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Rapid Report

Do helices in membranes prefer to form bundles or stay dispersed in the lipid phase?

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A recently developed computational technique, utilized to study different possible lipid/helix aggregates in a membrane layer, shows that hydrophobic helices have an appreciable preference for packing together rather than for staying dispersed in the lipid phase, preference stemming from the strength of the helix–helix interactions due to the hydrophobic nature of the helices.

Integral proteins in membranes are partially buried in the lipid phase where they adopt specific structures indispensable for the accomplishment of their function. Contrary to the situation which prevails in soluble proteins, information concerning the structure of membrane proteins is very limited, owing to the rarity of X-ray data. One structural information, however, based on early results on bacteriorhodopsin [1,2], and confirmed since by the recently obtained high-resolution structures [3–5], has led to generalize the hypothesis that the crossing of the lipid phase by a number of membrane proteins involves hydrophobic polypeptidic segments inserted as α-helices perpendicularly to the bilayer surface and forming more or less closely packed bundles. The factors leading to the formation of these structures have been discussed on the basis of a 'two-stage-model' [6], in which the first stage is the individual folding of the hydrophobic segments into helices autonomously stable in the lipid phase, and the second the formation of their appropriate assemblies. The intrinsic stability of the helical structure in the lipid phase compared to the random coil in water has been rationalized on the basis of the gross evaluation of the free energy balance involved [6,7]. The possible factors intervening in the second step have only been considered qualitatively. We present, in this note, the results of a quantitative evaluation of the essential energy contributions implied in the aggregation of helices in lipids and of the resulting implications concerning their tendency to bundle formation.

Explicitely, the question we address is whether the molecular interactions existing in the hydrocarbon region of a lipid layer favor the assembling of the helices or their dispersion among the lipids, and if so, to what an extent.

After defining three model systems representing different helix-lipid aggregates we calculate, in each case, the global stability of the various architectures, taking into account the lipid–lipid, lipid–helix and helix–helix interactions. The procedure utilized, developed in details elsewhere [8], treats a multicomponent membrane as a crystal-like two-dimensional array of repetitive unit cells and allows the optimization of the energy of a cell in the presence of all the others, including the conformational flexibility and translations and rotations of each molecule in the cell as well as the variation of the lattice parameters. Due to the crystal-like nature of the structure, the information contained in one cell so optimized is sufficient to charaterize the whole layer. The energy between cells includes all intermolecular energies and the energy inside a cell includes both the intermolecular energies between its molecules and the intramolecular (conformational) energy for each of them. These energies are computed as sums of electrostatic, Lennard-Jones and torsion components appropriately parametrized [9–12]. After energy minimization, a given molecule in the optimized unit cell can be characterized by its dissociation energy, DE, (energy necessary to extract the molecule from the system) which is the sum of its interaction energy with

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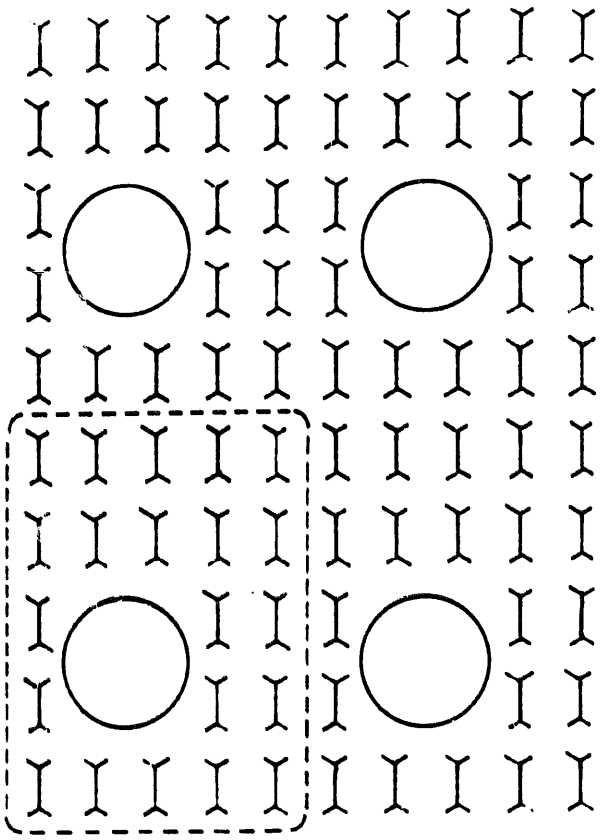

Fig. 1. Definition of the initial unit cell and of the layer in 'helix in lipids' (II).

all the other molecules in the cell and outside it and of the variation of its conformational energy upon extraction. The analysis and comparison of these dissociation energies for different aggregates allows a comparison of their stabilities.

