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Theoretical study of the packing of α-helices of poly(L-alanine) into transmembrane bundles. Possible significance for ion-transfer

Sylvie Furois-Corbin and Alberte Pullman

Institut de Biologie Physico-Chimique, Laboratoire de Biochimie Théorique associé au CNRS, 13, rue Pierre et Marie Curie, 75005 Paris (France)

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Energy optimizations are carried out on packages of N α-helices of poly(L-alanine) from $N = 3$–7, starting from an initial arrangement of the helices at the vertices of various polygonal prisms, in view of the possible formation of channel-making bundles in membranes. The results show: that, for each N, a number of stable packages exist; that the presence of one pair (and even two) of adjacent parallel helices in a package is not incompatible with its stability, due to the overcompensation of its unfavorable electrostatic energy by the sum of the corresponding favorable terms for the antiparallel pairs; and that some packages provide ready-made pores in their interior. The energy profile computed for Na^+ inside one of the pores (resulting from five helices) shows a favorable energy all the way through, in spite of the methyl groups protruding into the channel. Similarly one water molecule interacts favorably with this pore throughout.

Introduction

Although the role played by intrinsic membrane proteins in the transport of ions through lipids is well recognized the mechanisms involved are still largely a matter of speculation due, in particular, to the scarcity of precise structural and conformational information on the 'channels'. The last few years, however, have witnessed the onset of a breakthrough in this domain, starting with the obtention of a low-resolution map of the electron-scattering density of bacteriorhodopsin in the purple membrane [1] followed by the full determination of its amino-acid sequence [2–4]. Other sequence determinations are rapidly becoming available (e.g. for the sodium channel of *Electrophorus electricus* [5], the acetylcholine receptor [6–9] etc.), as well as electron spectroscopic images [10]. The early picture of bacteriorhodopsin showing seven rods of density perpendicular to the membrane thickness [1] is compatible with a folding of the molecule involving seven α-helical intramembrane segments [2] probably arranged in an irregular heptagonal packing [11]. The existence of intramolecular rods in a pentagonal arrangement detected in the acetylcholine receptor protein is equally compatible with models involving probably five α-helical portions of the different subunits [12]. The detection or suspicion of α-helical protein segments in other membrane proteins speaks in favor of their possible involvement in the formation of channels by making ap-propriate bundles [13]. Interestingly a set of 11 transmembrane α-helices possibly playing a role in electron transfer has also recently been detected in the photosynthetic reaction center complex of *Rhodopseudomonas viridis* [14]. Although this involves electron transfer while bacteriorhodopsin transfers protons, both proteins raise the problem of the role of transmembrane α-helix bundles.

In parallel to the study of the physiological ion-transducing proteins, a number of investigations have been devoted to the ability of synthetic polypeptides to induce conductance in black-lipid membranes [15–20] pointing, here also, among other possibilities, to the possible involvement of α-helical structures. Although the observations of Heitz and Spach might perhaps be explained by other structures or channel artefacts the question of the possible role of α-helices remains open. Alamethicin [21] provides another example of ion channel-forming molecules of α-helical structure to which can probably be added some of its analogs as well as a number of recently synthetized polypeptides of various length formed by repeating segments of purely hydrophobic sequences of aminoisobutyric acid and L-alanine amino acids [22].

Apart from recent calculations on the possible stable configurations of a pair of two α-helices made of 10 L-alanine residues [23], no systematic study of the packing properties of α-helices seems available, particularly in view of the formation of potential channel-making bundles in membranes. In view of the growing amount of evidence enumerated above pointing to the possible role of such structures in membrane conductance, we have deemed it interesting to start such a study using appropriate energy-minimization techniques recently developed in our laboratory [24]. In the first paper in this series [25] we have examined the factors governing the packing of pairs of poly-L-alanine α-helices of different lengths and, in particular, the influence of the length of the helix on the structure and energy characteristics of the most stable arrangement. We report, in the present paper, a study of the stability and structure of packages of N poly(L-alanine) helices for $N = 3$–7 and preliminary results on the energy 'profile' computed for a sodium ion in one of the potential pore-forming packings obtained. Finally the possibility of a water molecule to lie in the same channel will be considered.

