Regulation of surface-membrane enzymes by lipid ordering

A model based on allosteric transition theory

Pierre R. BLANQUET

Institut de Recherches sur le Cancer et Unité 124 de l'I.N.S.E.R.M., B.P. 311, 59020 Lille Cédex, France

(Received 29 December 1982/Accepted 9 May 1983)

This paper shows for the first time that effects of lipid ordering on the activity of surface-membrane enzymes can be interpreted in terms of the two-state allosteric transition theory. In this mathematical modelling, the conversion of an active molecule into a catalytically inactivated one of a given enzyme can be directly triggered by a change in the order parameter of lipids. Depending on the lipid order, the tendency for this conversion is either independent of the state of other enzyme molecules or co-operatively aided through intermolecular interactions that in turn depend on lipid ordering. The theory so developed is then applied to ($\text{Na}^+ + \text{K}^+$)-dependent adenosine triphosphatase (EC 3.6.1.3). On comparison of the relevant theoretical expression and published measurements, the fit to experimental results is shown to support the model. Furthermore, the model accounts for much of the data in the literature of the behaviour of this enzyme.

Membrane lipids have been shown to play an important role in the regulation of the activity of membrane-bound enzymes (Kimelberg, 1977; Sandermann, 1978; Ottolenghi, 1979; Farias, 1980; Sandermann, 1982). In particular, conclusive evidence has been gathered showing that the fluidity of the lipid bilayer is a physiological regulator for the co-operative behaviour of some surface-membrane enzymes (Ottolenghi, 1979; Farias, 1980; Sandermann, 1982). Clearly, effects on enzyme activity would become manifest if local or general conformational changes were involved in the rate-determining process of the overall reaction. However, there have as yet been no general theories aimed at rationalizing and accounting for the numerous findings concerning the dependence of the enzyme activity on physical state of lipids.

My intention here is to construct, in a simple way as possible, a plausible general model of the regulation of surface-membrane enzymes by lipid acyl-chain ordering on the basis of the two-state allosteric transition theory (Changeux *et al.*, 1967; Blanquet, 1978). In the General theory section below I describe, in terms of equilibrium statistical mechanics, how the lipid ordering may take part in controlling the transition between a catalytically active structural state and an inactivated state of a given enzyme. Since the most widely studied surface-membrane enzyme with regard to lipid-bilayer organization has been the ($\text{Na}^+ + \text{K}^+$)-

dependent ATPase (EC 3.6.1.3) (Kimelberg, 1977; Sandermann, 1978; Ottolenghi, 1979; Farias, 1980; Jørgensen, 1982), I then test the validity of the relevant theoretical expression by comparison with experimental results obtained with this enzyme. I also evaluate approximately some terms appearing in the formal expression by using published results, and compare the calculations with measurements performed on the ($\text{Na}^+ + \text{K}^+$)-dependent ATPase (Sinensky *et al.*, 1979).

General theory

As a theoretical prototype consider an amorphous mosaic of sparsely distributed protomers of a given enzyme making contact through a lipid boundary layer. The system is represented as a continuum of M repeating lipoprotein subunits (Fig. 1). The order parameter p of lipid acyl chains can be interpreted in terms of a simple geometric picture (Knowles *et al.*, 1976). If θ_i denotes the momentary angle between the direction of an acyl-chain segment and the normal to the bilayer, then the order parameter calculated separately for the carbon atom site i is related to the average orientation according to:

$$p = (1/2)(3\langle \cos^2 \theta_i \rangle - 1) \quad (1)$$

where the angular brackets indicate the time average. With this definition, the thermodynamic

Fig. 1. Distribution of protomers of $(\text{Na}^+ + \text{K}^+)$ -dependent ATPase and lipids adopted for simplification. Each surface membrane subunit is taken to contain a central (hatched) protomer of enzyme and a hexagonal boundary region of lipids.

average p corresponds to order parameter measured by e.s.r. and n.m.r. spectroscopy (Seeling & Niederberger, 1974).

