohr effect.

2. Mathematical model

If a protein (P) contains a Bohr proton, the reaction of the protein with its ligand (L) may be represented by the following reaction:

$$\begin{array}{ccc} \mathrm{PH} & \overset{K_L}{\rightleftharpoons} & \mathrm{PHL} \\ {\scriptstyle K_a}\Big\Uparrow\Big\Downarrow & & \Big\Uparrow\Big\Downarrow{\scriptstyle K_a'} \\ \mathrm{P} & \underset{K_L'}{\rightleftharpoons} & \mathrm{PL} \end{array} \qquad (1)$$

In the above equation, $K_L' = K_L K_a / K_a'$, where K_L

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and K_L' are the dissociation constants for a ligand, and K_a and K_a' are the dissociation constants for a proton.

Let us assume that these proteins are bound uniformly on the membrane. Then, the charge neutrality condition at the surface of the membrane leads to the following relation among the mobile and immobile ions:

$$q=[H^+]-K_w/[H^+]+\Sigma B(Z_B-\bar{r}_B)+\Sigma Z_I[I] +(1-\bar{Y})\rho_P+\bar{Y}\rho_{PL}=0, \quad (2)$$

where $[H^+]$, [B] and [I] are, respectively, the concentrations of proton, buffer, and salt, K_w is the water dissociation constant, Z_I, and Z_B are, respectively, the number of charges per salt and buffer ion when all the proton sites are occupied, and the sum extends over all the species present. In eq. 2, ρ_P and ρ_{PL} are the charge densities of unliganded and liganded proteins, respectively, and $\bar{r}_B$ is the average number of protons dissociated from a buffer ion at a given pH and obeys the following expression:

$$\bar{r}_B=\frac{\sum_{I=1}\prod_{j=1}^{I}(K_j/[H^+])}{1+\sum_{I=1}\prod_{j=1}^{I}(K_j/[H^+])} \quad (3)$$

where K_j is the jth acid dissociation constant. In eq. 2, $\bar{Y}$ is the fraction of liganded state of protein and obeys the following expression for the protein whose dissociation properties follow eq. 1:

$$\bar{Y}=\frac{[L]}{K_{app}+[L]} \quad (4a)$$

Here [L] is the concentration of a ligand (it can be a buffer ion), and K_{app} is the apparent dissociation constant for a ligand and takes the following form:

$$K_{app}=K_L(1+K_a/[H^+])/(1+K_a'/[H^+]) \quad (4b)$$

Note that as long as the magnitude of the terms involved in immobile ions in eq. 2 is greater than that of mobile ions (i.e., the first four terms), a microenvironmental effect is significant. In other words, a Donnan equilibrium potential, which exists on the interface of the two environments, becomes significant if the charge density of immobile ions is greater than that of mobile ions. The electrostatic partition coefficient, Λ, which is equal to $\exp(-E_D/RT)$ where E_D is a Donnan equilibrium potential, can be obtained from eq. 2 by substituting the relations

$$[H^+]_e=[H^+]_o\Lambda, \quad [I]_e=[I]_o\Lambda^{Z_I}, \quad [B_j]_e=[B_j]_o\Lambda^{Z_j} \quad (5)$$

where the subscripts e and o refer to the micro- and macroenvironments (i.e., the surface of membrane and the bath), respectively, and $[B_j]$ is the concentration of the jth dissociation species carrying a charge Z_j of a buffer B.

3. Numerical method

Let the system contain only one kind of salt, KCl. Further, let it contain phosphate buffer whose dissociated molecules are singly, doubly and triply charged ions, B_1, B_2 and B_3. Then, by substituting eq. 5 into eq. 2, we find:

$$q=([H^+]_o+[K^+]_o)\Lambda-(K_w/[H^+]_o+[Cl^-]_o+[B_1]_o)\Lambda^{-1} -2[B_2]_o\Lambda^{-2}-3[B_3]_o\Lambda^{-3}+(1-\bar{Y})\rho_P+\bar{Y}\rho_{PL}=0 \quad (6)$$

