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Thermodynamics and kinetics of protein incorporation into membranes

(hydrophobic effect/protein immobilization/lipid–protein interaction/hydrogen bonds/conformational entropy)

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ABSTRACT **The free energy and enthalpy of protein incorporation into membranes are calculated with special emphasis on the hitherto neglected effects of immobilization of protein and perturbation of lipid order in the membrane. The free energy change is found to be determined by the hydrophobic effect as the driving force for incorporation and the protein immobilization effect which leads to a considerable reduction of the free energy gained from the hydrophobic effect. For incorporation of a hydrophobic, bilayer-spanning α-helix, the free energy change obtained is of the order of −15 kcal/mol (1 cal = 4.184 J) in agreement with experimental results. The lipid perturbation effect yields only a small contribution to the free energy change due to an energy/entropy compensation inherent in lipid order. This effect dominates the enthalpy change, giving rise to values on the order of 100 kcal/mol with a pronounced temperature dependence around the lipid phase transition as observed experimentally. The kinetics of protein incorporation are even more strongly affected by the lipid perturbation effect, leading to an abrupt decrease of the rate of incorporation below the lipid phase transition.**

It is commonly accepted that the driving force for spontaneous incorporation of proteins into membranes is the hydrophobic effect. However, a number of other effects may support or counteract the hydrophobic effect. In general, the free energy of protein incorporation includes four contributions which arise from a change of (*i*) water structure, (*ii*) protein state, (*iii*) lipid state, and (*iv*) bonds between protein and water or lipid molecules.

The change of water structure represents the hydrophobic effect which originates in the reduction of the mobility of water molecules surrounding the protein. The change of the protein state comprises the external translational and rotational degrees of freedom which become immobilized upon incorporation of the protein and the internal degrees of freedom whose changes involve conformational changes and changes of internal bonds. The change of the lipid state accounts for the reduction of the mobility of the lipid molecules in a fluid membrane. This parallels the reduction of the mobility of water molecules in the hydrophobic effect and may therefore be called lipophobic effect of proteins. The change of bonds at the protein surface concerns mainly polar interactions such as hydrogen bonds and electrostatic interactions between polar amino acid residues and water molecules because the interaction energy between apolar residues and water or lipid molecules is relatively small.

The gain in free energy from the hydrophobic effect has been investigated thoroughly in the past (1–4). Estimates are also available for the free energy arising from the formation or breakage of hydrogen bonds and electrostatic bonds (3, 4) as well as from the change of protein conformation (5, 6). The protein immobilization effect has not been taken into account in previous work. This effect was studied in the context of enzyme–coenzyme binding where it was found to lead to a considerable reduction of the binding energy available from the hydrophobic effect (7, 8). The lipid perturbation effect also was not considered previously, although *a priori* there is no reason for this contribution to be small because a large number of lipid molecules may be affected by one protein molecule. From calorimetric measurements it is known that enthalpies up to hundreds of kcal/mol are involved in lipid–protein association (9–11).

In the present paper, the free energy and enthalpy of binding of a protein to a membrane are calculated, including the hitherto neglected effects of protein immobilization and lipid perturbation. The immobilization effect is estimated by a slight modification of the treatment in enzyme–coenzyme binding (8). The lipid perturbation effect is elaborated by adopting a previously developed continuum model for lipid–protein interaction (12–14). The results for the free energy and enthalpy of binding are compared with experimental data which have become available recently. The kinetics of protein incorporation are discussed along similar lines by estimating the contributions of the individual effects to the activation free energy and enthalpy.

Hydrophobic effect

The free energy gained from the hydrophobic effect upon incorporation of proteins into a lipid bilayer can be calculated in two ways: (*i*) on the basis of the amino acid sequence and the free energies of transfer of individual amino acid side chains from water into the vapor phase (15) or into hydrocarbons (16); or (*ii*) from the change of the protein/water interfacial area and a value for the free energy change per unit area (2). The second approach is used here. From studies of hydrocarbons and hydrophobic amino acids, the free energy per area was found to be 20–25 cal/(mol·Å^2) (1 cal = 4.184 J) (2, 17). The interfacial area is that area of a protein molecule that is accessible to water molecules. It can be estimated by describing the protein as a sphere or cylinder and multiplying the corresponding smooth area by a factor of 1.7 to account for the unevenness of the protein surface (2).

