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Minireview

Is the protein/lipid hydrophobic matching principle relevant to membrane organization and functions?

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Abstract Biological membranes are complex and well-organized multimolecular assemblies composed of a wide variety of protein and lipid molecular species. If such a diversity in protein and lipid polar headgroup structures may easily be related to a large panel of functions, the wide dispersion in acyl chain length and structure which the lipids display is more difficult to understand. It is not required for maintaining bilayer assembly and fluidity. Direct information on the lateral distribution of these various molecular species, on their potential specificity for interaction between themselves and with proteins and on the functional implications of these interactions is also still lacking. Because hydrophobic interactions play a major role in stabilizing membrane structures, we suggest considering the problem from the point of view of the matching of the hydrophobic surface of proteins by the acyl chains of the lipids. After a brief introduction to the hydrophobic matching principle, we will present experimental results which demonstrate the predictive power of the current theories and then, we will introduce the new and important concept of protein/lipid sorting in membranes. Finally, we will show how the hydrophobic matching condition may play a key role in the membrane organization and function.

Key words: Hydrophobic mismatch; Protein/lipid interaction; Protein/lipid molecular sorting; Structure/function relationship in membrane

1. Introduction

Biological membranes are complex and well-organized multimolecular assemblies composed of a wide variety of protein and lipid molecular species. Protein diversity is not surprising with regard to the wide number of functions membranes exhibit. Lipid polar headgroups, depending on whether they are charged or neutral, may modulate the activity of membrane proteins through interfacial electrostatic phenomena [1]. In contrast, the wide diversity in acyl chain length and structure that lipids display is more difficult to understand [2]. Such a diversity is not required to maintain bilayer assembly and fluidity. In addition, we still have no direct information on the lateral distribution of these various molecular species, on their potential specificity for interaction between themselves and with proteins and on the functional implications of these interactions.

Hydrophobic interactions play a major role in stabilizing membrane structures [3] and reconsidering the problem in terms of protein and lipid hydrophobic matching offers an attractive possibility. Any integral protein is characterized by a hydrophobic length d_P and a lipid bilayer by a hydrophobic thickness d_L directly related to the length of the acyl chains. Because any exposure of protein or lipid hydrophobic residues to water is unfavorable, one can predict that the free energy of a membrane will be reduced all the more as the hydrophobic matching condition $d_P \sim d_L$ is satisfied at best for the various protein and lipid components.

Introduced first by M. Bloom and O. Mouritsen in 1984 in their 'mattress' model of biological membranes [4], this principle of protein/lipid hydrophobic matching has since received considerable attention from both a theoretical and an experimental point of view [5–8]. In the present work, and after a brief introduction to the theory, we will first present experimental results which demonstrate its predictive power and then, we will introduce the new and important concept of protein/lipid sorting in membranes resulting from it. Finally, we will show how that the hydrophobic matching condition may play a key role in membrane organization and function.

2. Theoretical principles

Basic statements of the protein/lipid hydrophobic matching principle and its consequences on lipid organization are extensively described in many papers [4,9–15] and review articles [5–8]. In what follows, we will restrict our presentation to some of the notions which may be useful for a good understanding of the concept and its applications to membranes.

Let us first consider the consequences of a hydrophobic mismatch between an integral protein of hydrophobic length d_P and a lipid bilayer of mean hydrophobic thickness d_L on the acyl chain order (Fig. 1). Lipids in contact with that protein are expected to be ordered (Fig. 1A) or disordered (Fig. 1B) depending on whether d_L is respectively smaller or larger than d_P. The perturbation extends over a few lipid layers around the protein and the bilayer recovers its normal thickness via an exponential function of the form [13]:

$$d_L(I) = d_L + (d_P - d_L)\exp(-D(I)/\xi) \quad (1)$$

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Abbreviations: PC, phosphatidylcholine; diCn:0-PC, bis-saturated phosphatidylcholines, *n* being the number of carbon atoms in the acyl chains; diCn:1-PC, mono-unsaturated phosphatidylcholines, *n* being the number of carbon atoms in the acyl chains; RC, reaction center; LHCP, light harvesting antenna; BR, bacteriorhodopsin; MelB, melibiose permease; ER, endoplasmic reticulum; TMD, transmembrane domain