Consider that the enzyme protomers can exist in either catalytically active state X or state Y devoid of enzyme activity. The hypothesis is that the generation of enzyme activity is an all-or-none process. The two states are in equilibrium, and each allosteric protomer is subject to equilibrium constraint (Changeux *et al.*, 1967; Blanquet, 1978). Consider also, for example, that the transition from state X to state Y can be directly triggered by an increase in the degree of lipid ordering. It is reasonable to postulate that the work required to produce the structural changes in the acyl-chain array is linearly related to the order parameter p . The argument is based on thermodynamic con-

siderations. As a consequence, the total work Γ done on the protomers may be expressed as:

$$\Gamma = (\gamma_X \cdot M_X + \gamma_Y \cdot M_Y) p \quad (2)$$

where M_X and M_Y are the subunits of the two types. The constants γ_X and γ_Y may be regarded as being the work done on each protomer X and Y respectively when the probability of acyl chains being in the ordered state is 1 ($p = 1$).

One can then make use of pertinent basic thermodynamic equations to calculate the properties of the system. With eqn. (2) available, where $M_X = M - M_Y$, we find:

$$dE = T \cdot dS - p(\gamma_Y - \gamma_X) \cdot dM_X + (\mu + \gamma_Y p) \cdot dM \quad (3)$$

$$dG = -S \cdot dT - p(\gamma_Y - \gamma_X) \cdot dM_X + (\mu + \gamma_Y p) \cdot dM \quad (4)$$

where E is the energy of the system, G is the free energy of the transition from state X to state Y, T and S have their usual meanings, and μ is the chemical potential of a subunit. Mathematically, this problem is not essentially different from that of incompressible elastic systems (Hill, 1960). Let $\xi_X(T, p)$ and $\xi_Y(T, p)$ be the partition functions of state X and state Y respectively. Recall that the ratio of ξ_X to ξ_Y , the respective statistical weights of state X and state Y, reflects the relative stability of the two kinds of protomers (Hill, 1960). The canonical ensemble partition function of the system is:

$$Q(M_X, M, T) = \frac{M! \cdot \xi_X^{M_X} \cdot \xi_Y^{M-M_X}}{M_X! \cdot (M-M_X)!} \quad (5)$$

Let us write also the well-known thermodynamic relation:

$$G = -\frac{1}{\beta} \cdot \ln Q \quad (6)$$

with $\beta = N/RT$ and $R/N =$ Boltzmann constant. Since M is a very large number, the Stirling's approximation gives:

$$-\beta \cdot G = \ln Q = M \cdot \ln M - M_X \cdot \ln M_X + M_X \cdot \ln \left(\frac{\xi_X}{\xi_Y} \right) + M \cdot \ln \xi_Y - (M - M_X) \cdot \ln (M - M_X) \quad (7)$$

siderations. On the one hand, from the first postulate applicable to statistical-mechanical ensembles (Hill, 1960), one may replace the instantaneous average of the fraction of work required to trigger the conversion of a number of acyl chains in a given state by the average over the instantaneous fractions of work for each acyl chain, i.e. by the probability of observing each chain in this state. On the other hand, the order parameter has been shown to be linearly related to the probability of an acyl-chain segment being in one of the possible isomeric states (Seeling & Seeling, 1974). Let us still assume that the total work done on the M protomers is a large component the work required to produce the complementary structural changes in the acyl

Let us now perform the derivations:

$$-\beta \left(\frac{\partial G}{\partial M_X} \right)_{M, T} = \left(\frac{\partial (\ln Q)}{\partial M_X} \right)_{M, T} \quad (8)$$

We have:

$$\beta(\gamma_Y - \gamma_X) p = \ln \left(\frac{y \cdot \xi_X}{x \cdot \xi_Y} \right) \quad (9)$$

in which $x = M_X/M$ is the equilibrium fraction of X protomers and $y = M_Y/M$ is the equilibrium fraction of Y protomers. Therefore eqn. (9) may be expressed as:

$$\frac{x}{y} = \exp[\beta(u_Y - u_X)] \cdot \exp[-\beta(\gamma_Y - \gamma_X) p] \quad (10)$$

in which u_x is the average interaction free energy between any lipoprotein subunit containing a protomer X and its nearest neighbours (u_y has a similar signification for Y); $u_y - u_x$ is the free energy required to promote the transition from state X to state Y when a fraction of protomers is already in the state Y.

