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Molecular aspects of channel formation and ion transport through membranes

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Abstract – A summary is presented of theoretical studies aiming at a better understanding of the coupling properties of polypeptide α-helical segments, and of their aggregation into bundles of various sizes capable of enclosing channels apt to transport ions through biological membranes. The molecular aspects are stressed.

INTRODUCTION

Biological membranes surround the cells and their various compartments. They maintain the different domains within limits but also allow proper communication between them when needed, insuring the appropriate in and out flux of molecules and ions. Membranes are essentially made of lipids, long hydrocarbon chains (with polar heads) packed side-by-side all along their length, forming a tail-to-tail double layer with all the polar heads on one and the other side (Fig. 1).

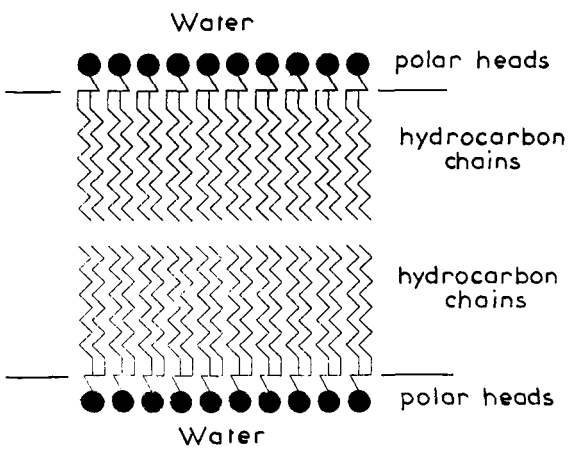

Fig. 1 :
A schematic view of a bilayer membrane.

Such a structure presents a very strong resistance to crossing by ions. To circomvent this resistance, nature uses special molecules or macromolecules which serve as carriers, channels or pumps. The carriers use "ionophilic" groups to capture and envelop the ion and carry it through the membrane inside a cage with a lipophilic exterior. Channels and pumps are made by special proteins which are partly inserted in the membrane.

We shall limit ourselves here to the consideration of ion channels, and to some of the problems they raise in which theoretical studies can be of use.

THEORETICAL STANDPOINT

The problems raised in the study of channel formation and fonctionning involve large or very large molecules or molecular systems in interaction and possibly undergoing conformational changes. In spite of the considerable progress accomplished lately, the standard -and quite accurate- methodologies of quantum chemistry available nowadays are not applicable. However, they can serve as the basis of development of accurate appropriate techniques adapted to the problems considered. Such techniques rely on the theory of intermolecular forces which expresses the energy of interaction of two molecular entities by a sum of contributions, electrostatic, repulsion, dispersion and polarization (ref. 1) :

$$E = E_{el} + E_{rep} + E_{disp} + E_{pol} \qquad (1)$$

where each component can be expressed as a sum of atom-atom (or bond-bond) interactions, each

term depending on the distance according to the nature of the force it corresponds to (for example 1/r for electrostatic terms, exponential for repulsion, $1/R^6$ for dispersion terms, etc). On the other hand each term contains parameters, or rather, constants, characteristics of the atoms or bonds involved. In the past the functions (or "potentials") utilized were often highly simplified, the constants choosen with some arbitrariness and some terms were dropped altogether. This situation has led pure theorists (and experimentalists alike) to a very widespread feeling of suspicion concerning the use of the so-called "empirical potential functions".

The state of the art, however, has considerably changed in the recent years for two reasons : on the one hand, the developments which have occured in perturbation theory have led to a clearer understanding of the different terms, in particular of their distance-dependence ; on the other hand, the constants involved in each term can now be carefully fitted so as to reproduce the results of accurate calculations on small interacting entities, results which themselves can be tested by gas-phase measurements. Similarly the variation in energy accompanying rotation about single bonds can be calculated as the variation of the interaction energy between the rotating fragments by a expression analogous to (1) supplemented by a torsion term, all parameters being similarly fitted by comparison with accurate conformational energy calculations.

A methodology based on these principles bas been developed in our Laboratory in the past few years and has resulted in the SIBFA (ref. 2 to 4) and FLEX (ref. 5, 6) procedures which allow the optimization of the energy of a system of molecules in interaction, including simultaneously the interaction energies and the conformational energy variations in the global optimization. The FLEX procedure, the most appropriate to deal with very large systems, was utilized for the problem considered below.