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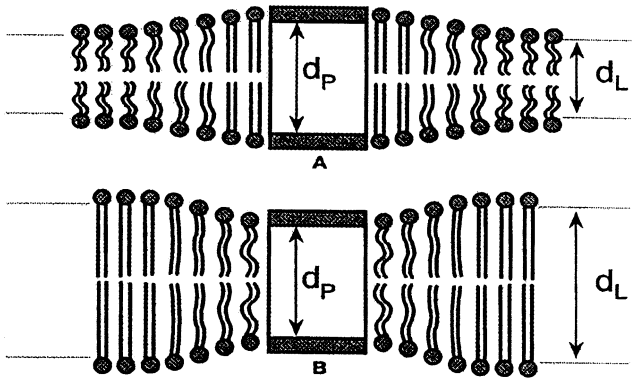

Fig. 1. Schematic representation of an intrinsic membrane protein of hydrophobic length d_P embedded in a lipid bilayer in which the unperturbed hydrophobic thickness d_L is smaller (A) or larger (B) than d_P. The influence of the protein, characterized by the coherence length ξ, extends over a certain distance from the protein surface and progressively vanishes so that the bilayer recovers its unperturbed thickness d_L.

($d_L(I)$ is the hydrophobic thickness of the lipid bilayer at distance $D(I)$ from the protein and ξ is the coherence length).

These local perturbations in acyl chain order are expected to result in an overall upward or downward shift ΔT in the mean phase transition temperature T_m of the lipids. Two approaches have been proposed to evaluate ΔT quantitatively. Although both take into consideration the differences which may exist between d_p and the hydrophobic thicknesses $d_{L,f}$ and $d_{L,g}$ of the lipids in the fluid and gel phases, respectively, they differ in their basic formulation. One is treated within a two-component solution theory and allows for phase separation. At low protein concentrations, linear effects of hydrophobic matching and direct hydrophobic-hydrophobic lipid/protein interactions are considered as main determinants of the phase behavior of the lipids [10]. It enables T_g and T_f, the temperatures corresponding respectively to the beginning and the end of the gel to liquid phase transition, to be calculated:

$$T_g(X_p) = T_m + X_p(1-K_d)RT_m^2/\Delta H_L \quad (2)$$

$$T_f(X_p) = T_m + X_p(K_d^{-1}-1)RT_m^2/\Delta H_L \quad (3)$$

(X_p is the protein mol fraction, T_m is the phase transition temperature of the pure lipid, R is the gas constant, ΔH_L is the transition enthalpy of the pure lipid and K_d is the distribution constant of the protein between the fluid and gel phases. $K_d = X_f/X_g$ and X_f and X_g are the protein mol fractions in the fluid and gel phases, respectively).

The other approach is based upon elastic models within the Landau-de Gennes theory which attribute the full excess free energy to an elastic distortion of the lipid phase. In the case of protein/lipid hydrophobic mismatch, the main elastic force results from an expansion or a compression of the lipid bilayer by the protein in the normal direction and changes in the hydrophobic thickness of the lipids in contact with the protein are derived from changes in the order parameter of their acyl chains [9,10]:

$$\Delta T = 16\xi^2(\phi_p/\pi\xi + 1)((d_m-d_p)/(d_{L,f}-d_{L,g}))X_p \quad (4)$$

(ξ, d_p, $d_{L,f}$, $d_{L,g}$ and X_p have their above meaning, ϕ_p is the perimeter of the protein, $d_m = 1/2(d_{L,f}+d_{L,g})$ is the mean hydrophobic thickness of the unperturbed lipid bilayer between the fluid and gel phases).

Worthy to note, these equations are valid for the condition of a low protein concentration in the lipids only, i.e. for proteins in the monomeric form and not intercorrelated.