Let us express $u_y - u_x$ by taking nearest-neighbour interactions between repeating lipoprotein subunits into account. Conceivably, interactions between nearest-neighbour subunits result from protein-protein, lipid-protein and lipid-lipid interactions, all of which should be influenced by the degree of lipid ordering (Marčelja, 1976). Let $w_{xx}(p)$, $w_{yy}(p)$ and $w_{xy}(p)$ be the nearest-neighbour interaction free energies. Let us also assume that each subunit has n nearest neighbours. According to the Bragg-Williams (mean-field) approximation (Hill, 1960; Strässler & Kittel, 1965), $u_y - u_x$ can be obtained by taking a random distribution of neighbours rather than their stochastic or fluctuating distribution:

$$u_y - u_x = n[\delta(p) - y \cdot \varepsilon(p)] \quad (11)$$

with

$$\delta(p) = w_{xy} - w_{xx}$$

and

$$-\varepsilon(p) = w_{xx} - 2w_{xy} + w_{yy}$$

On the other hand, because the activity of enzymes is generally measured under conditions in which it represents the maximal reaction velocity, the activity v is given by the relation $v = kx$, in which k is the pseudo-first-order rate constant for the reaction. Finally, then eqn. (10) becomes:

$$\ln \left(\frac{v}{k-v} \right) = \ln \{ \Omega(p) \cdot [\Lambda(p)]^{k-v} \} - L \cdot p \quad (12)$$

where $\Omega(p) = \exp[\beta n \cdot \delta(p)]$, $\Lambda(p) = \exp[-(\beta n/k) \cdot \varepsilon(p)]$ and $L = \beta(\gamma_y - \gamma_x)$. $\Omega(p)$ expresses the equilibrium constraint of a subunit in an environment of X subunits, $\Lambda(p)$ encompasses the character of the equilibrium constraint due to interactions between nearest-neighbour subunits, and the last term expresses an additional equilibrium constraint due to change of lipid ordering.

The lattice considered here possesses properties of translational symmetry. Concerning the case in which the equilibrium is shifted from state Y to state X for increasing values of p , therefore, no particular analysis has been, or need be, made. In this case it can be found easily that:

$$\ln \left(\frac{k-v}{v} \right) = \ln \{ \Omega(p) \cdot [\Lambda(p)]^v \} - L \cdot p \quad (13)$$

Eqns. (12) and (13) can be used to compute v as a function of p . The relative contributions of non-co-operative and co-operative responses are determined by $\Omega(p)$ and $\Lambda(p)$. Attempts might be made to calculate $\Omega(p)$ and $\Lambda(p)$ exactly from various models concerning the intermolecular forces. However, because of the mathematical difficulty of the problem, this rigorous approach would not be fruitful in practice. Moreover, each enzyme should exhibit a particular behaviour in this response. Hence approximate methods are clearly needed. In the Results and discussion section a simple approximate analytical solution is presented for $(\text{Na}^+ + \text{K}^+)\text{-dependent ATPase}$.

Results and discussion

Sinensky *et al.* (1979), using the e.s.r. probe technique, gave a description of the dependence of $(\text{Na}^+ + \text{K}^+)\text{-dependent ATPase}$ activity on the order parameter. These authors showed that increasing cholesterol content in surface membrane causes a decrease in this activity. I have compared these data with values calculated from eqn. (12). Before describing the model calculation, I first define the following two statements. 1. Each allosteric protomer of $(\text{Na}^+ + \text{K}^+)\text{-dependent ATPase}$ can take either a state Y, which prevents transition between the Na^+ - and the K^+ -induced form of the enzyme, or a state X, which must have a 'ping-pong' kinetic mechanism (Jørgensen, 1982) with respect to the alkali-metal cation substrates. 2. Cholesterol plays a physical role in modulating the lipid ordering, thereby affecting the $(\text{Na}^+ + \text{K}^+)\text{-dependent ATPase}$ activity. Studies have shown that the lipid ordering, but not a direct chemical effect of cholesterol, determines the activity of this enzyme when the cholesterol content varies (Sinensky *et al.*, 1979). It is worth emphasizing, nevertheless, that a number of subtle effects have been observed in phospholipid/cholesterol mixtures, which show monotectic behaviour. Thus there is evidence that cholesterol can interact selectively with certain lipid species, especially negatively charged phospholipids (Demel *et al.*, 1977). This is pertinent to the present assertion, as the activity of $(\text{Na}^+ + \text{K}^+)\text{-dependent ATPase}$ depends also critically on association with some specific lipids such as acidic phospholipids (Kimelberg, 1977; Sandermann, 1978; Brotherus *et al.*, 1980; Schmalzing & Kutschera, 1982). However, phospholipids of cellular membranes show no monotectic behaviour, and it is questionable whether these results have any biological relevance. Furthermore, re-examinations of some phospholipid/cholesterol systems did not reveal any preferential affinity of cholesterol for phospholipids (Blume, 1980). It is reasonable therefore to adopt the commonly used first approximation that cholesterol modulates only bilayer fluidity.