Standpoint and Method

The length required for an α-helix to span a membrane of average size of 30 Å is about 20 amino acids. We have shown, however, in our previous work [25] that a fair constancy in the properties of pairs of poly(L-alanine) α-helices is observed after the number of residues reaches $n = 13$, and have therefore adopted for this model investigation $n = 14$ (about 22 Å length), a number which should be sufficient to bring significant information for studying the problems stated above.

The right-handed poly(L-alanine) helices are set in the geometry given by Arnott [26]. Since the variations in energy obtained in allowing the side chain dihedral angles to vary were found very small in couples $(\text{L-Ala})_{10}$ [23] and $(\text{L-Ala})_{14}$ [25] helices, we have kept them fixed at the values of Ref. 26 in the optimization of the packages. Under these conditions only the interaction energy between the N helices of the package considered needs to be computed.

As in our previous work [25] we have used for the energy calculations a procedure developed in our laboratory [24] and applied successfully in studies of nucleic acid ligands [24] and nucleic acid-protein [27] interactions as well as in studies of the Gramicidin A channel [28]. The method being described in details in Ref. 24, we shall only recall that it relies on the calculation of intra- and intermolecular energies as a sum of electrostatic, Lennard-Jones, hydrogen-bonding, torsion and polarization components. The electrostatic component is computed using atomic monopoles appropriately optimized so as to reproduce with good accuracy the more accurately computed electrostatic properties of protein constituents [29] and the dispersion and repulsion energies are treated as a Lennard-Jones type interaction with the conventional 6–12 dependence, the case of hydrogen bonds being especially treated so as to account for the angular dependence of these interactions. Moreover the polarization energy on each species due to all the atoms of all the other species of the system is included. The energy optimization procedure uses analytical energy derivatives which require the analytical calculation of the forces and torques acting on each atom of the complex.

We shall call, in what follows, P_N a packing formed by N $(\text{L-Ala})_{14}$ α-helices. There is a large number of possibilities of starting configurations for each P_N. The greater N is, the more complex is the energy optimization procedure and it is obvious that even with a very powerful minimizer, starting from completely different configurations will not yield the same final conformation, due to the existence of many local energy minima. Thus one can obtain, for the same N, several packages of different energies, all corresponding to stable structures. The goal of this work, not being the search of the most stable arrangement among all possible P_N, will be limited to the examination of different stable structures for each N in view of an understanding of the packing properties and with the aim of investigating the possibility of such packings to form appropriate bundles. Thus we have adopted, for each N investigated, starting configurations where the helices are at a mutual distance short enough to give a good stability to the arrangement using the results of reference [25] and where, at the same time, the packing may allow the formation of a pore in its 'interior'. On this basis the helices are initially placed so that their pivots (the point situated on the helical axis at the center of its length) are distributed at the vertices of a regular polygon and the helical axes form the edges of a regular polygonal prism. For P_3, P_4 and P_5, irregular polygonal prisms have also been envisaged. In view of the finding [23,25] that the most stable configuration of a pair of poly(L-Ala) α-helices is achieved when the two helices are nearly antiparallel, we have based the building of the starting P_N on an alternate distribution of adjacent α-helices oriented upwards and downwards, from their N terminal to their C terminal. Thus when N is an odd number, the package comprises necessarily two adjacent parallel helices. We shall examine below the consequences of this situation.

We recall that all geometrical transformations, translocations and rotations of the helices [25], during the energy optimization procedure are made from the pivot of each helix. In what follows, helix numbered one, h_1, is kept fixed during the computations.

