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MINI-REVIEW

Selectivity of Lipid–Protein Interactions

Derek Marsh[1]

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Abstract

The spin label ESR and intrinsic fluorescence quenching methods of determining the selectivity of interactions of lipids with integral membrane proteins are summarized. The selectivity patterns of phospholipids, fatty acids, and steroids are reviewed for a variety of integral proteins. Where appropriate, correlations are established with biochemical assays of the effects of specific lipids on enzymatic activity and transport function.

Key Words: Lipid–protein interaction; lipid specificity; integral protein; lipid activation; lipid inhibition; spin label ESR; fluorescence quenching.

Introduction

The interaction of lipids with integral membrane proteins can be characterized biophysically by the number of lipid association sites on the protein and the relative association constants of the various lipids for these different sites. An exchange equilibrium is normally assumed between lipids, L and L*, competing for sites on the protein, P:

$$PL + L^* = PL^* + L \quad (1)$$

A relative association constant for lipid L* with respect to lipid L can then be defined by

$$K_r = [L^*]_b \cdot [L]_f / [L]_b \cdot [L^*]_f \quad (2)$$

where the subscripts b and f correspond to lipids associated with the protein, and free in the bilayer, respectively. Square brackets indicate concentrations, and activity coefficients have been taken as unity for the sake of simplicity.

[1]Max-Planck-Institut für biophysikalische Chemie, Abt. Spektroskopie, D-3400 Göttingen, FRG.

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The total number of association sites on the protein can be conveniently determined from the electron spin resonance spectra of spin-labelled lipids (see, e.g., Marsh, 1985, for a review). These numbers of sites are found to correspond roughly to the total intramembranous surface of the proteins so far studied. There is currently relatively little evidence for highly specific lipid sites on the protein, with a few notable exceptions such as β-hydroxybutyrate dehydrogenase or the specific phospholipid exchange proteins. This review is concerned primarily with the lipid specificity of the various sites on the intramembranous surface of the protein, and an attempt is made to correlate this with the effects of particular lipids on the biochemical activity of the protein.

Methods of Studying Lipid Specificity

There are two methods which have proved to be particularly useful in determination of the selectivity of interaction of lipids with integral membrane proteins. These are the electron spin resonance of spin-labelled lipids and the quenching of the intrinsic fluorescence of the integral proteins by either spin-labelled or brominated lipids (cf. Devaux and Seigneuret, 1985). The ESR method relies on the direct effects of the lipid–protein interaction on the lipid chain dynamics, and the fluorescence method requires the close proximity of the quenching lipid to the fluorescent group on the protein. Both methods are analyzed using the same model for lipid association with the protein, which was described in the previous section.

Spin Label Electron Spin Resonance

The electron spin resonance (ESR) spectra of spin-labelled lipids are sensitive to molecular motions on a time scale $< 3 \cdot 10^{-8}$ s. Since the lipid exchange at the protein surface is appreciably slower than this, the spectra of the spin-labelled lipids at the protein interface can be distinguished from those in the bulk lipid regions of the membrane. The ESR spectra from lipids labelled close to the terminal methyl ends of their chains frequently consist of two components, one of which corresponds to lipid chains with a reduced mobility resulting from direct interaction with the protein surface.

The relative amounts of the two spectral components can be quantitated by difference spectroscopy, combined with double integration of the individual spectra. The equation for lipid–protein association corresponding to the exchange equilibrium depicted by Eq. (1) is then

$$n_f^*/n_b^* = (1/K_r)(n_t/N_1 - 1) \quad (3)$$

where n_f^*/n_b^* is the ratio of fluid to motionally restricted lipid in the ESR spectrum, n_t is the total lipid/protein ratio in the sample, and N_1 is the number

of first-shell association sites on the protein. The approximation has been made that the concentration of the labelled lipid, L*, is small compared with that of the unlabelled lipid, L, and the relative association constant, K_r, is defined for the labelled lipid with respect to the unlabelled background host lipid.

Results of experiments in which the lipid/protein ratio is varied suggest that the selectivity arises from differences in the relative association constants rather than in the effective number of association sites (see, e.g., Marsh, 1985). Thus for experiments with two different spin-labelled lipids, A and B, in samples of the same lipid/protein ratio, the ratio of the relative association constants is given from Eq. (3) by

$$(n_f^*/n_b^*)_A/(n_f^*/n_b^*)_B = K_r(B)/K_r(A) \quad (4)$$

Hence the relative selectivities can be readily determined from the ESR spectrum alone.