The problem was to find an explicit expression for $\Omega(p)$ and $\Lambda(p)$. Comparative data furnish evidence for the allosteric nature of the inhibition by F^- ions of the $(Na^+ + K^+)$ -dependent ATPase (Farias *et al.*, 1970; Bloj *et al.*, 1973*a,b*). It was demonstrated that the relationship between the decrease of co-operative transitions for the inhibition by F^- and the increase of the membrane double-bond index could be interpreted as the change of interactions between the allosteric subunits of the enzyme, due to the decrease in the degree of membrane lipid ordering. Since the product $\Omega(p) \cdot [\Lambda(p)]^{k-v}$ encompasses the effects of the ordering change on nearest-neighbour interactions, attempts were made to evaluate $\Omega(p) \cdot [\Lambda(p)]^{k-v}$ from the correlations between the order parameter and co-operativity of the $(Na^+ + K^+)$ -dependent ATPase as previously reported (Farias *et al.*, 1970; Bloj *et al.*, 1973*a,b*; Sinensky *et al.*, 1979).

Let l be effector F^- for convenience endowed only with affinity towards state Y. One has the following equilibria:

and relations:

$$\frac{M_Y}{M} = \frac{y_1}{x_1 + y_1 + y_2} \quad (14)$$

$$K = \frac{y_1[l]}{y_2} \quad (15)$$

where K is a microscopic dissociation constant. On the other hand, the binding data plotted as $\ln[(1 - y_1)/y_1]$ versus $\ln[l]$ give h , the Hill coefficient (Farias *et al.*, 1970). Furthermore, the Hill equation constant, K_H , can be defined by the constant K , such as $K_H = K^h$ (Dahlquist, 1978). Since eqn. (12) may be expressed as:

$$\frac{v_1}{k - v_1} = \Omega(p) \cdot [\Lambda(p)]^{k-v_1} \cdot \exp(-L \cdot p_1)$$

we obtain, from eqns. (14) and (15):

$$\Omega(p) \cdot [\Lambda(p)]^{k-v_1} = \left[\frac{v_1}{k - v_1} - \frac{1}{K_H^{1/h}} \cdot \left(\frac{v_1}{k - v_1} \right)^{1/h} \right] \cdot \exp(L \cdot p_1) \quad (16)$$

Let p_0 be the order parameter that is required to promote an equilibrium displacement similar to that obtained when F^- binds to state Y for $p = p_1$. Eqn. (12) then becomes:

$$\ln \left(\frac{v_1}{k - v_1} \right) = \ln \{ \Omega(p) \cdot [\Lambda(p)]^{k-v_1} \} - L \cdot p_0 \quad (17)$$

Therefore, from eqns. (16) and (17):

$$\ln \left(\frac{v_1}{k - v_1} \right) = \ln \left[\frac{v_1}{k - v_1} - \frac{1}{K_H^{1/h}} \cdot \left(\frac{v_1}{k - v_1} \right)^{1/h} \right] - L \cdot (p_0 - p_1) \quad (18)$$

Fig. 2. Relationship between values of the Hill coefficient (h) for inhibition by F^- of $(Na^+ + K^+)$ -dependent ATPase and the lipid order parameter (p)

The results are obtained by plotting the cholesterol/phospholipid ratio both against values of h and p as previously determined (Kroes *et al.*, 1972; Bloj *et al.*, 1973*a,b*; Sinensky *et al.*, 1979).

Eqn. (18) can provide graphic prediction of co-operative variations of $v = v_1$ as a function of $p = p_0 - p_1$ when h varies on condition that there is some predictable relation between p and h . Since the membrane lipid fluidity is linearly related to the Hill coefficient for the inhibition by F^- of the $(Na^+ + K^+)$ -dependent ATPase (Bloj *et al.*, 1973*a*), values of h as a function of the cholesterol/phospholipid (C/P) ratio were graphically determined with two values of h (2 and 3.6) and their corresponding C/P ratios (0.95 and 1.8). These were estimated from the previously reported data for erythrocyte membrane-

bound enzymes (Kroes *et al.*, 1972; Bloj *et al.*, 1973*b*). In addition, the linear relationship between the values of p and the C/P ratios was estimated from the results obtained by Sinensky *et al.* (1979). A straight line drawn through the experimental points yielded $p = 0.672$ and $p = 0.712$ when C/P = 0.420 and C/P = 1.485 respectively. It was therefore possible to calculate approximately the variations of h knowing the values of p (Fig. 2).