Considering the simple case of a protein or protein segment spanning the bilayer in the form of an α-helix, the helix is described as a cylinder of radius $R_o \approx 5$ Å and height $h \approx 30$ Å, the thickness of the hydrophobic core of the bilayer. If the protein also forms a helix in water, the change in effective interfacial area is $2\pi R_o h \cdot 1.7 \approx 1{,}600$ Å^2. With a value of 22 cal/(mol·Å^2) for the free energy per area, the free energy change upon incorporation is $\Delta G_W \approx -35$ kcal/mol of helix, as obtained by others (4). If the protein in water adopts an unfolded conformation, the relevant area is that of a cylinder whose length

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is given by the number of amino acid residues, about 20, times the length of one residue, 3.7 Å, and whose radius is 3.2 Å in order to yield the same volume as the α-helix. The change in effective interfacial area then is 2,500 Å^2 leading to $\Delta G_W = -55$ kcal/mol.

Because the hydrophobic effect originates in the reduction of the mobility of water molecules, it is predominantly of entropic nature. The enthalpy change is relatively small (1), less than a few kcal/mol, and will be approximated by $\Delta H_W \approx 0$.

Hydrogen bonds and conformational changes

If hydrogen bonds between protein and water molecules are broken upon incorporation of the protein and not restored in the membrane, an energy of 5.8 kcal/mol of hydrogen bond is lost (18). To prevent this large loss of energy, protein molecules in the membrane adopt a conformation that allows the intramolecular formation of hydrogen bonds. This is optimal in an α-helical conformation. Hence, the hydrogen bonds are the cause for the frequently observed α-helical conformation of membrane-incorporated protein segments (4). This implies that hydrogen bonds do not contribute much to the free energy change of protein incorporation.

Considering the final conformation of the protein in the membrane as α-helical, the change in the internal degrees of freedom depends upon the protein conformation in water. If the protein in water is also helical, the internal degrees of freedom do not contribute to the free energy change. If, however, the protein in water adopts an unfolded conformation, internal degrees of freedom become lost upon incorporation. The corresponding free energy change has been estimated for helix–coil transitions on the basis of 3 degrees of freedom per residue, each with two possible conformations, and amounts to 1.2 kcal/mol of residue (5). For the example of a protein or protein segment of 20 residues, this leads to a free energy change of $\Delta G_c \approx 24$ kcal/mol. Such a value is comparable to the free energy difference between the folded and unfolded conformations due to the hydrophobic effect. For the unfolded protein, ΔG_W was about 20 kcal/mol more negative, so that the conformational effect ΔG_c is approximately compensated for. Hence, the free energy change upon incorporation is expected to be about the same for the helical as for the unfolded conformation in water. This implies, furthermore, that the transition in water from the folded to the unfolded conformation does not involve a considerable free energy change, as previously noted (4, 5).

Protein immobilization effect

The change in free energy due to the immobilization of external degrees of freedom of a protein upon incorporation may be estimated by analogy to the case of enzyme–coenzyme binding (8). The protein in water is treated as a freely moving particle, its free energy given by that of an ideal gas; in the membrane it is treated as completely immobilized with 0 free energy. The change in free energy of the translational degrees of freedom then results as $\Delta G_t = NkT \ln[V_{free}/(N\lambda^3)]$ for N protein molecules in a volume V_{free}, $\lambda = h/(2\pi mkT)^{1/2}$ denoting the de Broglie wavelength with m the protein mass and k and h the Boltzmann and Planck constants. For later comparison with binding energies defined for a 1 M standard state, ΔG_t has to be calculated for a 1 M protein solution—i.e., N is Avogadro's number for $V_{free} = 1$ liter. A bilayer-spanning protein of 20 amino acid residues has a molecular weight of about 2,000, so that at $T \approx 300$ K one obtains $\Delta G_t \approx 10$ kcal/mol. Under the same assumptions, immobilization of the rotational degrees of freedom yields approximately the same value (8). Thus, protein immobilization is found to involve an energy change $\Delta G_P \approx 20$ kcal/mol. The concomitant enthalpy change ΔH_P for the six degrees of freedom is $-3RT$ or $\Delta H_P \approx -2$ kcal/mol. Hence, the immobilization effect is also of predominantly entropic nature like the hydrophobic effect. These values hold for complete immobilization of the protein in the membrane, which is not attained (at least not for a fluid membrane). One expects that only one translational and two rotational degrees of freedom will be immobilized so that the above values for ΔG_P and ΔH_P are reduced by a factor of 1/2.