STRUCTURE OF CHANNEL-MAKING PROTEINS

Due to their insertion into lipids, it has proven extremely difficult to obtain crystals of membrane proteins appropriate for X-ray analysis, so that much less is known about their structure than about that of soluble proteins. One breakthrough in this domain started with the obtention of a low-resolution map of the electron scattering density of bacteriorhodopsin in the purple membrane (ref. 7) which allowed the building of a model of its membrane-spanning part as made of seven α-helical segments of the polypeptide chain crossing the bilayer roughly perpendicularly to the surface and closely packed together along the edges of a distorted heptagonal prism. On the other hand, in the case of the acetylcholine receptor protein, a considerable amount of experimental evidence (see ref. 8 for a review) leads to a model in which five "hydrophobic" α-helical polypeptide segments are arranged in a pseudo pentagonal prism around a central aqueous "pit" spanning the width of the membrane. Similar "bundles" of hydrophobic α-helical segments have been postulated to be involved in numerous channel-making proteins (ref. 9). Very recently, X-ray analysis of the photosynthetic reaction center complex of Rhodopseudomonas Viridis (ref. 10) has indeed detected the existence of 11 membrane-spanning hydrophobic helices. Although in this system the helices do not participate in channel formation, they confirm explicitely the role played by such structures in the crossing of membrane by proteins.

In parallel to the study of the physiological ion-transducing proteins, a number of observations heve indicated the capability of synthetic polypeptides to induce conductance in natural and artificial membranes, pointing to the possible involvment of α-helical structures (ref. 11).

Alamethicine, its analogs and similar synthetic peptides (ref. 12) are another example of ion channels most likely formed by bundles of essentially hydrophobic α-helices.

In view of gaining an understanding of the channel-making properties of such structures, a systematic study of the packing properties of hydrophobic α-helices and of their aptitude to form appropriate ion-conducting bundles was undertaken. An overview of the main results is presented below.

PAIRING OF HYDROPHOBIC α-HELICES

Taking the simplest hydrophobic aminoacid, alanine, poly-L-alanine was chosen first as model of a hydrophobic polypeptide α-helix to study the pairing properties of two such helices, varying their length from 6 amino acids to 26, number sufficient for a α-helix to cover 39 Å, more than the width of the lipid phase of a bilayer. This was done by optimization of the energy of a pair, in which one helix is fixed, the second being allowed to use its six degrees of freedom with respect to the first.

The essential results were the following (ref. 13, 14) :

1) Whatever the length considered, the most stable structure is a nearly antiparallel arrangement where the two helices are slightly inclined with respect to each other.

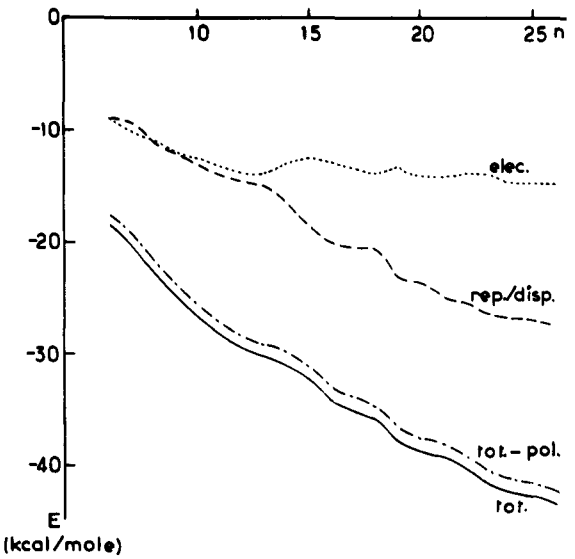

Fig. 2 : Evolution of the optimized interaction energy of a pair of α-helices of poly-L-alanine and its components in terms of the number of amino acids n.

TABLE 1. Interaction energy and its components (kcal/mole) of two $(L\text{-}Ala)_{14}$ α-helices in their optimal antiparallel and parallel arrangement.

