Biochimica et Biophysica Acta, 728 (1983) 179–185
Elsevier Biomedical Press

BBA 71545

ON THE RATE OF ASSOCIATION AND DISSOCIATION PROCESSES OF INTRINSIC MEMBRANE PROTEINS

CARLOS GUTIERREZ-MERINO

Departamento de Fisiología, Facultad de Medicina, Universidad de Extremadura, Badajoz (Spain)

Key words: Protein-lipid interaction; Membrane dynamics; Protein agregation; Membrane protein; Transport

An analysis of the possible energy barriers to be overcome during processes of association and dissociation of intrinsic membrane proteins within a biological membrane is presented. The lack of necessary thermodynamic data proscribes accurate quantitative calculation. Nevertheless, by introducing a simple model, in which a putative common transition state is proposed for both processes, it becomes possible to predict qualitatively that the dynamic equilibrium between different aggregation states of membrane proteins might come about very slowly within the membrane (it may even be blocked under appropriate conditions). This is an inference that arises independently from recent experimental data (Swanson, M.S., Quintanilla, A.T. and Thomas, D.D. (1980) J. Biol. Chem. 255, 7494–7502, and Gutierrez-Merino, C. and Biltonen, R.L., unpublished data) involving cytochrome *c* and Ca^{2+}-ATPase reconstituted systems, respectively. On the basis of this analysis there arises the possibility of unifying rationalization for the regulation of various membrane-mediated process such catalytic activity, transport processes, hormone responsiveness, and so forth, by physicochemical factors.

Introduction

Protein-protein interactions between different polypeptides of intrinsic membranes have been suggested to play an important role in many membrane-mediated processes such as ionic channel formation [1], active transport [2], energy transduction [3,4], enzymatic activity of membrane-bound enzymes [5], hormone-receptor interactions [6,7], cell membrane fusion [8], gap junction formation [9] and transmission of transmembrane messages [10] *.

Reconstitution of purified (detergent-solubilized) intrinsic membrane proteins into lipid membranes has constituted a current approach to the determination or confirmation of their functional properties and to the understanding of their regulation: see, for example, Refs. 11–13. However, large variability in the functional properties of reconstituted membrane particles using essentially similar protocols, has also been observed repeatedly [14–18].

Recently, Swanson et al. [19] have demonstrated that the physical properties of cytochrome *c* oxidase reconstituted systems appear to be dependent upon the state of the protein in the solubilized lipid-protein/detergent mixture prior to the removal of the detergent. Results obtained in our laboratory, using in this case reconstituted Ca^{2+}-ATPase of sarcoplasmic reticulum from rabbit skeletal muscle (unpublished data), have proven that this dependence is also valid for these systems and, thus, that it might be of general validity.

Although effects arising from changes in the

* Only representative references for each process have been chosen.

Abbreviations: DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DOPC, dioleylphosphatidylcholine; DSPC, distearylphosphatidylcholine; PE, phosphatidylethanolamine; DMPE, dimyristoylphosphatidylethanolamine; DPPE, dipalmitoylphosphatidylethanolamine.

0005-2736/83/0000–0000/$03.00

size distribution pattern of the reconstituted particular material cannot be definitively excluded from these studies, the data of Swanson et al. and ourselves strongly suggest that the state of aggregation of the protein within the reconstituted membrane reflects the state of aggregation of the protein in the solubilized mixture prior to detergent removal *.

This is quite an interesting result, because it implies that either association or dissociation of intrinsic membrane proteins within the membrane, or both, have to be rather slow processes. It is also and exciting result, because of its potential usefulness in the study of the dependence of the functional properties of intrinsic membrane proteins upon their aggregation state. However, no theoretical basis for this fact is evident in the literature. These reasons moved us to develop semi-quantitative calculations to look for the thermodynamic basis of the phenomena. The analyses presented here allow us to account for it qualitatively. Their implications with respect to the regulation of some relevant functions mediated by membrane proteins are briefly analyzed.