The relative association constant, K_r, determined from measurements at low spin label concentration, using Eq. (3), represents the mean value averaged over all N_1 first-shell sites. A generalized increase in selectivity can be distinguished from the existence of just a few highly specific sites by increasing the concentration of the specific lipid, and looking for saturation. This has been done for the association of cardiolipin with cytochrome oxidase (Powell *et al.*, 1985), and no evidence was obtained for the existence of unique specific sites.

For further discussion of the technical aspects of spectral subtraction and both experimental and theoretical verification of the two-component nature of the ESR spectra from lipid–protein systems, see Marsh (1987).

Fluorescence Quenching

Quenching of fluorophore fluorescence by spin-labelled or brominated phospholipids is essentially a contact interaction. Because the fluorescence lifetime of tryptophan is in the nanosecond range (typically 3–5 ns), i.e., much shorter than the residence time of a lipid on the protein, the fluorescence quenching is of the static type. For tryptophan residues in the Ca^{2+}-ATPase it has been found that approximately two lipid sites are sufficiently close to cause quenching of the average tryptophan (London and Feigenson, 1981; East and Lee, 1982). The fluorescence quenching is given by

$$(F - F_{min})/(F_0 - F_{min}) = (1 - X_L^*)^n \quad (5)$$

where X_L^* is the mole fraction of the quenching lipid, F and F_0 are the fluorescence intensities in the presence and absence of quencher, and F_{min} is the residual fluorescence when $X_L^* = 1$. The number of lipid sites around an average tryptophan was found to be $n = 2$ for lipids spin-labelled on the

8 C-atom, and $n = 1.6$ for lipids brominated at the double-bond position of oleic acid.

This lack of a one-to-one correspondence between the fluorescence quenching and the lipid binding makes it difficult to extract the relative association constant between labelled and unlabelled lipid directly. Instead separate fluorescence quenching experiments are carried out in which two different unlabelled lipids, say A and B, are competed against the labelled lipid (London and Feigenson, 1981). The assumption is made that the mole fractions of the two lipids, X_A and X_B, which give rise to the same fluorescence quenching correspond to equal degrees of association of the two lipids. The ratio of the relative association constants, $K_r(A:B)$, between the two unlabelled lipids is then obtained from the following equation, which is derived from Eq. (2) with the condition that the total lipid/protein ratio is high (such that the mole fraction of protein may be neglected):

$$1/X_A = K_r(A:B)/X_B + 1 - K_r(A:B) \tag{6}$$

The method requires reconstitutions with high concentrations of the labelled lipid, but by competing against two unlabelled lipids, the possible perturbing effects are cancelled. For further details see London and Feigenson (1981) and East and Lee (1982).

Results of Biophysical Studies

Fatty Acid Chain Dependence

The selectivity of interaction of phosphatidylcholines of different chain compositions with the Ca^{2+}-ATPase from sarcoplasmic reticulum has been investigated by the fluorescence quenching method (Caffrey and Feigenson, 1981). Reconstitutions were made in mixtures of a single spin-labelled phosphatidylcholine with unlabelled phosphatidylcholines of various chain lengths and degrees of unsaturation. It was found that the relative affinity was not dependent on chain composition for the various unsaturated symmetrical diacyl phosphatidylcholines tested. These were lipids with mono-unsaturated chains of lengths from C12 : 1 to C24 : 1, and with the di-unsaturated chain C18 : 2(9c, 12c), as well as with the *cis* and *trans* isomers of the C14 : 1, C16 : 1, and C18 : 1 chains. Although this implies that there is little preferential association of one phosphatidylcholine species relative to another, it must be remembered that the association constants are referred to the background lipid mixture, one component of which remains constant. The results therefore imply that, as far as the chains go, the strength of the lipid–protein interaction is similar to that of the lipid–lipid interaction in the bilayer milieu. The strength of the latter can be inferred from the chain-length dependence

Table I. Order of Selectivity of Spin-Labelled Lipids for Association with Integral Membrane Proteins[a]