For this estimation, the immobilized degrees of freedom were treated simply as being lost. Actually, they are subject to the binding potential which still allows some relative motion. In a more realistic approach one therefore may describe the immobilization of the translational degrees of freedom as the transition from a box of volume V_{free} to a smaller one of volume V_{bound}. The latter is given by the available membrane surface area multiplied by a length δz which measures the relative motion between the incorporated protein and the membrane in the direction of the membrane normal. The corresponding change in free energy, assuming ideal gas behavior of the protein in the boxes, is $\Delta G_t = NkT \ln[V_{free}/V_{bound}]$. This immobilization free energy is independent of the protein mass but depends on the lipid concentration. For a 0.1 mM lipid dispersion, assuming a membrane surface area of 50 Å^2 per lipid molecule and as an upper limit for the motion of the incorporated protein $\delta z \approx 1$ Å, one obtains $\Delta G_t \approx 8$ kcal/mol. The same procedure can be applied to the rotational degrees of freedom, volumes being replaced by the corresponding angular ranges. Assuming $\delta\phi_{bound} \approx 1°$ for the rotational motion of the incorporated protein and $\delta\phi_{free} = 360°$ leads to $\Delta G_r \approx 4$ kcal/mol per immobilized rotational degree of freedom. Hence, immobilization of a protein in a membrane involves a free energy change of $\Delta G_P \approx 16$ kcal/mol. The corresponding enthalpy change is 0, $\Delta H_P = 0$. These values are of the same order of magnitude as the above results derived on a less satisfactory basis.

Lipophobic effect

The free energy and entropy of the lipid perturbation induced by proteins upon incorporation are derived within the framework of a continuum model for lipid order (13, 14). The basic features of the model are the following. The lipid order is characterized by the orientational order of the hydrocarbon chains described by the order parameter S which is the average of the segmental order parameters along the chains. A protein molecule perturbs the lipid order by imposing a boundary order parameter S_o at the protein surface. With increasing distance from the protein surface the perturbation falls off exponentially with the lipid coherence length ξ until the unperturbed order parameter S_u is reached. Above the lipid phase transition, proteins order the lipids, hence $S_o > S_u$; below the transition they act disordering, $S_o < S_u$. For the free energy of lipid perturbation due to one protein molecule, at low protein concentration where overlap of perturbations from neighboring protein molecules can be neglected (14), one obtains

$$\Delta G_L = \nu^t Q \frac{8(T^h - T^t)}{T^t} \left(\frac{S_u - S_o}{S_u^t}\right)^2 \quad [1]$$

with

$$\nu^t = \left(\frac{\xi^t}{R_L}\right)^2 \left(2\frac{R_o}{\xi^t} + 1\right). \quad [2]$$

Here Q denotes the latent heat per lipid molecule at the phase

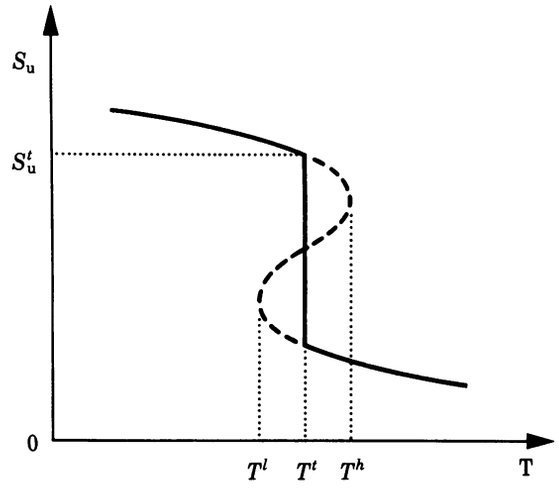

FIG. 1. Temperature dependence of the unperturbed order parameter S_u of a lipid bilayer, distinguishing stable (——) and metastable or unstable (---) states.