Part of this work has been presented at the 10th Meeting of the Spanish Biochemical Society, held at Santander, Spain (September, 1982).

The model

Consider the schematic diagram shown in the Fig. 1 as a representation of a typical association-dissociation equilibrium. A putative and simplified transition state has been introduced in the equilibrium. The diagram is intended to be a view of the membrane surface from a direction normal to it. The large circles represent cross-sections of the membrane protein subunits and the small ones cross-sections of the lipid molecules.

Let us now analyze association and dissociation processes separately. In what follows, the work to be applied against protein-protein interactions when displacing the equilibrium from I to III, or vice versa, will be initially disregarded and later explicitly considered on a qualitative basis **. Two main reasons justify this approach: (i) the lack of experimental data to make even a rough estimation of the work and (ii) its a priori logical variability from protein to protein.

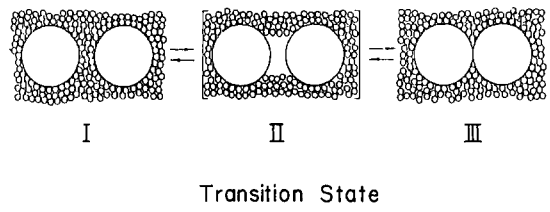

Fig. 1. Schematic diagram to illustrate the proposed model. See the text for additional explanations.

(A) Dissociation processes

For these processes, in going from the initial state (state III of the diagram) to the transition state, net work against the internal laternal pressure of the membrane has to be done. Considering recent findings of Conrad and Singer [21,22] and the work of Demel et al. [23], a value of 33 ± 2 dyn/cm for this internal lateral pressure, σ_i, will be assumed. On the other hand, the minimum separation, d, between the protein subunits in the transition state can be estimated as approx. 10 Å, which is the cross-sectional dimension of a lipid molecule [24] §. The entry of the second set of lipid

* It is to be noted that, in a paper published after this paper was first submitted for publication, Koningsberg [20] has reported that Ca^{2+}-ATPase from sarcoplasmic reticulum, once aggregated, does not seem to disperse upon incorporation into vesicles, an observation which is in line with those mentioned in this paper.

** The free energy terms due to alterations of protein-protein interactions when displacing the equilibrium from I to III or vice versa (see the diagram of Fig. 1) can be expressed more specifically as changes in the strength of these interactions, because of the change of the microscopic dielectric constant of the medium in between protein subunits when moving from I to II (association processes) and work to be done against attractive protein-protein interactions when moving from III to II (dissociation processes).

§ The separation distance, d, used in these calculations (one phospholipid headgroup diameter) can be questioned by arguing that a certain critical distance below this value could be sufficient. One should realize, however, that in this case it is necessary to take into consideration the energetic terms associated with, at least, lipid distortion as a part of the energetic barrier of the transition state. The outcome of

TABLE I

ACTIVATION ENERGIES FOR ASSOCIATION-DISSOCIATION PROCESSES CONFORMING TO THE MECHANISM PROPOSED

The protein diameters assume either a spherical shape for the protein molecule or that the protein is a cylinder whose cross-section at a plane parallel to the membrane surface has the dimensions shown in the table. The values of p have been calculated assuming that only the protein surface corresponding to a central angle of 60° has to be pelleted to reach the transition state (see diagram of Fig. 1). In the assocation $\Delta G^{\ddagger}$ values, the $\sum_{j=1}^{p} \Delta G_{f,j}$ term has been neglected, as suggested by its low value when compared to $\sum_{i=1}^{p} \Delta G_{b,i}$ (see the text and Table II). For the cases listed in the table, $\Delta G^{\ddagger}$ low and high values correspond to $\Delta G_b = -4$ and -10 kcal/mol lipid, respectively. In the dissociation $\Delta G^{\ddagger}$ is considered only the work against the internal lateral pressure of the membrane (see the text for additional details).