The computations including the ion were done for sodium. The procedure adopted is the following: one optimized packing was chosen on the basis of its energy and of the free space existing in its interior; Na^+ was placed successively in planes perpendicular to the helical axis of h_1 (which is the z axis of the system), and permitted in each plane two degrees of freedom (x and y) during the energy minimization. This was done first in maintaining the package rigid. The profile plotted thus involves simply the interaction energy of Na^+ with the whole fixed package. In a second set of calculations, the same optimizations were carried out, but allowing the package to reoptimize its structure at the same time. For these optimizations, all helices, except h_1, have six degrees of freedom and energy optimizations are carried out on the interaction energy of all the species including the ion present in the system for each plane investigated; the planes were spaced by 1 Å from the bottom to the top of the channel (to be sure not to miss possible extrema, a number of intermediate planes were explored in the sensitive regions). The interaction energy of Na^+ with the system made of the α-helices is given for each plane investigated, as $E_2 - E_1^0$, where E_2 is the total interaction energy summed over all the interacting species (the N helices and the cation) of the final complex and E_1^0 is the optimized energy of the corresponding starting package in the absence of the cation, calculated as the sum of the interaction energies between the N helices. The energy profile so obtained takes into account the loss in energy occurring upon deformation of the package and the concomittant gain in the energy of interaction. It is given as the plot of $E_2 - E_1^0$ in terms of the height z in the channel.

In a similar way we have explored essentially the possibility of a water molecule to lie in the pore. The methodology used is analogous to that used in the case of Na^+. In that case, all helices were kept fixed during the minimization procedure while the position and orientation of the water molecule was optimized in various regions of the channel.

Results

(A) Stable packages of N α-helices of poly(L-alanine) for N = 2–7

The results of the computations are presented in Fig. 1 and Table I. Fig. 1 gives schematically the shape of the optimized packages obtained from different types of starting configurations of the various P_N explored, limited to the projection of the pivots of each helix of the complex in a plane perpendicular to the helical axis of h_1. These representations are only an indication of the po-

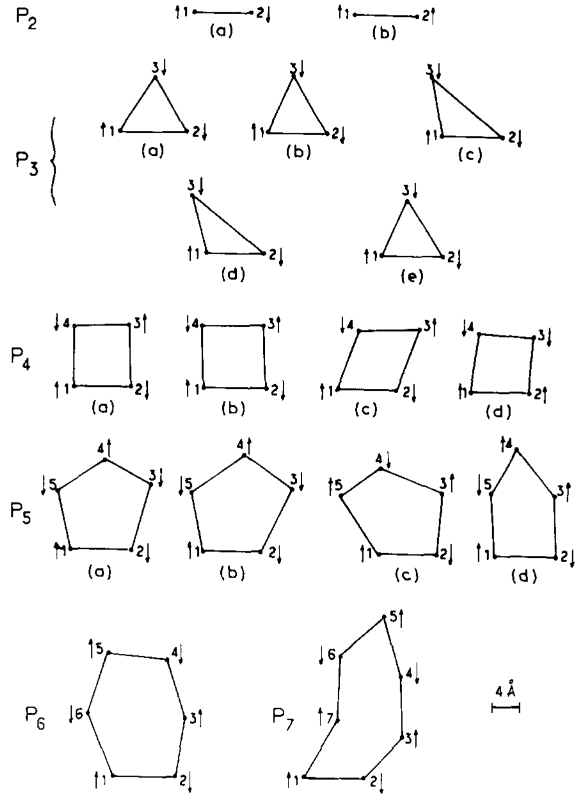

Fig. 1. Schematic representation of the relative positions and orientations of the N helices in various optimized P_N packages.

lygonal disposition of the packages, since neither the relative inclinations of the helices nor their relative shifts are indicated. These informations are given for typical packages in Table II and illustrated on a few examples in Fig. 2. On each schematic representation in Fig. 1 we have numbered the helices and indicated by an arrow their direction from the N terminal to the C terminal, so as to visualize easily the parallel and antiparallel pairs. To each representation is associated a letter distinguishing the various types of structures obtained for the same N. In Table I are given the values of the corresponding respective energies and of their most interesting components. As shown by the comparison of E_{tot} and E, the polarization terms by which they differ are weak and, moreover, E varies like E_{tot}. Thus the individual interaction energy components given in Table I without the polarization terms can be significantly used as such.