Finally, I compared the calculated and the measured (Sinensky *et al.*, 1979) values of the $(\text{Na}^+ + \text{K}^+)$ -dependent ATPase activity as a function of the order parameter when the value of h increased from 1.2 to 2.4 in the range of p from 0.676 to 0.700. As shown in Fig. 3, the variation of the calculated values of v coincides with the experimental points when there is some adequate relation between the arbitrary values of constants L and k .

In the special case $h = 1$ (for $p \leq 0.672$ in Fig. 2), on the basis of the proposed theory the state changes of $(\text{Na}^+ + \text{K}^+)$ -dependent ATPase are no longer governed by co-operative transitions. In other words, the tendency for the conversion of a particular active molecule into a catalytically inactivated one of the enzyme is independent of the state of other enzyme molecules [i.e. $\Lambda(p) = 1$]. On the other hand, owing to the relative strength of protein-protein interactions in comparison with the lipid-protein interactions, the decrease of the order parameter is shown to lead to a dominant protein-

protein force (Marčelja, 1976). As a consequence, it should be considered that the nearest-neighbour interaction free energies become less and less dependent on lipid ordering when the order parameter decreases [i.e. $\Omega(p)$ becomes approximately constant]. When p decreases for $h = 1$, therefore, eqn. (12) tends to express more and more an exponential increase of the enzyme activity. This is in agreement with the fact that the measured activity shows a marked increase when $p = 0.663$ (Fig. 3).

Care has to be exercised in drawing conclusions from the adequacy outlined above. Indeed the original data, considered *in toto*, were previously interpreted as indicating an exponential dependence of the activity on the order parameter (Sinensky *et al.*, 1979) (Fig. 3). This means that the agreement with experimental results can be obtained by more than one model. Only accurate additional measurements could distinguish between the alternatives. However, the present model is proposed with the added refinement of including the possible co-operative behaviour of the enzyme when one holds $p = \text{constant}$. In terms of the theory of membrane allosteric transitions (Changeux *et al.*, 1967; Blanquet, 1978), this means that the co-operative transition between the two hypothetical states of the enzyme protein can be also triggered by specific ligands; this is apparently the case for the inhibition by F^- ions. Therefore the fact that the present model gives a rather good agreement with the experimental results but also accounts for more complicated situations is indicative that it may be a correct representation of the molecule.

In addition to the above reasons, some more cognitive arguments are also in favour of the model considered here. In the last few years the usual way used to explain the influence of lipids on membrane enzymes such as $(\text{Na}^+ + \text{K}^+)$ -dependent ATPase has been based on the assumption that multiple lipid-binding-site kinetics can lead to co-operativity in enzyme activation (Ottolenghi, 1979; Sandermann, 1982). However, although these theories consider lipid-protein interactions to describe the relationship between the amount of lipids added to detergent-solubilized systems and enzyme activity, they do not take into account the fact that membrane bilayer lipids provide a continuum with a fluidity that makes both short-range and relatively extended interactions possible between protein-protein and lipid-lipid molecules (Blanquet, 1978). Furthermore, the explanation of the influence of lipids on the enzyme activity only in terms of lipid-protein binding equilibria requires the postulate of numerous different highly specific binding sites and/or of complex mutual-exclusion mechanisms for activators, inhibitors and substrates. From such a point of view, in particular, it would be rather difficult to imagine how membrane lipids are regulators for the

Fig. 3. Dependence of the activity (v) of $(\text{Na}^+ + \text{K}^+)$ -dependent ATPase on the lipid order parameter (p) —, Theoretical curve calculated from eqn. (18) for different values of h corresponding to values of p in the range from 0.676 to 0.700 (Fig. 2), for $K_H = 1.3 \text{ mM}$ (Bloj *et al.*, 1973b) and for arbitrary values of k and L ($k = 1$; $L = 0.506$); ----, exponential curve drawn through the experimental points (O) in accordance with the interpretation made by Sinensky *et al.* (1979).

allosteric inhibition by F^- of the $(Na^+ + K^+)$ -dependent ATPase.