Protein diameter (Å)	p	Association $\Delta G^{\ddagger}$ (kcal/mol)	Dissociation $\Delta G^{\ddagger}$ (kcal/mol)
20	4	16–40	9.6
30	6	24–60	14.4
40	8	32–80	19.1
50	10	40–100	24.0
70	14	56–140	33.6

molecules to form a shell around each protein molecule is being ignored at this stage, because this process can be either less disfavoured or even favoured. Using these values of σ_i and of d, the calculated work that proteins having different diameters must do against the lateral membrane pressure when undergoing the analyzed process can be calculated and the results are given in Table I. With these values, which probably represent minimum estimations, because the work against protein-protein interactions has been neglected, the rate constant, k, for the whole process can be obtained according to the kinetic theory of chemical reactions [26].

The results obtained for a protein of 50 Å diameter (corresponding to a protein subunit of approx. 100 kDa) are of special relevance because many intrinsic proteins performing relevant physiological functions, such as band 3 of erythrocytes [11], $(Na^+ + K^+)$-ATPase [12] and Ca^{2+}-ATPase [13], to cite only a few, are of approximately this size. For them, the work to be done against the lateral membrane pressure when dissociating through the indicated pathway is approx. 24 kcal/mol and, therefore, a half-time of 7.3 h can be derived for the dissociation.

(B) Association processes

In these cases, dissociation of part of the lipid annulus of the protein has to be assumed as a necessary intermediate step, thus leading to the same transition state already described for dissociation processes. The Gibbs free energy change in this step can be formulated as:

$$\Delta G^{\ddagger} = -\sum_{i=1}^{p} \Delta G_{b,i} + \sum_{j=1}^{p} \Delta G_{f,j} \qquad (1)$$

where p is the number of lipid molecules to be released to reach the transition state; $\Delta G_{b,i}$ is the Gibbs free energy of binding of an i-lipid molecule to the protein and $\Delta G_{f,j}$ is the Gibbs free energy change associated to the passage of a j-lipid molecule from the protein annulus to the bulk lipid.

In order to evaluate the first term of Eqn. 1, the value of the free energy of binding of each class of lipid to the protein is needed. Unfortunately, we lack the experimental results to allow a precise determination of this term. However, pioneering work by Warren et al. [27] and by Feigenson and colleagues [28,29] using Ca^{2+}-ATPase as a model intrinsic membrane protein has shown no evidence of preferential binding of any of the most frequently found lipids to this protein (i.e., ΔG_b (DPPC) $\simeq \Delta G_b$ (DMPC) $\simeq \Delta G_b$ (DOPC), (etc.). Brotherus et al. [30] have also determined relative affinity constants of different phosphatidylcholines (PC) and phosphatidylserines (PS) to $(Na^+ + K^+)$-ATPase and to cytochrome c oxidase. Their

this energy balance is rather difficult to predict due to the lack of experimental data, such as the molecular nature of this distortion. Nevertheless, it is to be recalled that the energy barrier to be overcome in the transition of a lipid molecule from all-*trans* to the Δtg kink is 2 kcal/mol [25]. Because several lipid molecules are likely to be involved, it can be inferred that these lipid distortions will make an important contribution to the activation energy of the whole dissociation process.

results have shown that $(Na^+ + K^+)$-ATPase binds negatively charged lipids (PS) preferentially, the ratio of binding constants, $K_b(PS)/K_b(PC)$, being approx. 4.5. It is to be noted that this is equivalent to a difference in ΔG_b of 1.5 RT, which is negligible with respect to the expected absolute values of ΔG_b, see below.

Under the assumption that these results may be generalized to all intrinsic membrane proteins, it can be written:

$$\sum_{i=1}^{p} \Delta G_{b,i} = p \cdot \Delta G_b \qquad (2)$$

Thus, this assumption reduces the problem to the estimation of ΔG_b and p.

The number of lipid molecules, p, to be dissociated from the surface of one protein subunit in order to reach the indicated transition state has to be dependent upon the size of one protein subunit. Table I summarizes some reasonable estimations of these numbers as a function of the size of the protein subunit.