Myelin Proteolipid	SA > PA > CL $\gtrsim$ PS > PG $\approx$ SM $\approx$ PC > PE	Refs. 1, 7
Na^+,K^+-ATPase	CL > PS $\approx$ SA $\gtrsim$ PA > PG $\approx$ SM $\approx$ PC $\approx$ PE	Refs. 2, 7
Cytochrome oxidase	CL > PA $\approx$ SA > PS $\approx$ PG $\approx$ SM $\approx$ PC $\approx$ PE	Refs. 3, 7
Acetylcholine receptor	SA > PA > PS $\approx$ PC $\approx$ PE	Ref. 4
F_0H^+-ATPase	SA > PA $\approx$ CL > PS $\simeq$ PG > PC > PE	Ref. 8
M-13 Coat protein	CL $\approx$ PA > SA $\approx$ PS $\simeq$ PG > PC $\approx$ PE	Ref. 9
Ca^{2+}-ATPase	CL > PS $\approx$ SA $\gtrsim$ PA $\gtrsim$ PG $\approx$ SM $\approx$ PC $\approx$ PE	Refs. 5, 7
Rhodopsin	CL $\approx$ PA $\approx$ SA $\approx$ PS $\approx$ PG $\approx$ SM $\approx$ PC $\approx$ PE	Refs. 6, 7

[a]CL = cardiolipin; PA = phosphatidic acid; PS = phosphatidylserine; PG = phosphatidylglycerol: PC = phosphatidylcholine; PE = phosphatidylethanolamine; SM = sphingomyelin; SA = stearic acid. References: 1. Brophy *et al.* (1984); 2. Esmann *et al.* (1985); 3. Knowles *et al.* (1981); 4. Ellena *et al.* (1983); 5. Hidalgo and Marsh (1987); 6. Watts *et al.* (1979); 7. Marsh *et al.* (1987); 8. Pringle and Marsh (1987); 9. Datema *et al.* (1987).

of the critical micelle concentrations involved in the lipid self-assembly (see, e.g., Cevc and Marsh, 1987). From this it can be deduced that the free energy of transfer of the lipid from water to the lipid bilayer or to the lipid–protein interface is approximately $1.1RT$ per CH_2 group, in either case.

An alternative approach to the study of the selectivity of chain interactions with the protein is to compare phospholipids with different numbers of hydrocarbon chains, using the spin label ESR method. Such comparisons are complicated by the potential selectivities between different lipid headgroups and the possibility of conformational differences in the polar groups between phospholipids and their lyso derivatives. However, for cytochrome oxidase reconstituted in dimyristoyl phosphatidylcholine it is found that there is little selectivity in interaction between spin-labelled cardiolipin or its monolyso derivative, or between spin-labelled phosphatidylcholine and spin-labelled lyso phosphatidylcholine (Powell *et al.*, 1987). This again suggests that there is little difference in the free energy of interaction of the lipid chains with each other and with the hydrophobic surface of the protein. For Na^+,K^+-ATPase-rich membranes from *Squalus acanthias*, it is also found that there is little difference in the fraction of spin-labelled cardiolipin which is associated with the protein, compared with that of spin-labelled monolyso cardiolipin (Esmann *et al.*, 1987). In the latter case, however, a selectivity is found for spin-labelled lyso phosphatidylcholine relative to spin-labelled phosphatidylcholine, possibly indicating the effects of a different conformation of the lyso derivative, as already mentioned.

Polar Headgroup Dependence

The relative selectivities of the different phospholipid species for a variety of different integral membrane proteins are given in Table I. These

data have been obtained from spin label ESR experiments, either on natural membranes or reconstituted lipid–protein systems. The only systematic series of studies to date using the fluorescence quenching method is for the Ca^{2+}-ATPase from sarcoplasmic reticulum. In this latter system it was found that there is virtually no selectivity between the different lipid headgroups (London and Feigenson, 1981; East and Lee, 1982), in agreement with the spin label results summarized in Table I. It would be of considerable interest to obtain results by the fluorescence quenching method with one of the numerous systems for which a well-defined specificity has been observed by ESR, and also to investigate the effects of chain composition in such a system.

As seen from Table I, the myelin proteolipid protein, Na^+, K^+-ATPase, F_0-ATPase, cytochrome oxidase, M-13 coat protein, and the acetylcholine receptor all display a well-defined specificity pattern for the different phospholipid species. The average relative association constants reach maximum values in the range $K_r = 4$–5 for these proteins. For Ca^{2+}-ATPase the specificity pattern is less pronounced and the selectivity is much weaker ($K_r < 2$) than for the previously mentioned proteins. For rhodopsin there is practically no selectivity whatsoever, with $K_r = 1$ for all lipids tested. It is clear from this that there is a different lipid specificity pattern for the different proteins. The selectivity is not simply a property of the lipid alone, but depends in detail on the particular protein amino acid composition, sequence, and structure.