Since eq. 6 is highly nonlinear in Λ, a Newton-Raphson procedure may be employed to obtain Λ. That is, we may obtain Λ using the relation,

$$\Lambda=\Lambda_t-q_t/q_t', \quad (7)$$

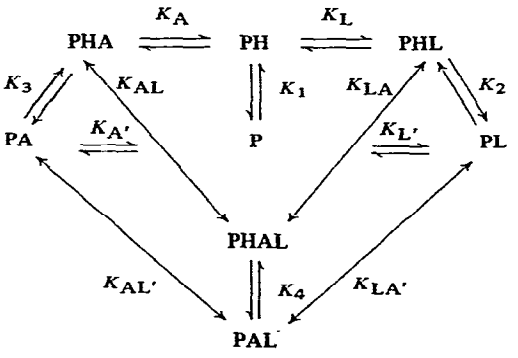
starting with an initial values Λ_t. Here q_t is obtained from eq. 6 by substituting Λ_t for Λ, and q_t' is obtained from eq. 6 by differentiating it with respect to Λ and substituting Λ_t for Λ. The differentiation of $\bar{Y}$ which appears in the expression of q' yields

$$\Lambda(d\bar{Y}/d\Lambda)=\bar{Y}(1-\bar{Y}) \times\left[\left(\frac{K_a/[H^+]}{1+K_a/[H^+]}-\frac{K_a'/[H^+]}{1+K_a'/[H^+]}\right)+Z_L\right] \quad (8)$$

where Z_L is the charge of the ligand. The total salt concentration in the bath is obtained by adding the ions coming from added salt, from buffer, and from the pH adjustment (i.e., KOH or HCl added to bring the solution to a desired pH).

There may exist an ion or molecule whose binding to a protein may induce a structural change and alter the net charge and pK_a of the protein.

The combined reaction of protein with its ligand and effector is then



(9)

We will also investigate the effect of an effector on the binding property for a ligand below.

4. Results and discussion

Fig. 1 shows how a noncooperative response (i.e., hyperpolic), a graded response (i.e., sigmoidal), and an 'all-or-none' response (i.e., hysteresis) occur in the binding function of biological membranes. As shown in this figure, if the phosphate concentration is about equal to or higher than the charge density of the membrane, the membrane behaves noncooperatively. If the phosphate buffer concentration is much lower than the net charge density of the membrane, however, an all-or-none response occurs. A graded response occurs in intermediate buffer concentrations.

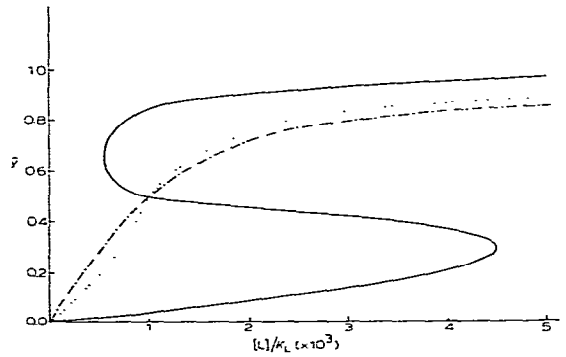


Fig. 1. The binding curve $\bar{Y}$ vs. the ligand concentration of a bath at pH 7 in the absence of added KCl and in the presence of phosphate buffer of concentrations 0.005 M (———), 0.05 M (— — —) and 0.1 M (·–·). The parameters used for the computation are $K_a = 10^{-10}$, $K'_a = 10^{-4}$ (see eq. 1 for the definition of K_a and K'_a), and the pK_a values of phosphate buffer were taken to be 2, 7 and 13. The net charge density of unliganded and liganded proteins were taken to be $\rho_P = -0.2$ M and $\rho_{PL} = 0.2$ M, respectively. The ligand carries no charge.