We have included in the results the parallel and antiparallel P_2 complexes because their fundamental features are at the basis of the packing of larger bundles: as indicated before, the antiparallel pair P_{2a} is by far the most stable one, due, as analyzed in Ref. 25, to the converging effects of the favorable electrostatic and Lennard-Jones components of the interaction. But it is seen that the pair of parallel helices can reach a stable optimal arrangement with a stabilization of −4 kcal/mol in the complex P_{2b} where the two helices are practically as close as in the antiparallel configuration: inter-axis distance 0.2 Å longer than in P_{2a}, very similar values of the tilt (about 15° with respect to parallelism and antiparallelism, respectively) and tip angles; in P_{2b} the electrostatic energy is repulsive in conformity to what is expected from the parallel orientation of the two macrodipoles [30,31], **but** this repulsion is overcompensated by an attractive Lennard-Jones component nearly as large as that of the antiparallel packing. It is to note that the present calculations are conducted in a medium of dielectric constant, ε, equal to 1. We have repeated them taking a fixed dielectric constant equal to 3, tentatively representing an upper limit for a lipid medium: for the same starting configuration of the complex, the two energy optimizations yield the same final configuration of the system. But when $\varepsilon = 3$ the antiparallel pair is destabilized by about 10 kcal/mol (decrease of favorable electrostatic attraction), while the parallel pair is stabilized by about 6.5 kcal/mol (decrease of unfavorable electrostatic repulsion). This implies that in a medium of dielectric constant appropriate for lipids, the antiparallel system does remain the most stable one but the difference in energy with respect to the parallel system is quite smaller than when $\varepsilon = 1$. Note that this dampening is maximized by the choice of an upper limit for ε.

A similar test on the effect of ε was carried out in the case of a P_3 packing where an energy optimization conducted from the same starting configuration with $\varepsilon = 1$ and $\varepsilon = 3$ yield the same final configuration of the system. With $\varepsilon = 3$ the two pairs of antiparallel helices are destabilized by about 8 kcal/mol while the pair of parallel helices is stabilized by about 7 kcal/mol, these variations coming from the electrostatic term. As a result the

TABLE I

ENERGY CHARACTERISTICS OF THE PACKAGES FOR $N = 2$–7

N, number of helices in the complex
E_{tot}, total interaction energy including the polarization terms
E, total interaction energy without the polarization terms
E_{anti}, interaction energy (without polarization) summed over all the pairs of antiparallel helices
E_{par}, interaction energy (without polarization) summed over all the pairs of parallel helices
E^{el}_{anti}, pure electrostatic component of E_{anti}
E^{el}_{par}, pure electrostatic component of E_{par}
The values in parenthesis for P_2 and P_{3e} correspond to a value $\varepsilon = 3$ for the dielectric constant. All others are for $\varepsilon = 1$. All energies are in kcal/mol.

N	Packing	E_{tot}	E	E_{anti}	E_{par}	E^{el}_{anti}	E^{el}_{par}
2	P_{2a}	−31.1 (−21.4)	−29.9	−29.9		−13.09	
	P_{2b}	−4.0 (−10.5)	−2.9		−2.9		11.22
3	P_{3a}	−54.0	−50.9	−47.8	−3.1	−26.3	11.0
	P_{3b}	−58.7	−55.9	−56.8	+0.9	−27.0	10.7
	P_{3c}	−55.9	−53.7	−59.0	+5.3	−26.4	6.0
	P_{3d}	−56.0	−53.9	−58.9	+5.1	−26.2	5.9
	P_{3e}	−66.7 (−50.7)	−59.6	−56.2 (−39.7)	−3.4 (−10.7)	−24.8	10.9
4	P_{4a}	−98.1	−94.2	−105.8	+11.6	−53.1	15.0
	P_{4b}	−98.7	−94.4	−105.8	+11.4	−52.2	14.9
	P_{4c}	−108.5	−104.1	−106.4	+2.3	−48.7	15.5
	P_{4d}	−75.4	−71.6	−65.8	−5.8	−35.4	15.1
5	P_{5a}	−113.6	−107.9	−119.6	11.7	−58.7	24.0
	P_{5b}	−114.3	−108.6	−120.7	12.1	−60.0	26.1
	P_{5c}	−113.4	−107.2	−123.5	16.3	−66.4	29.2
	P_{5d}	−119.9	−113.5	−129.0	15.5	−67.5	30.5
6	P_6	−163.1	−156.6	−185.4	28.8	−96.4	35.4
7	P_7	−187.3	−177.1	−206.3	29.2	−109.6	53.1