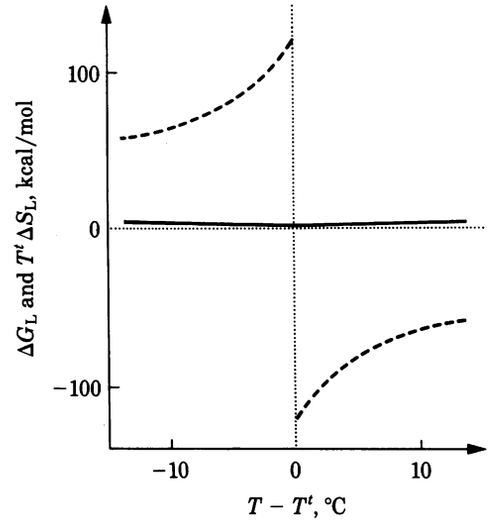

FIG. 2. Temperature dependence of the free energy ΔG_L (---) and the entropy $T^t\Delta S_L$ (——) of lipid perturbation by a bilayer-spanning α-helix.

transition, and T^t, T^h, and S_u^t are specified in Fig. 1. ν^t represents the number of lipid molecules within the coherence length at $T = T^t$, which may be called boundary lipids. Protein and lipid molecules are described as cylinders of radius R_o and R_L, respectively. Assuming S_o to be temperature independent and $S_u(T)$ to vary symmetrically around the phase transition as shown schematically in Fig. 1, the perturbation entropy $\Delta S_L = -\partial\Delta G_L/\partial T$ results as

$$T^t\Delta S_L = 2\nu^t Q\left(\frac{T^h - T^t}{(T^h - T^t) + |T^t - T|}\right)^{1/2}\frac{S_u - S_o}{S_u^t}. \quad [3]$$

If S_o lies midway between the unperturbed order parameters of the fluid and ordered phases at $T = T^t$, ΔG_L is symmetric with respect to the phase transition and ΔS_L is antisymmetric as shown in Fig. 2. The pronounced temperature dependencies of ΔG_L and ΔS_L represent a pretransitional behavior as known from response functions such as the lateral compressibility of membranes (14). By analogy, they should be extended over a temperature range of a few degrees on both sides of the phase transition.

An intuitive feeling for the above result is obtained by considering the case $S_o = 0$—i.e., a protein in the ordered lipid phase reduces the lipid order at its surface to that of the fluid phase. In the limit $T << T^t$ where S_u varies proportionally to $(T^t - T)^{1/2}$, Eq. 1 yields

$$\Delta G_L = \left(\frac{3}{4}\right)^2 \nu^t Q\frac{T^t - T}{T^t}. \quad [4]$$

This relationship states that the perturbation free energy per protein molecule is given by the product of the latent heat Q per lipid molecules as a measure of the amount of energy involved, the number of boundary lipids ν^t as a measure of the number of lipid molecules involved, and a temperature factor which vanishes at $T = T^t$. This factor expresses an energy/entropy compensation which is a characteristic feature of first-order phase transitions. Incorporation of a protein molecule into a membrane at $T < T^t$ therefore involves a large increase in lipid enthalpy and entropy due to fluidization of a large number of boundary lipids but only a small increase in free energy.

For a quantitative estimate we again consider a bilayer-spanning α-helix. With $R_o = 5$ Å, $R_L = 5$ Å, and $\xi^t = 15$ Å (14), Eq. 2 yields $\nu^t = 15$ for the number of boundary lipids in the membrane plane or $\nu^t = 30$ per protein in the bilayer. Experimental evidence suggests that, at $T - T^t$, $S_o \approx 0.5$ independently of the kind of protein (19). The same value is assumed for $T < T^t$, so that, at $T = T^t$, S_o lies midway between the unperturbed order parameters $S_u^t \approx 0.8$ and 0.2 of the ordered and fluid phases, respectively. For the latent heat, the value of dimyristoyl phosphatidylcholine is used, $Q = 5.4$ kcal/mol (20); for the temperature factor, the theoretical value $(T^h - T^t)/T^t \approx 1/160$ is used (14). With these values, Eqs. 1 and 3 yield, at $T = T^t$, $\Delta G_L \approx 1$ kcal/mol and $T^t\Delta S_L \approx \mp 122$ kcal/mol, the minus and plus signs referring to temperatures immediately above and below T^t, respectively. At 10°C above or below T^t, assuming $S_u = 0.1$ or 0.9, the free energy change is increased to $\Delta G_L \approx 2$ kcal/mol, whereas the entropy change is decreased to $T\Delta S_L \approx \mp 64$ kcal/mol (Fig. 2).