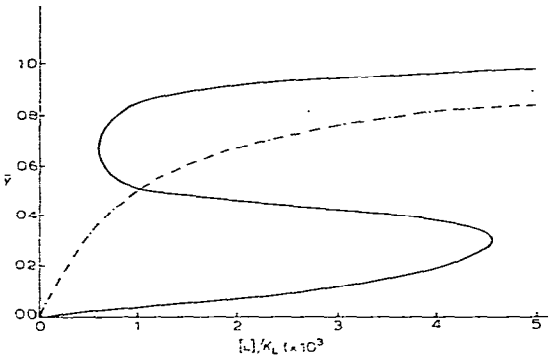


Fig. 2. Dependence of the binding curve on the charge density of the membrane at pH 7 in the presence of 5 mM phosphate buffer. Here, the external condition and the parameters used for the computation are the same as the 5 mM buffer case in Fig. 1. (·———) Computed with $\rho_P = 0.2$ M and $\rho_{PL} = +0.2$ M; (— — —) computed with $\rho_P = -0.02$ M and $\rho_{PL} = +0.02$ M; (·–·) computed with $\rho_P = -0.002$ M and $\rho_{PL} = +0.002$ M.

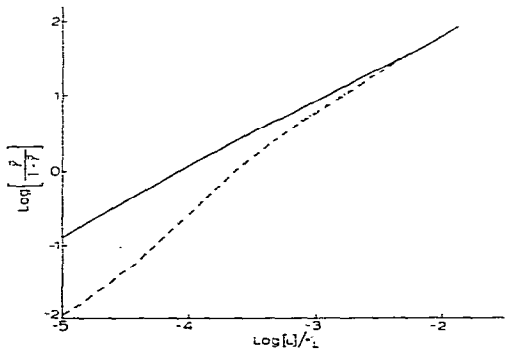


Fig. 3. Hill plot showing various types of cooperativity. Here, the external condition and the parameters used for computation are the same as the solid line in Fig. 2. Charge densities: (———) $\rho_P = +1.0$ M and $\rho_{PL} = +0.2$ M; (— — —) $\rho_P = +0.2$ M and $\rho_{PL} = +0.2$ M; (—·—) $\rho_P = 0.0$ M and $\rho_{PL} = +0.2$ M.

In Fig. 2 we show how the charge density influences the shape of the binding curve. An external condition and the parameters used for the solid line are the same as those of the all-or-none case in Fig. 1. Note that if the charge density is decreased 10-fold, the binding curve becomes a graded shape (i.e., a sigmoidal shape) and if the charge density is further decreased 10-fold, the binding curve exhibits a noncooperative shape (i.e., a hyperbolic shape). Thus, the charge density strongly influences the cooperativity of the biological membranes.

Fig. 3 shows the relationship between the Hill plot and the net charge of the liganded and unliganded proteins bound to the membrane. Here, the net charge density of unliganded and liganded proteins are, respectively, taken to be +1.0 and +0.2 M for the solid, line +0.2 and +0.2 M for the dashed line, and 0.0 and +0.2 M for the dashed-dotted line, respectively. Note that the membrane is not cooperative if the charge densities are the same for the liganded and unliganded proteins. If the charge density of the unliganded state is higher than that of the liganded state, then negative cooperativity occurs. The positive cooperativity occurs if the charge density is higher for the liganded state. Thus, this figure shows how negative-, positive-, or noncooperativity occurs in the biological membrane depending on the charge density of unliganded and liganded states.

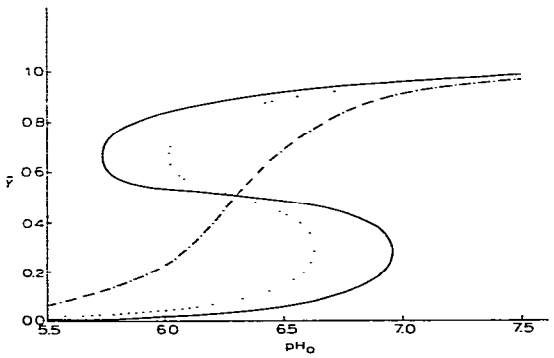


Fig. 4. Effects of salt on $\bar{Y}$ vs. pH_0 plot. Here, the parameters used for the computation are the same as the solid line in Fig. 2 with the exception of the KCl salt concentration. The ligand and phosphate buffer concentrations are fixed at 5 mM each. Concentration of KCl: 0.001 M (———), 0.01 M (— — —) and 0.1 M (— · —).