To be used, Eqs. 1–4 require the knowledge of thermodynamic and structural parameters which concern both the lipids and the proteins. ξ characterizes the distance over which the protein perturbs the lipids. A value of 1.5 nm was proposed, from theoretical arguments [16]. However, from Monte Carlo simulations, it appears that ξ is not a constant parameter but depends on d_p, ϕ_p, the protein/lipid hydrophobic mismatch (ξ is minimum in the absence of mismatch) and temperature [13]. Thus, ξ shows a dramatic peak at the T_m of the lipids [13,16] and in certain cases may take larger values than previously anticipated [13]. It also turns out from these studies that in contrast to what was previously assumed [16], ξ may differ from ξ_L, the correlation length of the pure lipid bilayer [13,17]. A crucial point is how to estimate d_p and d_L, two parameters which depend on both the structure and conformation of proteins and lipids and the thermodynamic parameters of hydrophilicity and hydrophobicity which characterize their various constitutive residues. In other words, where should the boundaries between the hydrophilic and hydrophobic domains of transmembrane proteins and lipid bilayers be settled? For membrane proteins, high resolution structures are scarce and by themselves would not allow for the boundaries to be positioned accurately. In the absence of accurate structural information, d_p may be estimated from hydropathy profiles. Helical structures are assumed to be oriented parallel to the bilayer normal and to contribute 0.15 nm per amino acid residue. More structural data are available for the lipids. However, hydrated bilayers are characterized by a steep, but not infinite, interfacial polarity gradient from the bulk of the water phase ($\varepsilon \sim 80$) to the bulk of the acyl chains ($\varepsilon \sim 2$),

Table 1
Hydrophobic lengths $d_{L,f}$ and $d_{L,g}$ of hydrated bilayers of diCn:0-PCs and diCn:1-PCs

Acyl chains	$d_{L,f}$ ±0.1 nm	$d_{L,g}$ ±0.1 nm	d_m
C12:0	1.95	2.7	2.3
C14:0	2.3	3.15	2.7
C16:0	2.6	3.6	3.1
C18:0	2.95	4.05	3.5
C14:1	2.3		
C16:1	2.65		
C18:1	3.0		
C20:1	3.35		

For the saturated species in the fluid phase, $d_{L,f}$ was obtained from the X-ray diffraction data of Lewis and Engelman [21] by removing 1.1 nm (2×0.55) to the phosphate to phosphate transbilayer distance (see text). In the gel state, $d_{L,g}$ was obtained from the X-ray diffraction data published for diC14:0-PC [22,23] and diC16:0-PC [23], still by removing 1.1 nm to the phosphate to phosphate transbilayer distance. For the four lipids, $d_{L,g}$ was also determined through direct calculation assuming elongated acyl chains oriented at 30° with respect to the bilayer normal. Consistently, the $d_{L,g}$ values so obtained may be deduced from the corresponding $d_{L,f}$ values as $d_{L,g} \sim 1.37 d_{L,f}$. d_m is the mean hydrophobic thickness $(d_{L,f}+d_{L,g})/2$. For the unsaturated species, $d_{L,f}$ was deduced from X-ray [19,21] and molecular dynamics simulations [20] data as above. $d_{L,f}$ values relate to lipids with a nearly constant molecular area of 0.66 nm^2 for the saturated species and 0.68–0.70 nm^2 for the unsaturated ones.

through the polar headgroup region [18–20], and the question of the positioning of the hydrophilic/hydrophobic boundary still remains. It seems reasonable to place the boundary where water ceases to be detected in the bilayer. In the fluid Lα phase and thanks to recent high resolution X-ray and neutron diffraction studies [19] and molecular dynamics simulations [20], this boundary may be positioned quite accurately at −0.55 nm from the Gaussian distribution function of the phosphate group. Because most of the available X-ray diffraction data refer to lipids in the fluid phase, $d_{L,g}$ is usually estimated from $d_{L,f}$. In previous works, this was done by considering lipid with their main axis oriented parallel to the bilayer normal [10]. Here, we propose to consider the tilted configuration which, as a matter of fact, is what prevails for most of the saturated phospholipids in the gel state and which can also exist in proximity to proteins. The $d_{L,f}$ and $d_{L,g}$ values so re-evaluated for a set of bis-saturated and unsaturated phosphatidylcholines (PCs) (diCn:0- and diCn:1-PCs, n being the number of carbon atoms in the acyl chains) is shown in Table 1. Details on the way they were obtained from published X-ray diffraction data are provided in the legend.

3. Experimental verification of the theory

3.1. Influence of proteins on the physical state of lipids

Eqs. 2–4 are easily accessible to experimental verification by studying the gel to liquid phase transition temperature of lipids having incorporated an integral membrane protein. They predict a linear variation of T_m with X_p and ΔT to be nil for $d_p = d_m$. This provides an alternative and elegant way to estimate d_p from the mean hydrophobic thickness d_m of the host lipids.