Total free energy and enthalpy change

The total change of free energy upon incorporation of a protein into a membrane is $\Delta G° = \Delta G_W + \Delta G_P + \Delta G_L$, the sum of the contributions from the hydrophobic effect, the protein immobilization effect, and the lipophobic effect. The binding energy $\Delta G°$ determines the binding constant $K_b = \exp(-\Delta G°/RT)$ for the equilibrium $P + L_\nu \rightleftarrows (PL_\nu)$ with the assumption that a complex of ν lipid molecules represents a binding site for a protein molecule (21). For incorporation of a hydrophobic, bilayer-spanning α-helix the individual contributions determined above are plotted in Fig. 3, together with the resulting value for $\Delta G°$. The largest contribution arises from the hydrophobic effect, $\Delta G_W \approx -35$ kcal/mol, causing spontaneous incorporation. The immobilization effect also yields a large contribution, $\Delta G_P \approx +16$ kcal/mol, and leads to a considerable reduction of the free energy gain. The contribution from the lipid perturbation effect is relatively small, $\Delta G_L \approx +2$ kcal/mol. Hence, the total free energy change, $\Delta G° \approx -17$ kcal/mol, is determined essentially by the hydrophobic effect and the counteracting immobilization effect. This result holds irrespective of whether the protein in water is folded in an α-helix or unfolded because the free energy change due to the conformational change is compensated for by the increased hydrophobic effect. Furthermore, this value of $\Delta G°$ is relatively independent of temperature and exhibits only a weak minimum at the lipid phase transition, where $\Delta G° \approx -18$ kcal/mol.

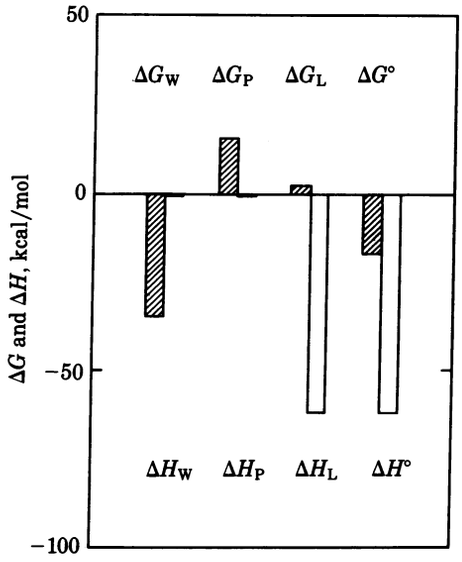

FIG. 3. Contributions from the hydrophobic effect (subscript W), the immobilization effect (P), and the lipophobic effect (L) to the change in free energy (hatched) and enthalpy (open) upon incorporation of a hydrophobic, bilayer-spanning α-helix into a membrane above the lipid phase transition.

It is interesting to estimate the limiting length of a hydrophobic helix for which spontaneous incorporation ceases. With decreasing length of the helix the hydrophobic effect decreases proportionally to the helix length, whereas the immobilization effect is independent of the molecular weight of the protein. Because, for a bilayer-spanning helix ΔG_W is about twice as large as ΔG_P and of opposite sign, the total free energy change $\Delta G°$ vanishes approximately for a helix that spans only one monolayer. In this case, open hydrogen bonds in the middle of the bilayer may further hinder incorporation.

The contributions to the total enthalpy change $\Delta H°$ and the resulting value for $\Delta H°$ are included in Fig. 3. Here, the contributions from the hydrophobic effect and the immobilization effect are vanishingly small, $\Delta H_W \approx \Delta H_P \approx 0$, and the lipid perturbation effect dominates, $\Delta H° \approx \Delta H_L \approx -62$ kcal/mol at 10°C above T^t. Therefore, $\Delta H°$ has a pronounced temperature dependence dictated by ΔS_L (Fig. 2). Approaching the phase transition, $\Delta H°$ becomes larger and, immediately above T^t, $\Delta H° \approx -121$ kcal/mol. At the phase transition, $\Delta H°$ changes sign and $\Delta H° \approx +123$ kcal/mol immediately below T^t.