packing resulting from the energy optimization with $\varepsilon = 3$ is less stable than for $\varepsilon = 1$, remaining nevertheless quite stable ($E_{tot} = -50.74$ kcal/mol) but it shows a smaller difference between the respective interaction energies of parallel and antiparallel helices than when $\varepsilon = 1$ (see E_{par} and E_{anti} in Table I for the P_{3e} packing). It is likely that this situation facilitates the formation of packages including parallel α-helices in membranes, a configuration sometimes imposed by the necessity of the folding of a long continuous chain into a succession of membrane-crossing segments (e.g. in bacteriorhodopsin).

It might be objected that the energy of interaction of the hydrophobic methyl groups of the α-helices with the lipids is not explicitely included. However, this introduction is not likely to modify the preceding conclusions since this term, stabilizing whatever be the helix orientation, should not be very different according to the orientation to the methyl groups.

Let us examine the packages with more than two α-helices, in the light of the results of Table I and Fig. 1. The P_3 complexes are those for which we have tried the largest number of arrangements. They present a particular interest due to the presence of necessarily two adjacent parallel helices. These are taken as being h_2 and h_3 in all cases. The most stable packing we have obtained is P_{3e}. P_{3a} and P_{3b} are the optimized structures obtained from two starting configurations where the three helices form an equilateral triangle but in a different way: in the starting of P_{3a} the edges are 9 Å long and in the starting of P_{3b}, constructed from