On the other hand, the ΔG_b of lipid binding to phospholipase A_2 has been estimated to be equal to $-(6.8 \pm 1.7)$ kcal/mol lipid [31]. It is to be noted that the interaction of phospholipase A_2 with membrane-like structures does not appear to involve the catalytic centre directly, but rather a hydrophobic region of the protein, the so-called interface recognition site [31]. This is, to the best of my knowledge, the only case for which ΔG_b has been determined. The high affinity of intrinsic membrane proteins for lipids, as in the case of phospholipase A_2, suggests that this value can be as a good reference one. Using ΔG_b values from -4 to -10 kcal/mol lipid, the first term of the Eqn. 1 can be estimated and the results are included in Table I.

The value of $\Sigma \Delta G_{f,j}$ has to be dependent upon the physical state of the annular and of the bulk lipid. Let us derive some calculations taking egg lecithin as a model lipid mixture. At room temperature, egg lecithin lipids are mostly in the liquid crystalline state (such will be the case for most of the lipids of mammalian membranes); therefore, we will take this state as the state of the bulk lipid for estimation of ΔG_f. It is evident that this value also depends upon the lipid composition and that it can be written:

$$\sum_{j=1}^{p} \Delta G_{f,j} \simeq p \cdot \sum_{k} f_k \cdot \Delta G_f^{(k)} \qquad (3)$$

where k refers to each class of lipid molecules in the lipid mixture; f_k is the fraction of the k-class of lipid molecules in the mixture and $\Delta G_f^{(k)}$ is the value of ΔG_f for the k-class of lipid molecules at the chosen temperature. The values of $\Delta G_f^{(k)}$, see Table II, have been approximately estimated as half the ΔG of melting of the k-lipid molecule, since recent findings [34,35] support the idea that the state of the lipid in the protein annulus is somewhere inbetween the gel and liquid crystalline states.

Nevertheless, it turns out that this assumption is not restrictive at all, since the derived value of $\Sigma \Delta G_{f,j}$ is negligible when compared to the value of $\Sigma \Delta G_{b,i}$, even if $\Delta G_f^{(k)}$ is taken equal to the value of ΔG of the lipid melting (see Tables I and II).

The high values obtained for $\Delta G^{\ddagger}$ show that the association of intrinsic membrane proteins might be a very slow process (see Table I). Moreover, in certain cases it may be considered as a forbidden one.

TABLE II

GIBBS FREE ENERGY OF MELTING OF THE RELEVANT LIPID COMPONENTS OF EGG LECITHIN

The listed lipids constitute 63% of the total egg lecithin lipids and they are the ones having thermodynamically well-defined melting processes. ΔG_m is Gibbs free energy of melting at room temperature, approximately 22°C, in kcal/mol [32]. f is the fraction of the lipid in the egg lecithin lipid mixture [33].

	ΔG_m	f
DPPC	0.55	0.20
DSPC	1.15	0.10
DOPC	−1.25	0.18
PE	0.6–1.0 [a]	0.15

[a] These values of ΔG_m correspond to DMPE and DPPE, respectively. Since the presence of unsaturated lipids in PE mixtures will decrease these values even further, these are, then, maximum estimations.

Final remarks

These calculations show that, whatever may be the thermodynamically most favoured protein aggregation state when an intrinsic membrane protein is embedded in a biological membrane, the achievement of the equilibrium aggregation state from 'a priori' established non-equilibrium situations might be a rather slow process, prohibited from a practical view point in certain cases. It immediately follows that the large variability of the functional properties of reconstituted vesicular structures can be at least partially accounted for by the variability of the protein concentration in the solubilized form in the reconstitution mixture, a variable previously uncontrolled.

Although free-energy terms coming from the work to be done against protein-protein interactions have been neglected in the above calculations, due to the evident difficulties in making a reliable estimation of them at present, it should be pointed out that the consideration of these interactions cannot simultaneously reduce the activation energies for association and dissociation processes to a negligible level but, on the contrary, whatever their sign, they will increase one of these activation energies.