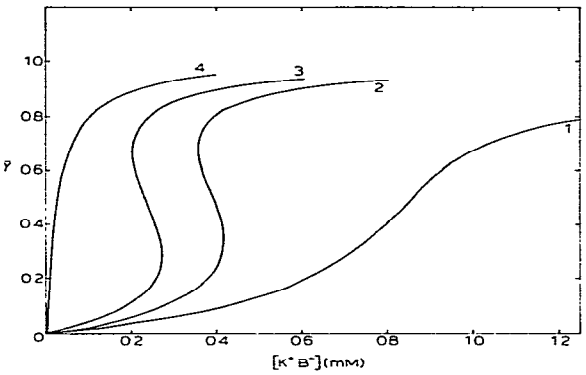


Fig. 5. Effects of an activator on $\bar{Y}$ in the presence of 2 mM buffer ($pK_a = 2$, 7 and 13) and in the absence of added salt at pH 7. Here, a ligand is a salt (K^+B^-) whose anion is a buffer with $pK_a = 2$ and 7. The parameters used for computation are $K_1 = 10^{-10}$, $K_2 = 10^{-4}$, and $\rho_P = -0.01$ M, $\rho_{PL} = +0.00$ M and $K_L = 0.1$ M. It is assumed that only a doubly charged anionic form of the ligand can bind to the protein. Other parameters used for this computation are: $K_3 = 10^{-4}$, $K_4 = 1$, $K_L = K_{LA} = K_{AL} = K_A$ and $\rho_{PL} = \rho_{PAL} = \rho_{PA}$. An activator is assumed to be a salt (K^+B^-) whose anion is a buffer with $pK_a = 2$ and 6, and only B^{2-} is assumed to bind to the protein. Curves: (1) 0 mM, (2) 0.05 mM, (3) 0.1 mM, (4) 0.5 mM activator.

In Fig. 4, we show effects of salt on the $\bar{Y}$ vs. pH_0 curve. As shown in this figure, $\bar{Y}$ depends strongly on the pH of the medium and also on the salt concentration. Note that at high salt concentration $\bar{Y}$ exhibits a positive cooperativity and at low salt concentration $\bar{Y}$ exhibits an all-or-none response.

Fig. 5 shows effects of an activator on the ligand binding curve at pH 7 in the presence of 2 mM phosphate buffer. Here, it is assumed that a ligand is a salt (K^+B^-) whose anions is a buffer having $pK_a = 2$ and 7 and that only a doubly charged anionic form can bind to the protein. Note that the binding curve becomes hyperbolic at a high activator concentration, all-or-none in the intermediate values of the activator concentrations, and sigmoidal in the absence of the activator concentration.

Excitable membranes exhibit complex cooperative phenomena, in that their biological activities

depend upon threshold concentrations of regulatory ligands [4]. That is, the binding curve of an excitable membrane exhibits an all-or-none response with certain regulatory ligands. In the past, the all-or-none response was theoretically explained by Changeux et al. [3,4] by using a model which makes use of the presence of regulatory sites, the capacity of the macromolecules to undergo reversible changes of conformation, and the organization of the macromolecular repeating units into highly ordered structures. It is, however, well known that the proteins bound to biological membranes are not highly ordered and are not necessarily allosteric proteins. For example, acetylcholinesterase, which is a key enzyme in excitable membranes, is not an allosteric enzyme.

The cooperativity proposed in this paper is based on the microenvironmental effect created by the charged proteins on the surface of the membrane. When the proteins on the membrane are negatively charged, the pH of the microenvironment is lower than that of the solution. When the proteins are positively charged, however, the pH of the membrane surface is higher than that of the solution. Since the binding constant of most of the regulatory proteins depends on pH (i.e., they possess Bohr protons), it is not surprising that excitable membranes exhibit complex cooperative phenomena.

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