The model systems presently used are composed of two species representing, respectively, the hydrophobic α-helical segments and the hydrocarbon part of the lipids: the helices are made of the simplest hydrophobic amino acid, alanine, and comprise 14 residues, a number sufficient to insure the constancy of the optimal structure of two interacting helices [13,14], the chains comprise 14 saturated carbons in an initially all-*trans* conformation. Three modes of co-packing of the helices and the chains were considered:

(I) 'separated phases', made of two separate regions; one comprising helices only (Ia), the other hydrocarbon chains only (Ib).

(II) 'helices in lipids', where the helices are dispersed among lipids in such a way that each helix is surrounded by three shells of lipids (see Fig. 1).

(III) 'lipids in helices', where individual or a few lipids are interspersed within a layer essentially made of helices.

In Ia (helices only), the unit cell is defined by two antiparallel helices and the layer is constructed by translating this cell in the plane perpendicular to the helix axes to form a hexagonal lattice with appropriate parameters. The cell energy is minimized with respect to the parameters of the lattice, the rotations of the first helix in each cell, the translations and rotations of the second helix and the angles of torsion in each helix. (It was verified that the inclusion of more helices in the cell does not modify significantly the results). In Ib (lipids only), the unit cell contains one chain and the lattice is built by translating the center of the chain in a plane perpendicular to its axis. The cell energy was first minimized in allowing the three rotations of the chain and the variation of the lattice parameters, then all single bond rotations. (Again the inclusion of more chains in the cell does not modify the results).

In II ('helices in lipids'), one cell was constructed as including one helix parallel to the normal to the layer and twenty-one chains parallel to the helix axis. The layer is such that any helix is surrounded by three shells of chains.

In III ('lipids in helices'), two possible arrangements were considered. In 111a the cell is built by placing four helices and four chains in such a way that, after the formation of the total layer, a pair of chains is surrounded by five helices (see Fig. 2a). IIIb corre-

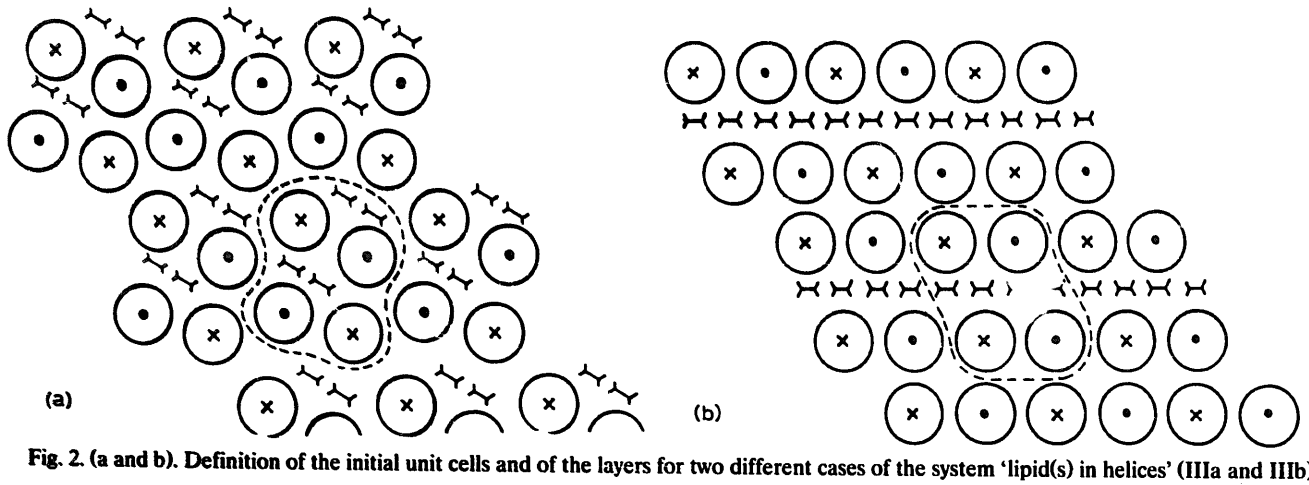

Fig. 2. (a and b). Definition of the initial unit cells and of the layers for two different cases of the system 'lipid(s) in helices' (IIIa and IIIb).

TABLE I

Average dissociation energies (kcal/mol) of one helix, DEH and of one lipid, DEL, from each layer and their mean, MDE

II and III stand, respectively, for 'helix in lipids' and 'lipid(s) in helices'.

	DEH	DEL	MDE
I	72.1 *	48.4 **	60.3
II	55.8	41.8	48.8
IIIa	74.1	27.9	51.0
IIIb	74.0	29.9	52.0

* From system Ia (pure helices).
** From system Ib (pure lipids).

sponds to a cell made of a different combination of four chains and four helices so as to give rise to a layer in which one sheet of chains is sandwiched between helix aggregates.