In particular, it is interesting to note that neither the tetraacyl phospholipid cardiolipin, nor the single-chain free fatty acid, is invariably the lipid which displays the highest selectivity. As discussed in the previous section, this indicates that the number of hydrocarbon chains is not the overriding factor in the lipid selectivity. Sphingomyelin, which contains a potential hydrogen bond donor (the amide group), does not exhibit a preferential selectivity relative to the corresponding glycerolipid analogue, phosphatidylcholine. In addition, phosphatidylethanolamine, which contains a potential hydrogen bond donor in the headgroup, also exhibits no specificity (or even a decreased specificity) relative to phosphatidylcholine.

The highest selectivity is observed for the negatively charged phospholipids, but this does not bear a simple relation to the net charge on the headgroup, since no specificity is observed for phosphatidylglycerol over phosphatidylcholine, for example. Experiments on the ionic strength dependence show that the selectivity for certain negatively charged lipids cannot be completely screened out by high concentrations of salt. This is the case for phosphatidic acid and phosphatidylserine interacting with Na^+,K^+-ATPase (Esmann and Marsh, 1985), for cardiolipin interacting with cytochrome oxidase (Powell *et al.*, 1987), and for phosphatidic acid and stearic acid interacting with the myelin proteolipid protein (Horvath *et al.*, 1987). On the

other hand, the selectivity of stearic acid for the Na^+,K^+-ATPase can be screened completely by salt. For the myelin proteolipid protein, most of the selectivity displayed by phosphatidylserine can also be screened by salt.

The lipid selectivity is also affected by pH titration if the pK_a's of the groups involved are in the accessible range. Both phosphatidic acid and stearic acid display a well-defined titration behavior in their interaction with the Na^+,K^+-ATPase, with effective pK_a's of 6.6 and 8.0, respectively (Esmann and Marsh, 1985). In the singly protonated states, both lipids display no preferential selectivity relative to phosphatidylcholine, although phosphatidic acid still bears a net negative charge. For the myelin proteolipid protein, the fractions of motionally restricted stearic and phosphatidic acids titrate with an effective pK_a of 7.6, for both lipids, and phosphatidylserine also begins to titrate at pH's greater than 8 (Horvath *et al.*, 1987). In the protonated state stearic acid displays a selectivity which is still greater than that for phosphatidylcholine and similar to that of phosphatidylserine in the singly negatively charged state. The selectivity for phosphatidic acid in the singly negatively charged state, at low pH, is yet greater than that for both the latter lipids. Clearly the selectivity depends on the details of both the lipid and protein structure (and most probably on the state of hydration), and cannot be explained solely in terms of simple electrostatics.

The selectivity of cytochrome oxidase for cardiolipin decreases slightly at high pH, presumably as a result of titration of lysine or hystidine residues on the protein (Powell *et al.*, 1987). Similarly, a slight increase in the degree of association of phosphatidylglycerol was observed with the Ca^{2+}-ATPase on titrating from pH 7.5 to 5.6 (London and Feigenson, 1981), although, in both these latter cases, the effect of titrating the protein is considerably less than that of titrating the lipid, suggesting that the groups titrated may be somewhat removed from the lipid association site.

Removal of the charge on cardiolipin by methylation of the phosphate groups leads to a decreased affinity for cytochrome oxidase (Powell *et al.*, 1987). The decrease is similar to that obtained by screening at high ionic strength, and still represents a significant selectivity relative to phosphatidylcholine. Covalent modification of cytochrome oxidase by citraconic anhydride, which reverses the charge on the lysine side chains, also decreases the selectivity for cardiolipin.

Steroids

Steroid molecules can, in principle, occupy substitutional sites for phospholipids at the lipid–protein interface. It has also been suggested that, for the Ca^{2+}-ATPase, they may also occupy sites at the dimer interface which are not available to phospholipids (Simmonds *et al.*, 1982, 1984).