Available data concern the reaction center (RC) and light harvesting antenna (LHCP) proteins from *Rhodopseudomonas sphaeroides* [9], bacteriorhodopsin (BR) from *Halobacterium salinarium* [24] and melibiose permease (MelB) from *Escherichia coli* (Dumas, F., Tocanne, J.F., Leblanc, G. and Lebrun, M.C., submitted). These various proteins differ in size. From hydropathy profiles, they show d_P values of 2.8, 3.1 [9], 3.1 [24] and 3.0 nm (Dumas et al.), respectively. All affected the T_m of saturated PCs of different chain lengths (diCn:0-PC) and at a low and fixed protein concentration X_p, the changes in $T_m(\Delta T)$ were found to correlate with the hydrophobic mismatch $(d_P - d_m)$, both in magnitude and sign ($\Delta T > 0$ for $(d_P - d_m) > 0$ and $\Delta T < 0$ for $(d_P - d_m) < 0$).

BR ($d_P = 3.1$ nm) showed no influence on diC16:0-PC ($d_m = 3.1$ nm), illustrating the interesting case $\Delta T = 0$ for $d_P = d_m$ [24]. Data published for rhodopsin reconstituted in a set of diCn:0-PCs (protein/lipid molar ratio, 1/60) [25] may be re-analyzed in the same spirit. The protein had no influence on diC14:0-PC ($d_m = 2.7$ nm) while it increased and decreased the T_m of shorter and longer lipids, respectively. The rotational correlation time of the protein was minimum in diC14:0-PC and diC15:0-PC ($d_m = 2.9$ nm). This was interpreted by the authors as indicating a monomeric state for the protein in these two lipids and aggregation in the others, linked to an absence of hydrophobic mismatch [25]. Rhodopsin exhibits seven long α-helices whose mean hydrophobic length d_P is difficult to appreciate from hydropathy profiles. A d_P value of 2.7–2.9 nm may be deduced from the above observations. It matches well with the 2.8 nm proposed for the protein [26] and used in recent modelization of its three-dimensional structure [27].

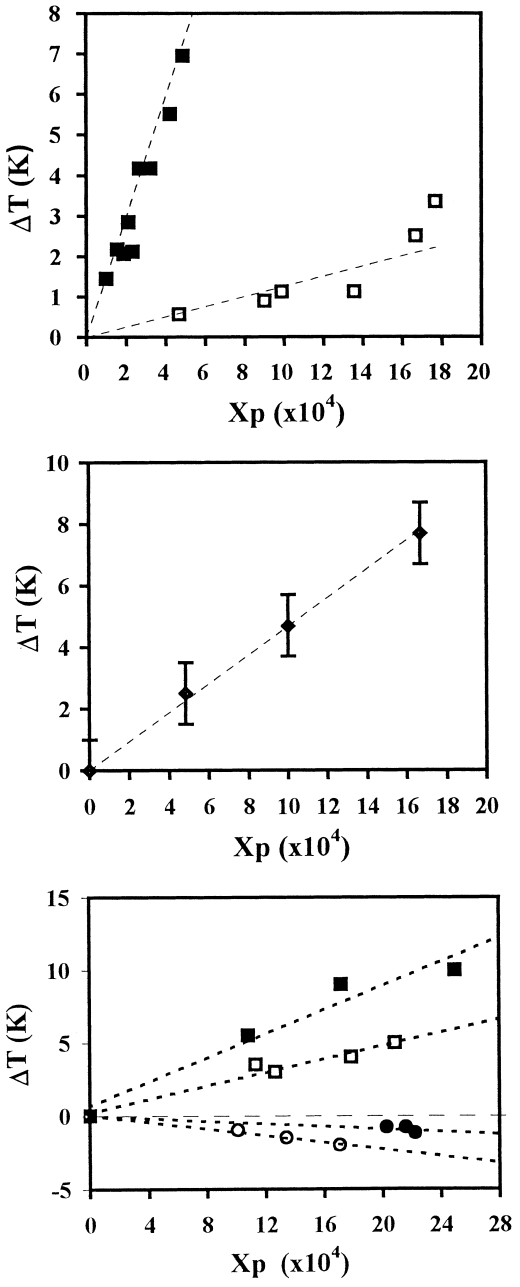

Fig. 2. Changes (ΔT) in the gel to liquid phase transition temperature of various disaturated PC species supporting an increasing concentration X_p of various membrane proteins. (A) RC (■) and antenna (LHCP, □) proteins in diC13:0-PC vesicles. Reproduced from [9] with permission. Line 1 was calculated using Eq. 4 with $\xi = 1.5$ nm, assuming a monomeric protein distribution. Line 2 was also calculated with $\xi = 1.5$ nm but assuming two-dimensional LHCP aggregates of an average size of 50 molecules [9]. (B) BR in diC12:0-PC. Reproduced from [24] with authors' permission. (C) MelB in diC12:0-PC (■), diC14:0-PC (□), diC16:0-PC (●) and diC18:0-PC (○) (Dumas et al., submitted). For BR and MelB, best fitting of Eq. 4 to the experimental data was achieved with $\xi = 1$ nm.