This analysis allows one to predict which physical parameter changes can induce rapid changes in the distribution of the aggregation state of intrinsic membrane proteins and, thus, which physical parameters are expected to play an important role in the control of the functions performed by these proteins whenever these functions depend upon their aggregation state (see the Introduction). Let us now discuss this point in more detail.

A first important conclusion derived from this study is that the contribution of lipid conformational transitions to the activation energy, $\Delta G^{\ddagger}$, of the processes considered here appears to be negligible over the temperature range 22–37°C when compared to other contributions (see Tables I and II). Therefore, entropic energy terms such as those mentioned by Israelachvili [36] are not expected to play a major role in the rate of association and/or dissociation processes of intrinsic membrane proteins in this temperature range in mammalian cells, though they are expected to play an important role in the equilibrium distribution between different aggregation states of the protein, as Israelachvili noted in Ref. 36.

In addition, from the analysis presented in this paper it is derived that the most important energetic barriers in the processes considered here are:

(a) Lateral internal pressure of the membrane and protein-protein interactions, for dissociation processes;

(b) Dissociation of lipid molecules bound to the protein and protein-protein interactions, for association processes.

However, because intrinsic membrane proteins carry out their function deeply embedded within the lipid bilayer and also because there seem to be only small differences in the values of ΔG_b measured for several of the most frequently found lipids (see above) it appears unlikely that, under physiological conditions, appreciable changes in the free energy contribution to $\Delta G^{\ddagger}$ coming from the dissociation of lipids bound to the protein can be rapidly induced to regulate the rate of association of intrinsic membrane proteins. It is to be noted, though, that marked changes in the lipid composition of the membrane, such as those induced by aging [37], can promote large changes in this free energy contribution to $\Delta G^{\ddagger}$. Therefore, this study indicates that the regulation of the rate of aggregation of intrinsic membrane proteins in membranes in processes such as those mentioned in Refs. 1–10 lies entirely in changes of the strength of protein-protein interactions. One of the most simple ways to achieve this is by changing the net electrical charge of certain regions of the protein molecule. It is well known that the binding of ions (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, etc.) and phosphorylation play important regulatory roles in the control of the ATPases, where protein-protein interactions have been suggested to be necessary for the energetic coupling of ATP hydrolysis and the transport process: see, for example, Refs. [2,38,39].

It should also be emphasized that more sophisticated ways of altering protein-protein interactions might operate in biological membranes. Large conformational changes of a membrane protein upon binding of a water-soluble ligand or substrate have been shown to occur in, at least, plasma membrane ($Na^{+} + K^{+}$)-ATPase and Ca^{2+}-ATPase of the sarcoplasmic reticulum [38,40,41].

It is conceivable that, as a consequence of the induced conformational changes, one or several charged amino acids of the protein previously exposed to the aqueous solvent move into the lipid bilayer. A change in the environment of electric charges from a medium of high dielectric constant (water) to another of low dielectric constant (lipid) has such a multiplicative effect on the potential energy of repulsion (or attraction) between adjacent protein subunits that it may act as a trigger of the association (or dissociation) processes considered in this paper. In this sense, I would like to add that the electric potential established across the membrane is a force that energetically favours the jumping of adequate electric charges in and out of each side of the bilayer; from this it follows that the electrical potential should control the dynamics of the association $\rightleftharpoons$ dissociation equilibrium of intrinsic membrane proteins and the lifetime of the transition state of these processes. Many transport processes are known to operate in an electrogenic manner [42–45]. At this point, it is to be noted that the postulated transition state may be used in the concept of a transient channel for ions or metabolites across the membrane.