Comparison with experimental results

The binding constant for insertion of melittin into bilayers of dimyristoyl phosphatidylcholine has been reported (21). The membrane-incorporated part of melittin can be regarded as an example for a hydrophobic, bilayer-spanning α-helix, and therefore a binding energy of $\Delta G° \approx -17$ kcal/mol would be predicted. This value may decrease by a few kcal/mol because of a charged residue in the hydrophobic segment (3, 4). The experimental result is $\Delta G° \approx -9$ kcal/mol and thus in good agreement with the theoretical estimate. The same result for $\Delta G°$ was obtained at two temperatures above and below the lipid phase transition. This again is consistent with the theoretical prediction. Another example is cytochrome b_5, for which approximately the same binding energy, $\Delta G° \approx -11$ kcal/mol, follows from experimental data (22). The hydrophobic segment of cytochrome b_5 is larger than that of melittin, but its conformation is not well known.

According to the theoretical estimate, the limiting case for spontaneous incorporation is given by a helix that spans one monolayer. An example of such a case is gramicidin A, whose 15 residues adopt a π_6 helix about 16 Å long (23). Nonetheless, gramicidin A is known to incorporate readily into membranes as a monomer. The solution to this puzzle lies in the extraordinary high hydrophobicity of the gramicidin A amino acid sequence, which is about a factor of 2 higher than the average hydrophobicity of membrane-incorporated protein segments (24). Hence, the hydrophobic effect is again of the same magnitude as for a bilayer-spanning helix of average hydrophobicity, thus guaranteeing spontaneous incorporation. The unique sequence of gramicidin A would thus be a necessary requirement for its spontaneous incorporation into membranes.

Another case for which binding studies are available is that of so-called amphipathic helices such as the apolipoproteins or glucagon. These are considered to associate with membranes by forming α-helices whose hydrophobic side penetrates into the membrane whereas the hydrophilic side remains in contact with water. Compared to the case of a hydrophobic helix, the hydrophobic effect is thus reduced by a factor of 1/2. Analogous to the case of a monolayer-spanning hydrophobic helix of 10 residues, $\Delta G°$ is expected to vanish for an amphipathic helix of 20 residues. Indeed, such a helix was found to behave ambiguously (25) because it penetrated spontaneously into membranes of dimyristoyl phosphatidylcholine up to a temperature of 40°C but not at higher temperatures, indicating that the binding energy is close to 0. The polypeptide glucagon consists of 29 residues so that part of the reduction of the hydrophobic effect, compared to a hydrophobic helix, is compensated for by the increased length of the amphipathic helix. The theoretical estimate would be $\Delta G_W \approx (1/2)\cdot(3/2)\cdot(-35) \approx -26$ kcal/mol leading to $\Delta G° \approx -9$ kcal/mol at $T \approx T^t$. The experimental result is $\Delta G° \approx -7$ kcal/mol at $T \approx T^t$, with a slight increase at lower temperatures (11, 26). Thus, the theoretical estimates are reasonable also for amphipathic helices.

For the enthalpy change, experimental results for a hydrophobic helix are not available but, for the amphipathic helices of apolipoproteins and glucagon, $\Delta H°$ has been measured by mixing calorimetry (10, 11). In both cases $\Delta H°$ was found to behave qualitatively as shown in Fig. 2 for the lipid perturbation effect. This provides direct evidence for the dominating role of the lipid perturbation effect in the enthalpy change. The numerical results for the association of apolipoprotein AII with dimyristoyl phosphatidylcholine were $\Delta H° \approx -250$ kcal/mol and +100 kcal/mol immediately above and below T^t (10), respectively; for glucagon, $\Delta H° \approx -150$ kcal/mol and +80 kcal/mol (11). The values for glucagon are comparable to those calculated for a hydrophobic helix. Because the number of perturbed lipids for glucagon is close to that for a hydrophobic helix (26), this implies that the lipid perturbation effect is quantitatively described by the theory presented. The difference in the absolute values of $\Delta H°$ immediately above and below T^t may result from an asymmetry of $\partial S_u/\partial T$ or from different values of S_o above and below T^t. The enthalpy change observed for apolipoprotein AII is larger than that for glucagon, as expected because this protein is larger than glucagon and perturbs more lipid molecules. For the apolipoproteins a proportionality between $\Delta H°$ and the change in helicity upon association with membranes was observed (10). Because the helicity reflects the length of the membrane-penetrating part of the proteins and this length should be proportional to the number of perturbed lipids, this observation is consistent with the theoretical result that $\Delta H° \approx \Delta H_L$ is proportional to the number of perturbed lipids.