The linear variations of ΔT with X_p observed in Fig. 2 may be accounted for with Eq. 4, using ξ values of 1.5 nm for the RC and LHCP proteins [9] and 1.0 nm for BR [24] and

MelB (Dumas et al.), in the expected range. Introducing $\xi = 1.0$–1.5 nm in Eq. 1 leads to the prediction that the thickness of at least ~ 5–7 lipid annulus would be significantly perturbed by the proteins. Consistently, time-resolved fluorescence depolarization and energy transfer experiments carried out on BR/diC14:0-PC vesicles indicated that such a protein significantly influences the acyl chain order of the lipids up to a distance of 4.5 nm from its surface, i.e. over 5–6 lipid annulus [28].

Finally, it is worth noting that the ratio of the ΔT values obtained for a given protein re-incorporated in two different lipids L1 and L2 may be reduced to an expression in which now the sole unknown parameter is d_P:

$$(\Delta T_{L1}/X_{p,L1})/(\Delta T_{L2}/X_{p,L2}) =$$

$$((d_{m,L1}-d_p)/(d_{m,L2}-d_p))\cdot((d_{L2,f}-d_{L2,g})/d_{L1,f}-d_{L1,g})) \quad (5)$$

This stands on the reasonable approximation that the acyl chain-mediated conformational changes of the protein are of a small amplitude and without noticeable consequence on both its perimeter ϕ_p and the coherence length ξ. When applied to MelB and using the data in Fig. 2C, this approach yielded a d_p value of 3.0 ± 0.9 nm for the protein, similar to that of 3 nm estimated from hydropathy profiles (Dumas et al.).

3.2. Phase preference and molecular sorting of lipids by proteins

In lipid mixtures and as a direct consequence of the hydrophobic matching condition, a transmembrane protein is expected to be solvated by the lipid species capable of best matching its hydrophobic surface. This theoretical prediction of lipid sorting by proteins, resulting from computer simulations [14], is supported experimentally by data obtained with various proteins recombined in lipid mixtures and which suggest phase preference or lipid sorting depending on whether the lipids undergo lateral phase separation or form homogeneous mixtures. Thus, fluorescence quenching experiments showed a preference of the (Ca^{2+}, Mg^{2+})ATPase for diC16:0-PC in the fluid phase over the gel phase [29]. In a diC14:1-PC/diC18:1-PC 1/1 mixture, the protein showed an activity (17.9 i.u./mg) above that (13.9 i.u./mg) obtained upon averaging the activities found in diC14:1-PC (3.7 i.u./mg) and diC18:1-PC (24.1 i.u./mg), suggesting a preference of the protein for the unsaturated long chain lipid [30]. Fluorescence energy transfer experiments indicated that the pulmonary surfactant protein SP-C reconstituted in surfactant lipids was excluded from gel phase palmitoyl lipids and preferred shorter chain and unsaturated lipids below the bulk lipid phase transition temperature [31]. In mixtures of lipids in the fluid state and using pyrene-labelled phospholipids with different chain lengths (excimer to monomer fluorescence intensity ratio measurements), lactose permease showed a preference for those lipids whose hydrophobic length best-matched its hydrophobic surface [32]. In PC, MelB showed a minimal and optimal activity in diC12:0-PC and diC16:1-PC, respectively (vide infra) (Dumas et al.). In mixtures of these two lipids (Fig. 3), an averaged activity was measured up to 50 mol% diC16:1-PC while for higher concentrations, the activity was as found in diC16:1-PC alone (unpublished data), strongly suggesting a preference of the protein for this lipid. Straightforward demonstration of phase preference was obtained with BR in diC12:0-PC/diC18:0-PC mixtures. This work combined computer simulations and fluorescence resonance energy transfer experiments using 7-nitrobenz-2-oxa-1,3-diazol-4-yl-labelled lipids as the donors and the retinal group of BR as the acceptor. diC12:0-PC and diC18:0-PC exhibit strong non-ideal mixing over a wide temperature range, with the co-existence of nearly pure diC12:0-PC and diC18:0-PC domains in the gel-gel and fluid-gel states. As mentioned above, these two lipids can match or mismatch the hydrophobic length of the protein via a mechanism of chain extension or compression, depending on whether they are in the gel or fluid states. Consistently, the theoretical and experimental data showed mechanisms of phase preference or lipid sorting. Phase preference was observed at low and moderate temperatures, in the gel-gel and fluid-gel co-existence regions, where BR was found located exclusively in the solid or fluid diC12:0-PC-rich domains. Lipid sorting was detected at a high temperature, in the mixed fluid phase, where the protein was preferentially surrounded by diC18:0-PC at the expense of diC12:0-PC [33].