	E_i	E_{elec}	$E_{rep/disp}$	E_{pol}
↑↓	- 33.9	- 15.9	- 16.8	- 1.3
↑↑	- 0.8	14.3	- 14.0	- 1.2

2) A constancy appears in the packing characteristics when the helices reach a length of 13 aminoacids. Above this length, the values of the 6 parameters characterizing the optimal position of the second helix with respect to the first become practically stationnary.

3) The variation of the optimal energy of a pair and that of its components upon lengthening the helices (fig. 2) indicates that both the electrostatic term and the repulsion-dispersion component of the energy favor the interaction, contributing about equally and increasing in the same fashion up to about 10 amino acids, wherefrom the second one becomes dominant while the first reaches an asymptot above 13 amino acids. The polarization component of the total energy is very small and essentially constant.

4) The packing mode is akin to the "knobs into holes" insertion mode developed on geometrical grounds *(ref. 15), where the methyl groups of one helix fit into the holes made by methyls on its partner,* allowing a close approach of the axes (about 7.9 Å in the constant structure). This structure explains the situation observed above : for the short helices, there can be only a few such imbrications, the effects of the ends are more important than for the long helices. After n=13 the essential pattern of the interaction is well set and the packing mode becomes constant.

An important result concerns the preference for the antiparallel mode of packing : such a preference of α-helices for an antiparallel arrangement is often considered as the consequence of the electrostatic interactions of the net dipoles of the two helices which are roughly parallel to the helix axes (ref. 16). In fact the above observations on the relative contributions of the components of the binding energy indicate that the origin of the energy preference for antiparallelism in hydrophobic helices is not due solely to an electrostatic effect but, rather, to a combination of it with the dispersion attraction. An illustration of the situation is given in Table I which compares the optimal antiparallel and parallel arrangements of two polyalanine α-helices containing 14 aminoacids. It shows the near-equality of the two main attractive energy contributions with a slight advantage for the second one in the antiparallel pair. In the parallel pair, the electrostatic component is unfavorable, as expected on the basis of the dipole-dipole interaction. However this is compensated by the second term and the polarization energy so that the pair is slightly stable. Such a stability will even increase in longer helices where the electrostatic term has reached its asymptote while the second term increases with the number of methyl groups.

BUNDLES OF N HYDROPHOBIC α-HELICES APT TO FORM CHANNELS

The possibility of formation of bundles of hydrophobic α-helices capable of including a channel in their interior was then considered (ref. 14). N polyalanine α-helices were placed initially on the edges of a polygonal prism from N=3 to 7. In order to possibly represent the folding of one single polypeptide chain crossing the membrane N times (as in bacteriorhodopsine (ref. 7)) the helices were placed alternately

up-down-up-down-etc along the edges of the prism. From this starting point, the package was allowed to optimize its conformation by energy minimization, one helix being fixed and each of the N-1 others free to use its 6 degrees of freedom with respect to the first one. Regular and distorted polygonal prisms were used as starting points. Note that, due to the large number of variables and the large number of local minima, this procedure can rarely yield the most stable bundle for a given N but is perfectly apt to find possible stable structures, thus to fulfill the aim of the study. In order to be able to go up to N=7, the length of the constituent helices was taken as 14 amino acids a number which was shown to insure the constancy in the pairing properties.