Both positive and negative selectivities, relative to phosphatidylcholine, have been found for the association of spin-labelled steroids with different integral proteins. Spin-labelled androstanol displays a pronounced selectivity ($K_r = 4.3$) in its interaction with the acetylcholine receptor (Ellena *et al.*, 1983), whereas the same steroid associates only to a similar extent as phosphatidylcholine ($K_r = 1$) with both cytochrome oxidase (Knowles *et al.*, 1981) and rhodopsin (Watts *et al.*, 1979). The degree of association with the Na^+,K^+-ATPase (Esmann *et al.*, 1985) and with the myelin proteolipid protein ($K_r = 0.34$; Brophy *et al.*, 1984), on the other hand, is considerably less than that of phosphatidylcholine. A spin-labelled analogue of cholesterol has also been found to display a negative selectivity ($K_r = 0.65$) with respect to phosphatidylcholine in its association with the Ca^{2+}-ATPase (Silvius *et al.*, 1984).

Competition experiments between unlabelled cholesterol and brominated phosphatidylcholine in the fluorescence quenching of the Ca^{2+}-ATPase have shown that cholesterol does not compete for the same sites as phosphatidylcholine on the protein (Simmonds *et al.*, 1982). However, dibromocholestan-3β-ol was found to be capable of efficiently quenching the protein fluorescence, from which it was concluded that there were additional sites on the protein which were accessible to the steroid but not to the phospholipids. Presumably some of the sites detected by the spin-labelled steroid would also correspond to those inaccessible to phosphatidylcholine, although the lipid/protein titration of Silvius *et al.* (1984) suggests that at least part of the sites can be competed for by phosphatidylcholine. The existence of specific steroid sites of course opens up the possibility of interesting functional implications.

Results of Biochemical Studies

In several cases it is difficult to distinguish between the requirement of a particular lipid for the activity of a given protein and the less specific role of different lipids in the technology of reconstitution. For this reason a lipid specificity cannot necessarily be argued from the success or otherwise in reconsistuting a particular functional property. Correlations which often can be made more reliably are those with inhibition.

Fatty Acid Chain Dependence

Several membrane enzymes, including the Ca^{2+}-ATPase (Hesketh *et al.*, 1976; Hidalgo *et al.*, 1978), cytochrome oxidase (Fajer *et al.*, 1987), and rhodopsin (O'Brien *et al.*, 1977; Baldwin and Hubbell, 1985) are found to function less efficiently when the lipid chains are in the gel phase than when

they are in the fluid liquid crystalline phase. In the case of both the Ca^{2+}-ATPase and rhodopsin it appears that there is also a chain-length dependence of the activity in the fluid phase. For the Ca^{2+}-ATPase an optimum bilayer thickness is required to sustain ATPase activity (Moore *et al.*, 1981; Caffrey and Feigenson, 1981; Johannson *et al.*, 1981). This is in contrast to the selectivity studies reported in the previous section, in which there was found to be no preference of the Ca^{2+}-ATPase for lipids of a particular chain-length. However, as cautioned above, it is to a certain extent difficult to extrapolate from the results of reconstitution to the absolute requirement for a particular state of fluidity of the lipid chains.

Phospholipid Headgroup Dependence

Several studies have found a more efficient reconstitution of the activity of delipidated Na^+,K^+-ATPase in the presence of certain acidic lipids (Kimelberg and Papahadjopoulos, 1974; Wheeler *et al.*, 1974; Palatini *et al.*, 1977; Mandersloot *et al.*, 1978; Cornelius and Skou, 1984). This correlates qualitatively with the results on the phospholipid spin label selectivity reported above. However, in some of these cases phosphatidylglycerol was found to be effective, although the enzyme displays no selectivity for this lipid. In other studies a purely nonspecific potentiation of activity by negative charges was suggested (de Pont *et al.*, 1978), and in yet a further study an active Na^+,K^+-ATPase preparation was obtained with phosphatidylcholine as the sole lipid (Hilden and Hokin, 1976). These different results illustrate the difficulty, referred to above, in distinguishing between the technology of reconstitution and a true specific requirement of a particular lipid for activity.

A study of the reactivation potential of the mitochondrial H^+-ATPase by various dioleoyl phospholipids has found an effectivity scale in the order phosphatidylglycerol > phosphatidic acid > phosphatidylcholine (Brown *et al.*, 1985). Although this corresponds qualitatively with the selectivity of the protein for negatively charged lipids, the behavior of phosphatidylglycerol is again anomalous. This lipid is particularly effective in activation, whereas it does not display a particularly strong selective association with the H^+-ATPase (cf. Table I).