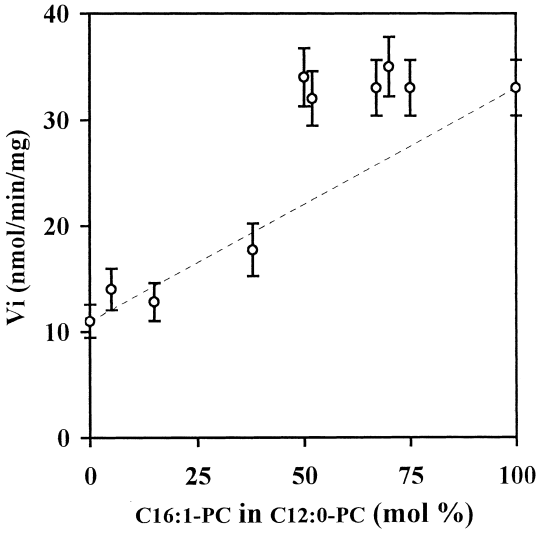

Fig. 3. Transport activity of MelB in diC12:0-PC/diC16:1-PC mixtures.

4. Hydrophobic mismatch and protein activity

With respect to proteins, the excess of energy due to a hydrophobic mismatch with the lipids is expected to have consequences on their conformation and/or aggregation state [4,6,34,35] and therefore on their activity. Many studies have shown that the activity of membrane proteins is sensitive to the acyl chain length of the supporting lipid bilayer [8]. However, any conclusion that hydrophobic mismatch is responsible for the observed effects should stand on systematic comparisons of d_P and d_L values. This was done in the case of MelB only (Dumas et al.). Nevertheless, we will also consider those proteins whose activity versus acyl chain lengths showed a bell-shape profile, an argument strongly in favor of mismatch. Thus, when reconstituted in diCn:1-PCs, the (Ca^{2+}, Mg^{2+})ATPase from rabbit muscle sarcoplasmic reticulum [30,36–40] and the (Na^+, K^+)ATPase from porcine kidneys [41] showed optimal activity at a lipid chain length of around

18–20 carbon atoms, shorter or longer acyl chains supporting lower activity. In both cases, addition of decane which is known to increase the bilayer thickness [42] increased or decreased the activity when the enzymes were reconstituted in the shorter or longer lipids, respectively. Interestingly, the amount of decane required to restore optimal (Ca^{2+}, Mg^{2+})ATPase activity in the short chain lipids was almost exactly equivalent to increasing the chain length to 20 carbon atoms [37], thus supporting the conclusion that hydrophobic mismatch was responsible for the loss in protein activity. The activities of the human erythrocyte hexose transporter in diCn:1-PC [43], of the leucine transport system of *Lactococcus lactis* [44] and *Pseudomonas aeruginosa* [45] in phosphatidylethanolamine/PC mixtures, of cytochrome *c* oxidase and the (F1,F0)ATPase complex in diCn:1-PC [46] were also found to be chain length-dependent, with maximum activity around 16–18 carbon atoms. MelB affords the first example in which the influences of the protein on the lipids and of the lipids on the protein were analyzed both quantitatively and by reference to the d_P and d_L parameters (Dumas et al.). As shown above, the gel to liquid phase transition temperature of diCn:0-PCs was affected by the protein in a way which could be accounted for with the theory and used to estimate its hydrophobic length d_P. When reconstituted in diCn:1-PCs, MelB showed optimal activity in diC16:1-PC and lower activities in shorter and longer lipids. With respect to the hydrophobic matching condition, diC16:1-PC in the liquid phase exhibits a hydrophobic thickness of 2.6 nm, slightly lower than the 3 nm estimated for the protein. Actually, due to various local constraints of free volume reduction [47] and compression-expansion [48–50], acyl chains in contact with the protein are expected to elongate, explaining why maximum activity and therefore best matching was found in diC16:1-PC and not in diC18:1-PC.