For the case of the dissociation of intrinsic membrane proteins, the two physical factors mentioned above may play regulatory roles in physiologically relevant conditions to control its rate. Let us recall that factors altering the physical state of the lipid molecules within the membrane will alter the lateral internal pressure, because of the dependence of the compressibility of the lipid phase upon the lipid state [46]. Temperature [46], cholesterol [47] and anesthetics [48], to cite only a few, are such factors. Mechanical actions on the membrane structure, like a mild sonication treatment, able to disrupt its integrity by creating packing defects or any type of void spaces within the bilayer, will enhance the rate of intrinsic membrane protein disaggregation or allow it to proceed if it had been blocked by the lateral internal pressure. By contrast, from the results of the analysis presented here, any effect of these mechanical actions on the rate of association of intrinsic membrane proteins must be directly related to the probability of the appearance of void volumes in between protein subunits in such a way that a spatial disposition like that of the transition state of Fig. 1 is attained, and this event is unlikely to occur with high probability in these systems.

I wish to emphasize that the calculations and the rationale of this paper have been developed considering that most of the bulk lipid of the membrane is in the liquid crystalline state, as is the case for the subcellular mammalian membranes studied under physiological conditions [49]. For the case in which most or all the lipid is in the gel state, to reach the transition state of Fig. 1, the diffusion of lipids out of the protein annulus (in association processes) or the diffusion of lipids to occupy the void volume in contact with the protein (in dissociation processes) may become rate-limiting due to the low diffusion coefficient of lipids in the gel state [25].

Finally, it should be noted that this study allows us to rationalize hormone refractoriness from a new perspective, at the least for those cases in which the formation of patches of receptors aggregated upon hormone binding has been demonstrated (see for example Ref. 6), as it opens the possibility that the dissociation of the receptors might be the rate-limiting step in the regeneration of hormone reponsiveness.

Acknowledgements

This work was supported by a National Institutes of Health Grant GM-26894 to R.L. Biltonen and by a Grant from the Comision Asesora Científica y Técnica (Proyecto num. 588) to the author of this paper.

References

1 Neher, E. and Stevens, C.F. (1977) Annu. Rev. Biophys. Bioeng. 6, 345–381
2 Ikemoto, N., Miyao, A. and Kurobe, Y. (1981) J. Biol. Chem. 256, 10809–10814
3 Stoeckenius, W. (1980) Acc. Chem. Res. 13, 337–344
4 Casadio, R. and Stoeckenius, W. (1980) Biochemistry 19, 3374–3381
5 Ross, E.M. and Gilman, A.G. (1980) Annu. Rev. Biochem. 49, 533–564
6 Zidovetzki, R., Yarden, Y., Schlessinger, J. and Jovin, T.M. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 6981–6985
7 Conti-Tronconi, B.M. and Raftery, M.A. (1982) Annu. Rev. Biochem. 51. 491–530
8 Ahkong, Q.F., Blow, M.M.J., Botham, G.M., Launder, J.M., Quirk, S.J. and Lucy, J.A. (1978) FEBS Lett. 95, 147–150

9 Nicholson, B.J., Hunkapiller, M.W., Grim, L.B., Hood, L.E. and Revel, J.P. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 7594–7598

10 Cuatrecasas, P. (1974) Annu. Rev. Biochem. 43, 169–214

11 Kasahara, M. and Hinkle, P.C. (1977) J. Biol. Chem. 252, 7384–7390

12 Kagawa, Y. (1978) Biochim. Biophys. Acta 505, 45–93

13 Racker, E. (1973) Biochim. Biophys. Res. Commun. 55, 224–230

14 Korenbrot, J.I. (1977) Annu. Rev. Physiol. 39, 19–49

15 Oldfield, E., Gilmore, R., Glaser, M. Gutowsky, H.S., Hshung, J.C., Kang, J. C., King, T.E., Meadows, M. and Rice, D. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 4657–4660