The attractiveness of the present much more unconstrained view is that, by contrast, it fits rather well with the numerous data about the behaviour of effectors and substrates. It can also include the possibility that the motional-freedom regulation of enzyme molecules by lipid ordering could be through protein-lipid binding-site kinetics. Indeed, the protein-lipid association rate constants are limited by the rate of lateral diffusion of lipids, which in turn depends on bilayer physical state (Lee *et al.*, 1973; Sandermann, 1982). In addition, this view allows one to consider, as assumed by Monod *et al.* (1965) for the V allosteric systems, that the two types of allosteric protomers have the same affinity towards Na^+ and K^+ and differ only in their catalytic activity. On this basis, for example, it is easy to understand why titration with K^+ alone gives hyperbolic curves, whereas in the presence of Na^+ the titration curves become sigmoid-shaped (Hegvyvary & Jørgensen, 1981). This would mean that K^+ exerts an effect on the equilibrium displacement only if the two types of protomers differ in their catalytic activity.

Finally, I must point out that an extrapolation of this mathematical modelling to other membrane-bound enzymes might prove possible. Although only a few membrane systems are known to exhibit a co-operative behaviour (Ottolenghi, 1979; Farias, 1980; Sandermann, 1982), the present theory does offer a quite general interpretation of the dependence of enzyme kinetics on acyl-chain mobility, whether it is co-operative or not.

References

- Blanquet, P. R. (1978) *J. Theor. Biol.* **70**, 345–399
- Bloj, B., Morero, R. D., Farias, R. N. & Trucco, R. E. (1973a) *Biochim. Biophys. Acta* **311**, 67–79
- Bloj, B., Morero, R. D. & Farias, R. N. (1973b) *FEBS Lett.* **38**, 101–105
- Blume, A. (1980) *Biochemistry* **19**, 4908–4913
- Brotherus, J. R., Jost, P. C., Griffith, O. H., Keana, J. F. W. & Hokin, L. E. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 272–276
- Changeux, J. P., Thiéry, J., Tung, Y. & Kittel, C. (1967) *Proc. Natl. Acad. Sci. U.S.A.* **57**, 335–341
- Dahlquist, F. W. (1978) *Methods Enzymol.* **68**, 270–299
- Demel, R. A., Jansen, J. W. C. M., van Dijck, P. W. M. & van Deenen, L. L. M. (1977) *Biochim. Biophys. Acta* **465**, 1–10
- Farias, R. N. (1980) *Adv. Lipid Res.* **17**, 251–282
- Farias, R. N., Goldemberg, A. L. & Trucco, R. E. (1970) *Arch. Biochem. Biophys.* **139**, 38–44
- Hegvyvary, C. & Jørgensen, P. L. (1981) *J. Biol. Chem.* **256**, 6296–6303
- Hill, T. L. (1960) *Introduction to Statistical Thermodynamics*, 2nd edn., Addison-Wesley, New York
- Jørgensen, P. L. (1982) *Biochim. Biophys. Acta* **694**, 27–68
- Kimelberg, H. K. (1977) in *Dynamic Aspects of Cell Surface Organization* (Poste, G. & Nicolson, G. L., eds.), vol. 3, pp. 205–293, North-Holland, Amsterdam, New York and Oxford
- Knowles, P. F., Marsh, D. & Rattle, H. W. E. (1976) *Magnetic Resonance of Biomolecules: An Introduction to the Theory and Practice of NMR and ESR in Biological Systems*, pp. 194–196, John Wiley and Sons, London, New York, Sydney and Toronto
- Kroes, J., Ostwald, R. & Keith, A. (1972) *Biochim. Biophys. Acta* **274**, 71–74
- Lee, A. G., Birdsall, N. J. M. & Metcalfe, J. C. (1973) *Biochemistry* **12**, 1650–1659
- Marčelja, S. (1976) *Biochim. Biophys. Acta* **455**, 1–7
- Monod, J., Wymann, J. & Changeux, J. P. (1965) *J. Mol. Biol.* **12**, 88–118
- Ottolenghi, P. (1979) *Eur. J. Biochem.* **99**, 113–131
- Sandermann, H. (1978) *Biochim. Biophys. Acta* **515**, 209–237
- Sandermann, H. (1982) *Eur. J. Biochem.* **127**, 123–128
- Schmalzing, G. & Kutschera, P. (1982) *J. Membr. Biol.* **69**, 65–76
- Seeling, J. & Niederberger, W. (1974) *J. Am. Chem. Soc.* **96**, 2069–2072
- Seeling, A. & Seeling, J. (1974) *Biochemistry* **13**, 4839–4845
- Sinensky, M., Pinkerton, F., Sutherland, E. & Simon, F. R. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4893–4897
- Strässler, S. & Kittel, C. (1965) *Phys. Rev. A* **139**, 758–760