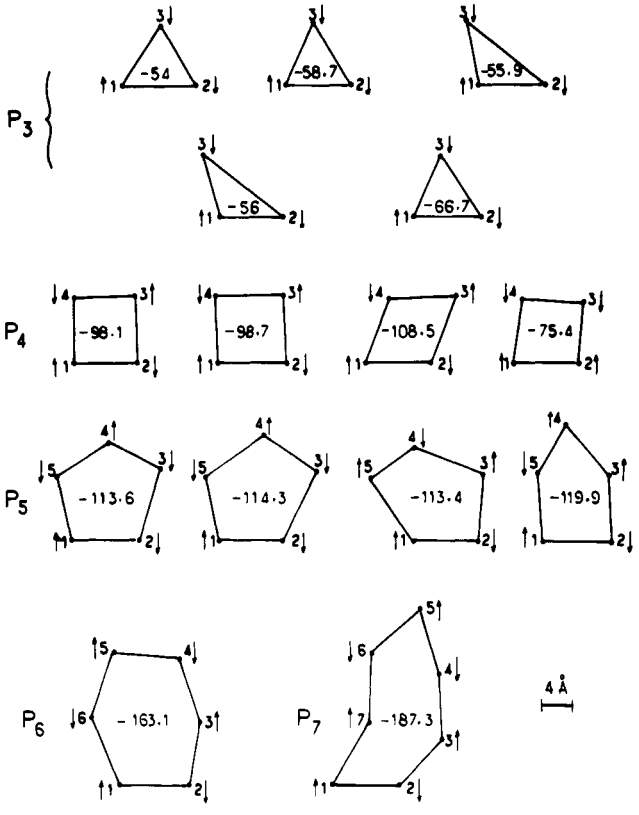

Fig. 3 :
Stable bundles of N=3 to 7 α-helices of $(L\text{-Ala})_{14}$ (sections through the centers of mass of the helices).

The main results were the following (see ref. 14 for details) (see fig. 3) :

- a number of appreciably stable structures exist for each N, with relatively small differences in energy
- the presence of a pair of adjacent parallel helices does not prevent a bundle to be stable, a feature due to the fact that the unfavorable electrostatic interaction between two such helices is never larger numerically than the sum of the favorable such interactions over all the pairs of antiparallel helices in the bundle.
- some of the structures obtained display inner cavities of different dimensions which indicate the possibility of them serving as channel. It is the case in particular for the packages of five helices particularly P5b (vide infra).

EFFECT OF BULKY SIDE CHAINS ON THE HELIX INTERFACES

The hydrophobic membrane segments of the proteins which have been sequenced to date indicate the presence of a number of bulky hydrocarbon side chains. Their possible effect on the packing properties has been investigated by substituting leucines for alanines at the interfaces between the helices and reoptimizing the couples and bundles with N=5 (ref. 17). For the pairs, the results have shown that the presence of the leucines does not modify the essential conclusions enumerated in section 4, namely the preference for a nearly antiparallel arrangement, the existence of a stable parallel one, the same evolution of the packing energies and their components with the length of the helices and the same reasons for the preference of the antiparallel over the parallel couple.

Differences are essentially in the increased distance between the helical axes at equilibrium, and in the details of the reorientation of the interfacing side chains upon interaction. (This was negligible in the polyalanine bundles).

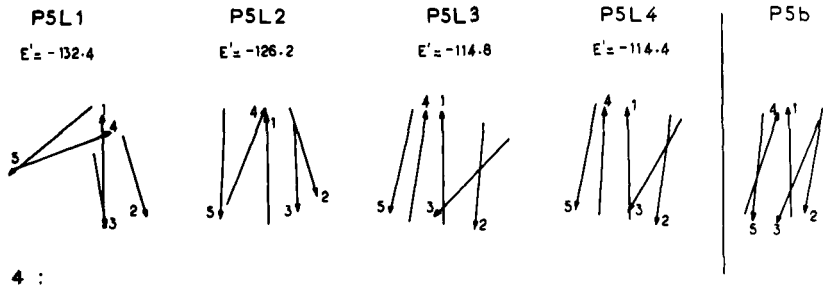

Fig. 4 :
The relative disposition of the axes of the five helices in four stable bundles (P5L1 to P5L4) containing interfacial leucines. P5b is given for comparison. Energies of interaction in Kcal/mole.

For the bundle of five helices, starting from different pentagonal prisms yielded four quite stable structures of very similar stabilities (see fig. 4), the essential difference between them residing in the shape of the hole enclosed by the packages which varies from conically shaped and irregular to more cylindrical. Details of the differences observed in the dimensions and shape of the inner pore limited by the five helices show explicitely how a modulation of the size and shape of a "channel" can be associated with tilting and/or sliding of the helices. Such modulations are at the basis of various models for opening/closing of channels but were never supported by calculation.

ABILITY OF A PURE HYDROPHOBIC BUNDLE TO SERVE AS ION-CHANNEL

The dimensions of the hole surrounded by the five α-helices in the P5b polyalanine package suggested an exploration of the possibility of a sodium cation to lie and possibly to pass through such a system of entirely hydrophobic helices. For this study (ref. 14), Na^+ was placed in successive planes regularly and closely spaced perpendicular to the z axis of the system, and its energy of interaction with the entire package was optimized, letting the ion reach its optimal position, in the package first maintained rigid, then letting the helices reoptimize their relative positions under the influence of the ion.