16 Knowles, A.F. and Racker, E. (1975) J. Biol. Chem. 250, 3538–3544

17 Bennett, J.P., McGill, K.A. and Warren, G.B. (1980) Current Topics Membranes Transp. 14, 127–164

18 Okamoto, H., Sone, N., Hirata, H., Yoshida, M. and Kagawa, Y. (1977) J. Biol. Chem. 252, 6125–6131

19 Swanson, M.S., Quintanilla, A.T. and Thomas, D.D. (1980) J. Biol. Chem. 255, 7494–7502

20 Konigsberg, P.J. (1982) Biochim. Biophys. Acta 685, 355–366

21 Conrad, M.J. and Singer, S.J. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 5202–5206

22 Conrad, M.J. and Singer, S.J. (1981) Biochemistry 20, 808–818

23 Demel, R.A., Geurts van Kessel, W.S.M., Zwaal, R.F.A., Roelofsen, B. and Van Deenen, L.L.M. (1975) Biochim. Biophys. Acta 406, 97–107

24 Huang, C. and Mason, J.T. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 308–310

25 Thompson, T.E. and Huang, C. (1978) in Physiology of Metabolic Disorders (Andreoli, T.E., Hoffmann, J.F. and Fanestil, D.D., eds.), Ch. 2, p. 32, Plenum, New York

26 Glasstone, S., Laidler, K.J. and Eyring, H. (1941) The Theory of Rate Processes, McGraw-Hill, New York

27 Warren, G.B., Toon, P.A., Birdsall, N.J.M., Lee, A.G. and Metcalfe, J.C. (1974) Biochemistry 13, 5502–5507

28 London, E. and Feigenson, G.W. (1981) Biochemistry 20, 1939–1948

29 Caffrey, M. and Feigenson, G.W. (1981) Biochemistry 20, 1949–1961

30 Brotherus, J.R., Griffith, O.H., Brotherus, M.O., Jost, P.C., Silvius, J.R. and Hokin, L.E. (1981) Biochemistry 20, 5261–5267

31 Soares de Araujo, P., Rosseneu, M.Y., Kremer, J.M.H., Van Zoelen, E.J.J. and De Haas, G.N. (1979) Biochemistry 18, 580–585

32 Lee, A.G. (1977) Biochim. Biophys. Acta 472, 237–281

33 Bangham, A.D., Hill, M.W. and Miller, N.G.A. (1974) in Methods in Membrane Biology (Korno, E.D., ed.), Vol. 1, pp. 1–68, Plenum, New York

34 Moore, B.M., Lentz, B.R. and Meissner, G. (1978) Biochemistry 17, 5248–5255

35 Rice, D.M., Meadows, M.D., Scheinman, A.O., Goñi, F.M., Gomez-Fernandez, J.C., Moscarello, M.A., Chapman, D. and Oldfield, E. (1979) Biochemistry 18, 5893–5903

36 Israelachvili, J.N. (1977) Biochim. Biophys. Acta 469, 221–225

37 Katz, S.S. (1981) J. Biol. Chem. 256, 12275–12280

38 De Meis, L. and Vianna, A.L. (1979) Annu. Rev. Biochem. 48, 275–292

39 Verjovski-Almeida, S. and Silva, J.L. (1981) J. Biol. Chem. 256, 2940–2944

40 Kuriki, Y., Halsey, J., Biltonen, R. and Racker, E. (1976) Biochemistry 15, 4956–4961

41 Epstein, M., Kuriki, Y., Biltonen, R. and Racker, E. (1980) Biochemistry 19, 5564–5568

42 Wilson, D.B. (1978) Annu. Rev. Biochem. 47, 933–965

43 Bakker, E.P. and Harold, F.M. (1980) J. Biol. Chem. 255, 433–440

44 Rosier, R.N., Tucker, D.A., Meerdink, S., Jain, I. and Gunter, T.E. (1981) Arch. Biochem. Biophys. 210, 549–564

45 Zimniak, P. and Racker, E. (1978) J. Biol. Chem. 253, 4631–4637

46 Marcelja, S. and Wolfe, J. (1979) Biochim. Biophys. Acta 557, 24–31

47 Wolber, P.K. and Hudson, B.S. (1981) Biochemistry 20, 2800–2810

48 Mountcastle, D.B., Biltonen, R.L. and Halsey, M.J. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 4906–4910

49 Melchior, D.L. and Steim, J.M. (1976) Annu. Rev. Biophys. Bioeng. 5, 205–238