Kinetics of protein incorporation

The kinetics of protein incorporation can be treated along lines similar to those derived above for the thermodynamics. In general, incorporation proceeds through an activated state whose free energy $\Delta G^\dagger$ determines the incorporation rate $k \approx \exp(-\Delta G^\dagger/RT)$. One might assume that in the activated state the protein molecules are immobilized at the membrane surface but still largely in contact with water. Hence, the hydrophobic effect is weak, whereas the immobilization effect contributes to $\Delta G^\dagger$. Immobilization of internal degrees of freedom and breakage of hydrogen bonds are expected to further increase $\Delta G^\dagger$. Considering the lipophobic effect, we assume that no strong perturbation of lipids is required in the activated state at $T > T^t$, but at $T < T^t$ the ordered lipids have to be completely fluidized. This corresponds to a boundary order parameter $S_o = 0$ so that the contribution ΔG_L to $\Delta G^\dagger$ is given by Eq. 4. As a consequence, $\Delta G^\dagger$ increases drastically below T^t but remains continuous at $T = T^t$ due to the energy/entropy compensation inherent in the lipid phase transition. Therefore, the incorporation rate should decrease abruptly below T^t and, in an Arrhenius plot, one would obtain a break at T^t with a low activation energy above T^t and a high activation energy below T^t. Qualitatively, such a behavior has been observed experimentally (25, 27).

It should be mentioned that another type of behavior has also been observed, showing a maximum of the incorporation rate at the phase transition (25, 28). Such a behavior can be interpreted as a consequence of cooperative fluctuations of lipid order around the phase transition (14).

A similar treatment may be applied to protein activity (29). The activity of membrane proteins often exhibits a break at T^t with a low activation energy above T^t and a high activation energy below T^t (30, 31). One is tempted to interprete this behavior by postulating that protein activity requires a fluid lipid environment. By analogy to the case of incorporation, the activation energy below T^t is then dominated by the lipid perturbation effect leading to the break at T^t and the high activation energy below T^t. Thus, the lipid perturbation effect is of increased relevance for the kinetics of protein incorporation and protein activity compared to the thermodynamics of binding because, below the phase transition, the lipid perturbation in the activated state is stronger than in the equilibrium state.

Conclusion

The free energy governing the incorporation of a protein or protein segment into a membrane is determined by essentially two effects: the hydrophobic effect and the protein immobilization effect. The lipophobic effect due to lipid perturbation yields only a small contribution because of an effective energy/entropy compensation. The immobilization effect leads to a considerable reduction of the free energy gained from the hydrophobic effect so that the binding energy for a hydrophobic α-helix spanning the bilayer is of the order of -15 kcal/mol helix.

Such a relatively low binding energy has consequences on the insertion of proteins into and across membranes for which the helical hairpin hypothesis was proposed (4). In this model, due to its driving force for incorporation, a leader sequence is able to pull polar parts of a protein into the membrane in the form of a hairpin. Because the leader sequence is an example of a hydrophobic bilayer-spanning α-helix, the above estimate for the free energy applies, and thus the driving force for incorporation is considerably smaller than suggested on the basis of the hydrophobic effect alone (4). On the other hand, a low binding energy offers the possibility to regulate incorporation by other effects of low energy such as the electrostatic interaction between charged amino acid residues and a membrane potential (32, 33).

The lipid perturbation effect dominates the enthalpy change associated with protein incorporation. This effect is distinguished from the other effects by a characteristic temperature dependence around the lipid phase transition which permits its experimental verification. The influence of the lipid perturbation effect is more strongly pronounced in the kinetics of protein incorporation and in protein activity in which, due to its temperature dependence, it can provide a sensitive regulation mechanism.

I am indebted to J. Owicki for his proposal for the improvement of the immobilization effect.

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