If the influence of the protein on the lipids may be interpreted in a straightforward manner in terms of acyl chain flexibility and order, the consequences of hydrophobic mismatch on protein activity are more difficult to analyze. Compression-expansion of the acyl chains [48–50] and more subtle effects like splay-distortion [49], surface tension [49] and line tension of the lipids in contact with the protein [50] may independently or in combination trigger an aggregation of the proteins or changes in their conformation [48]. Thus, the changes in activity of (Ca^{2+}, Mg^{2+})ATPase in diCn:1-PCs were shown to be correlated to its aggregation state, optimal activity being associated with minimal aggregation [39]. For large polytopic proteins and in particular for those endowed with carrier activity, conformational changes may be thought of first as corresponding to modifications in the relative orientation of the transmembrane segments. Thus, an increase in the concentration of lactose permease (a 12 α-helices transmembrane protein from *E. coli*) in lipids has recently been shown to result in a continuous decrease in transport activity in a manner that correlated with an increase in the average tilt angle of the helices with respect to the bilayer normal [51]. In the same respect, the orientation with respect to the bilayer normal of a synthetic α-helical peptide of 19 amino acids was shown to depend on the lipid acyl chain length and to be optimal for 18–20 carbon atoms [52]. In the various lipids tested, MelB remained in the monomeric state and displayed a similar affinity for its sugar substrate (Dumas et al.), suggesting that changes in transport activity were to be related to changes in the orientation of the transmembrane segments of the protein.

5. Relevance of the hydrophobic matching condition to biomembranes

There is no direct experimental evidence that the concept of hydrophobic mismatch operates in biological membranes. However, as already mentioned in Section 1, transmembrane proteins and lipids exhibit a quite wide variety of hydrophobic lengths and it is clear that random distribution of these various molecular species in membranes would be accompanied by a high degree of hydrophobic mismatch, an energetically unfavorable situation. The lipid and protein compositions of membranes being what they are, stabilization of these multimolecular assemblies may be achieved via a general mechanism of protein and lipid sorting based on the preference of membrane proteins for the lipid species or the lipid phases which can best match their hydrophobic surface. Such an idea has recently been introduced to explain the sorting and targetting of membrane proteins in the course of their synthesis and maturation, from the endoplasmic reticulum (ER) to the plasma membrane, via the Golgi apparatus and also their retention in the various membrane compartments to which they belong. This stands on the observation that the average length of the transmembrane domains (TMDs) of plasma membrane proteins, ~20 amino aid residues, is five amino acids longer than the average length, ~15 residues, of proteins from the Golgi [53–55] and that the lipid composition varies from one membrane compartment to another [54–56]. Along the way, from the ER to the plasma membrane, the concentrations of cholesterol and sphingomyelin and sphingoglycolipids increase causing an increase in the membrane thickness. Indeed, sphingolipids form longer cylinders than glycerophospholipids [56] while cholesterol, by increasing the lipid acyl chain order, increases the bilayer thickness [54,55]. Typically, addition of 30 mol% cholesterol to 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine at 25°C increases the bilayer thickness from 2.6 to 3 nm [57]. Furthermore, an increase in the concentration of cholesterol in phospholipids is accompanied by a process of lateral phase separation between a cholesterol-poor liquid phase and a thicker cholesterol-rich liquid-ordered phase [58] and there is evidence indicating a preference of cholesterol for sphingolipids [56]. On these grounds, a lipid sorting model has been proposed to explain how the Golgi enzymes remain in the Golgi apparatus while plasma membrane proteins are exported to the cell surface [54,55]. It states that along the secretory pathway, plasma membrane and Golgi proteins first co-exist in cholesterol-poor bilayers but that plasma membrane proteins segregate away as the cholesterol and sphingolipid concentrations increase and lipid domains form.