For the Ca^{2+}-ATPase substituted with dioleoyl phospholipids, it was found that the zwitterionic lipids, phosphatidylcholine and phosphatidylethanolamine, supported a higher ATPase activity than the negatively charged phospholipids, phosphatidylglycerol, phosphatidylserine, and cardiolipin (Bennett *et al.*, 1978). Phosphatidic acid was able to support even less activity. These results on selective activation contrast with the direct measurements of lipid–protein association (see Table I), which have revealed little selectivity between the different lipid species.

The erythrocyte passive sugar transport protein, when reconstituted into phospholipids of the same chain composition, displays differences in turnover number depending on lipid headgroup which are in the order phosphatidylserine > phosphatidic acid > phosphatidylglycerol ≫ phosphatidylcholine (Tefft *et al.*, 1986). Unfortunately, there is currently no corresponding information on the specificity of association of phospholipids with this protein.

Nonbilayer-Forming Lipids

Although the formation of nonbilayer phases has little direct bearing on the specificity of interaction of lipids with the protein, it is possible that lipids which form such phases may selectively affect activity by changing the properties of the lipid environment in the immediate vicinity of the protein. The active Ca^{2+} uptake is potentiated by the presence of nonlamellar-forming lipids, such as unsaturated phosphatidylethanolamines, in reconstituted vesicles containing the Ca^{2+}-ATPase (Navarro *et al.*, 1984). In a rather more indirect way, nonlamellar-forming phosphatidylethanolamines were found to support coupled cytochrome oxidase activity, since they favored the formation of reconstituted systems which contained only one, unidirected cytochrome oxidase molecule per vesicle (Madden *et al.*, 1984).

Fatty Acids

Fatty acids have been found to be among the class of noncompetitive inhibitors which have a local anaesthetic-like effect on the acetylcholine receptor. The free fatty acids can block the agonist-induced ion flux, without giving rise to receptor desensitization (Andreasen *et al.*, 1979). This property correlates extremely well with the selectivity of the receptor for spin-labelled fatty acids (see above and Table I, above). The site of action suggested for such noncompetitive blockers from the inhibition studies (Heidmann *et al.*, 1983) correlates extremely well with the spin label results.

Fatty acids have also been found to be inhibitors of the Na^+,K^+-ATPase (Ahmed and Thomas, 1971), which again correlates very well with the specificity for spin-labelled fatty acids observed by ESR spectroscopy.

Cardiolipins

Cardiolipin is a characteristic lipid component of the inner mitochondrial membrane and has been implicated in the activation of several mitochondrial enzymes, including the phosphate carrier (Kadenbach *et al.*, 1982), the ADP-ATP translocator (Beyer and Klingenberg, 1985), complex III (Ragan and Racker, 1973), and cytochrome oxidase (Robinson *et al.*, 1980), of which the most intensively studied is probably cytochrome oxidase. Cardiolipin is

not absolutely essential for the activity of cytochrome oxidase, since it has been possible to substitute all of the endogenous lipid by dimyristoyl phosphatidylcholine and still maintain a high oxidative activity (Watts *et al.*, 1978; Powell *et al.*, 1985). Nevertheless, cardiolipin has been found to copurify with the enzyme and to be particularly effective in its reconstitution (Awasthi *et al.*, 1971; Yu *et al.*, 1975), which correlates rather well with the lipid selectivity observed by ESR. A preferential concentration of the negatively charged cardiolipin in the immediate vicinity of cytochrome oxidase might be expected to enhance the electrostatic interaction with cytochrome *c*, thereby increasing activity. It is also significant that cardiolipin appears to associate with many mitochondrial proteins, although it must be noted that a preferential association is also observed with certain proteins from membranes which do not contain appreciable quantities of cardiolipin (cf. Table I).

Conclusions

Well-defined specificity patterns are observed for the association of lipids with a variety of integral proteins, whereas certain proteins, viz. rhodopsin and the Ca^{2+}-ATPase, display relatively little specificity. In certain, but not all, cases the lipid selectivity correlates with the requirement of certain lipids for activity. This is more particularly true for inhibition. Other lipid effects than just direct lipid–protein association can, however, contribute to activation. Conversely, it is not possible to infer specific lipid–protein associations on the basis of activation by a particular lipid.

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