Table I summarizes the results for the four systems characterized by the averaged dissociation energies for a helix molecule in the unit cell (DEH) and the same for a molecule of lipid (DEL). Averaging accounts for the fact that every molecule of each species in the optimized unit cell differs somewhat from its congener as illustrated on the example of Fig. 3. The values of MDE, average of DEH and DEL, represent on a comparative basis the overall stabilities of the systems.

These results indicate that case I, where the helices and the lipids are packed completely separately is, by far, the most stable arrangement. This is followed by case III (a or b), where some lipids are interspersed among aggregates of helices, while case II, in which

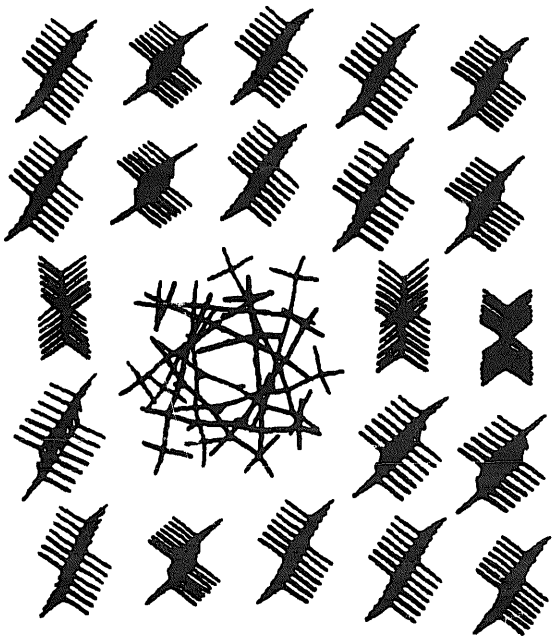

Fig. 3. The inequivalence of the lipid chains in the optimized unit cell of system II. View from above.

one helix is isolated in the midst of lipids, is the least favored. Layer I is favored both by the helices and by the lipids as seen in the values of the helix and lipid dissociation energies, both the largest in that case: the helices prefer to be in their own aggregate and so do the lipids, although with a lesser strength. In mode III where the lipids are interspersed between helices, DEH is about the same as in the separate phase but DEL is appreciably smaller than in that phase. In other words, the intercalation of some lipids in the cluster of helices at most deepens slightly the potential well of a helix in the cluster, but is unfavorable from the point of view of the lipids.

The comparison of the values of the helix dissociation energies DEH in the three types of layers shows that the major element responsible for the preference observed is the strength of the helix–helix interactions: while DEH is about the same in I and III, its value is strongly decreased in II where no helix-helix interaction is present. The helix-lipid interaction energy is clearly not large enough to compensate for the corresponding loss in stability, as shown by comparing the values of DEL, appreciably decreased in II with respect to that in the pure lipid cluster.

Two remarks may be added concerning the dependence of these conclusions on the model adopted:

(i) Since the helix-helix interaction between hydrophobic transmembrane helices of sufficient length is dominated by the attractive Van der Waals/London component of the energy which increases with the number [13] of methylene groups of the hydrophobic residues, the replacement of the polyalanine helices by hydrophobic segments resembling more closely the sequences observed in real membrane proteins (including valines, leucines, isoleucines, etc.) would increase the helix–helix interactions (see Ref. 15 for an explicit example). A similar effect would occur upon replacement of the 14-membered helices by longer segments comprising the 20 residues necessary to span the width of the hydrocarbon layer in a membrane (see Ref. 13). (Although, in both cases, the lipid–helix interactions would also be increased by terms of a similar nature but smaller, the balance should remain in the same direction).

(ii) The above conclusions which favor the assembling of helices, are based on enthalpy differences. It has been suggested that entropic effects would favor the separation of helices, an admittedly very crude estimate indicating a value "in the range of 1 to 10 kcal/mol" for the separation of a pair of helices [7]. It may be noted that the enthalpy differences obtained here seem sufficiently large to overcome even the largest estimate of the entropic contribution.

To the question raised at the beginning, it may be answered presently thus that, within the limits of the model adopted, the interactions which exist in the

hydrocarbon region of a membrane (lipid–lipid, lipid–helix, helix–helix) concur to favor the assembling of helices over their dispersion in the lipid phase. The separate assembly of the helices and of the lipids in two distinct phases is by far the most favored, the second best arrangement being the packing with partial lipid insertion among helices, the helix–helix interactions playing a leading role in the preferences observed. Overall, the preference for packing inside the membrane appears as an intrinsic property of the hydrophobic helices due to their amino acid composition which, not only allows a favorable interaction with the lipid phase as commonly considered, but also insures the strength of the lateral interactions between the helices necessary to form sufficiently stable functional structures when needed.

Let us, finally, point out that, since the present results have been obtained using helices containing pure hydrocarbon side chains, a further conclusion of this study is that the involvement of local hydrogen bonds and/or salt bridges between helices is not indispensable for bringing them together (although their presence brings an increment of bundle stability [15,17]).

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