This concept of protein sorting via lateral partitioning between co-existing lipid domains is now supported experimentally by the results obtained with BR in binary lipid mixtures, in which a mechanism of phase preference was clearly put forward [33]. It is also supported by results indicating that the length, and not the composition, of the single TMDs of resident glycosylation enzymes of the Golgi apparatus may be an important parameter to prevent these proteins moving beyond the Golgi. Thus, lengthening the TMD of sialyl-transferase, an enzyme of the mammalian *trans* Golgi, gradually

increased the cell surface expression of this protein to a level approaching that of a control cell surface protein provided that five or more (nine) extra amino acid residues were added [59]. Similarly, insertion of four isoleucine residues in the TMD of β-1,4-galactosyltransferase, another enzyme of the Golgi, overrided the Golgi retention signal and directed the enzyme to the plasma membrane [60]. The concept also applies to tail-anchored proteins which normally reside in the ER and which are bound to membranes by a hydrophobic tail close to the C-terminus and which have most of their sequence as a cytosolically exposed N-terminal domain. For example, addition of five extra amino acids to the tail anchor of cytochrome b_5 led to re-localization to the plasma membrane [61]. Lengthening the hydrophobic segment of the protein UBC6 from 17 to 21 amino acids resulted in re-targetting from the ER to the Golgi complex while a further increase in length to 26 amino acids directed this protein to the plasma membrane [62].

The reverse effect was shown to occur with plasma membrane proteins. Expression of a type I plasma membrane protein with a synthetic TMD of 23 leucines led to its localization to the cell surface, but shortening the TMD to 17 leucines resulted in its accumulation in the Golgi [59]. Similarly, decreasing the number of hydrophobic residues in the TMD of the influenza virus neuraminidase shifted this protein from the plasma membrane to the Golgi and the ER [63].

The mechanism of protein sorting described above is reminiscent of that proposed for the apical and basolateral transport pathways of proteins and is based on the existence of cholesterol- and sphingolipid-rich lipid domains in membranes, the so-called lipid rafts [64,65]. The question of whether lipid rafts are concerned by the hydrophobic matching condition is to be addressed too and in this respect, the MAL proteolipid may afford an interesting track. MAL is a non-glycosylated and highly hydrophobic integral membrane protein of 16 kDa [66] expressed in T-lymphocytes, myelin-forming cells and polarized epithelial cells and is systematically found associated with lipid rafts. It has recently been shown to be necessary for normal apical transport and accurate sorting of the influenza virus hemagglutinin in MDCK cells, a pathway involving cholesterol- and sphingolipid-enriched membrane domains [67]. Interestingly, hydropathy profiles show the presence of four potential α-helices, on average 23 amino acids long [66]. This would correspond to an hydrophobic length d_p of 3.4 nm, much longer than that expected for a Golgi protein but which could match thick cholesterol- and sphingolipid-rich lipid domains. This might provide a physical basis for the involvement of MAL in the raft-dependent transport pathway of proteins from the Golgi to the cell surface.

6. Conclusions and perspectives

The question was: is the hydrophobic matching principle relevant to biological membranes? The answer is: 'more than likely, yes'. In model-reconstituted systems, it appears to be an operational concept grounded on sound theoretical simulations capable of accounting for firmly established experimental data. The various arguments described in Section 5 point to its relevance to biological membranes, with particular emphasis on the important question of protein sorting and targetting between the various cell membrane compartments. If the condition of hydrophobic mismatch can be easily generated in reconstituted sytems for measuring its consequences on lipid structure and protein activity, this situation is energetically unfavorable and is not believed to prevail in biological membranes. Instead, it is suggested that in any membrane, the hydrophobic mismatch inherent to the protein and lipid composition may be released by a process of protein aggregation or, more interestingly, via a general mechanism of protein/lipid sorting. Sorting may be understood at various levels: (i) a macroscopic level corresponding to the residence of certain proteins in a given membrane or to the lateral partitioning of proteins between co-existing lipid domains within the same membrane, (ii) at a molecular level corresponding to the preference of proteins for certain lipids and (iii) even at a submolecular level, corresponding to the selection by a protein of the lipid species capable of matching each of its hydrophobic transmembrane segments selectively. This concept of hydrophobic mismatch-dependent protein/lipid sorting is particularly attractive due to its inherent self-organizing character. It leads us to consider membranes as supramolecular assemblies whose organization is related at the most intimate level to the very chemical structure of the various protein and lipid components. If the concept opens new avenues in membranology, it is also clear that along with the necessity to develop new methodologies capable of depicting membrane organization in situ and at the molecular level, much biochemical and biophysical investigation is still required before the relationships between composition, structure and function in biological membranes will be fully elucidated.

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