The most striking result, schematized in fig. 5, is that the interaction energy of Na^+ with the channel is favorable all the way through, due to the favorable interaction with the carbonyl oxygens lining up the channel wall, and in spite of the presence of the internal alanine methyl groups. The labilization of the channel improves the profile, due to small displacements of the helices which facilitate the ion-carbonyl interactions without costing much energy.

A similar exploration carried out with a molecule of water showed that a favorable interaction occurs with the carbonyls of the channel wall and that the space is everywhere sufficient to accomodate the molecule in different orientations in spite of the methyl groups.

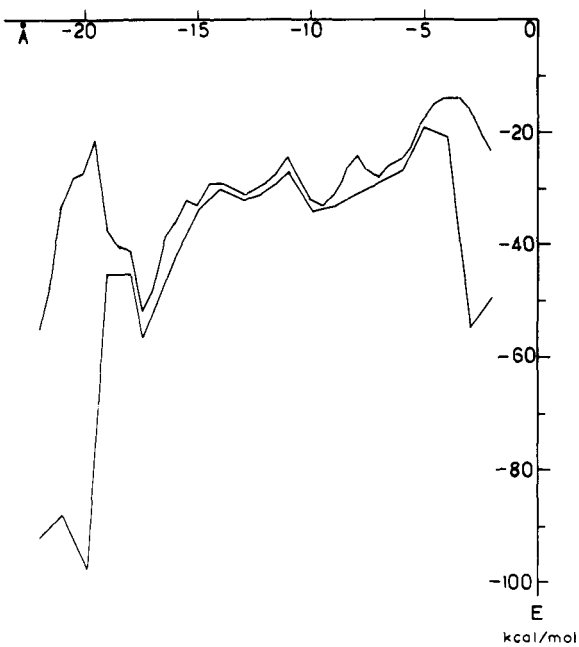

Fig. 5 :
"Energy profile" of a sodium ion in pure polyalanine bundle.

Upper curve : structure rigid,
lower curve : structure free
(see text for details).

EFFECT OF PRESENCE OF POLAR SIDE CHAINS ON INNER WALL OF THE CHANNEL (refs. 18, 17)

In view of coming closer to the situation in membrane proteins where sequence determination indicates the presence, in the hydrophobic segments, of a few polar side chains (non ionic), the effect of the presence of such groups was considered by replacing the methyl groups on the α carbon of the inner wall of the channel by serines $-CH_2OH$. The essential findings were the following :

- Reoptimization of the structure of the polyalanine package carrying serines (allowed to optimize their side chains) did not lead to a strong modifications in the overall structure of the package. The largest changes are small relative sliding of two helices to allow some serine OH groups to form hydrogen bonds to another serine or to a neighbour carbonyl group. Similar results were obtained when serines were substituted on the inner wall of the channel-making bundle of five leucine-containing helices.

- The energy profile for Na^+, recalculated as before in the serine-substituted package (letting the serine side-chains optimize their structure under the influence of the ion) shows a deepening of the energy all along its length, deepening due to the interaction of the ion with the oxygen atom of the serine hydroxyl groups which use the lability of the side chain to turn towards the ion so as to ensure the best possible interaction at every height.

- In an analogous fashion, the serine groups facilitate interaction with water, favoring various hydrogen bonding patterns.

Such properties (attractive power of the end group combined with the flexibility of the side-chain) are equally characteristic of other polar residues (ref. 19) and seem likely to play a general role in the channelling of ions and/or water.

CONCLUSION AND PERSPECTIVE

The studies summarized above aimed at a preliminary understanding of the essential features concerning the packing of hydrophobic α-helices and their ability to form ion channels. They have put into evidence important molecular aspects of the coupling properties, of the formation of stable packages and on their possible interaction with ion and water : role of the hydrocarbon side chains in the interaction energies, differences in the shape of the packages generated by the presence of large such chains, role of polar side chains due to their nucleophilic character and/or hydrogen-bonding capacity and to their flexibility around their single bonds, laying the basis for more elaborate investigations of the channels made by membrane proteins. Such investigations are presently under way, taking into account more precise sequences and the explicit effect of the lipid and water phases.

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