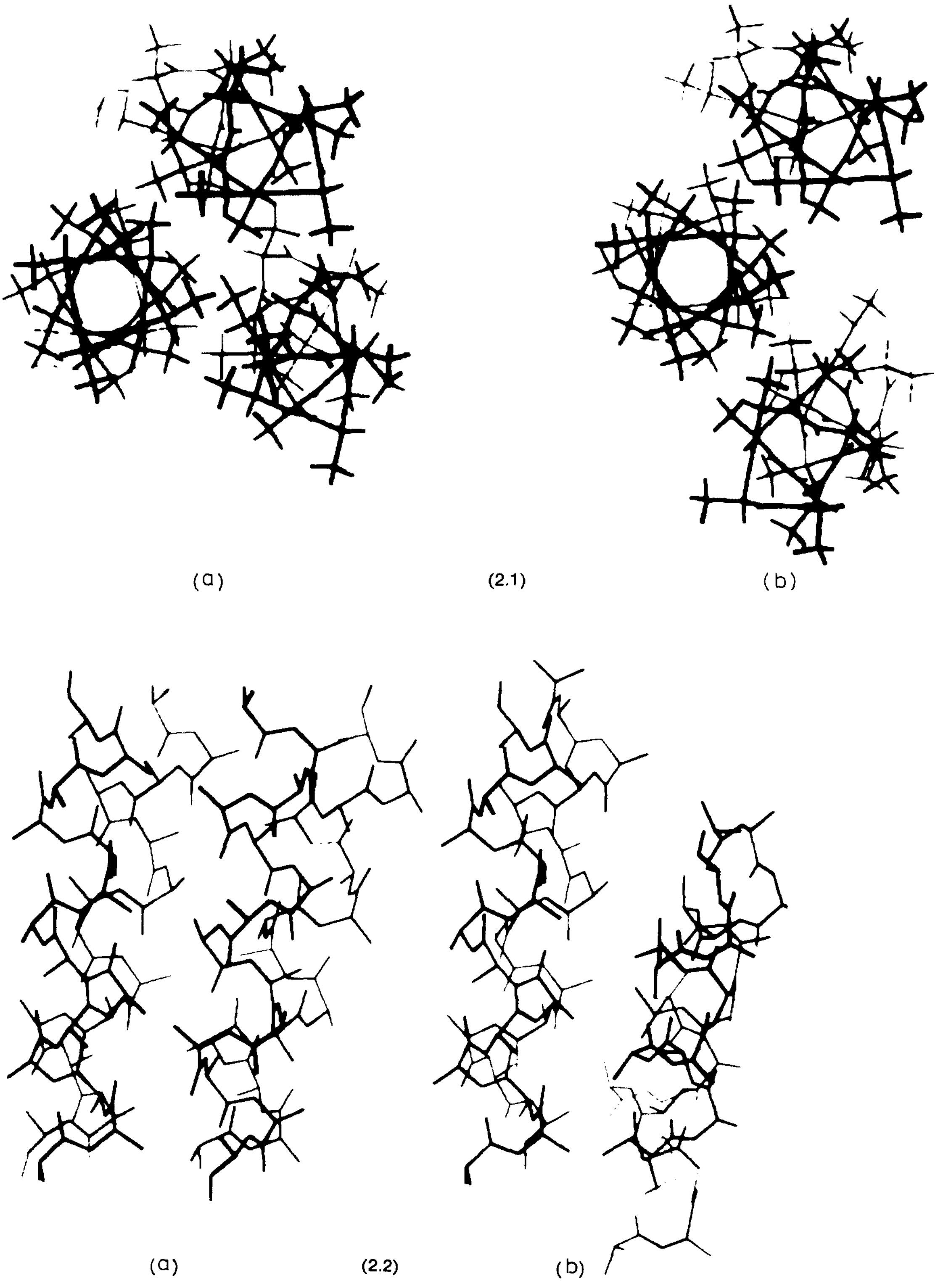

Fig. 2. View in three dimensions of some packages. (1) top view of (a) P_{3b} and (b) P_{3d}; (2) 'front' view (without hydrogen atoms) of (a) P_{4b} and (b) P_{4d}; (3) view (without hydrogen atoms) of P_7, from the N terminal of h_1.

P_{2a}, they are 7.9 Å long. In P_{3a} the interaction energy between the parallel helices stabilizes this pair but this is achieved at the expense of the interaction energy of the two other pairs of antiparallel helices (E_{anti} much less favorable than in all the other P_3). In P_{3b} the interaction energy of the pair of parallel helices is nearly zero (0.88 and −0.25 kcal/mol without and with the polarization terms, respectively), leading thus to an unfavorable situation.

The P_{3c} package is the optimized configuration calculated from a starting complex where the pair 1–2 (antiparallel helices) is that of P_{2a} and the position of h_3 is deduced from that of h_2 by the simultaneous rotation and shift corresponding to one helical step of an α-helix (see Ref. 25). Thus the starting 312 angle is 100°. In the resulting optimized configuration P_{3c}, this angle has become 102°9 and h_3 is significantly shifted in z. Screwing this helix upwards by one helical step [25] and optimizing the energy of the resulting structure yields the P_{3d} packing where the 312 angle is 105°6. In the optimized structures P_{3c} and P_{3d} the two parallel helices are farther apart than in the other P_3 explored: the Lennard-Jones attractive component of their interaction energy becomes very weak and the corresponding positive electrostatic component governs the value of their interaction. On the other hand the favorable interaction energy of the antiparallel helices is quite strong (compare E_{anti} in P_{3c} and P_{3d} with $2E_{anti}$ in P_2a).

TABLE II

DISTANCE TO h_1 (R), SHIFT (d), TILT (β) AND TIP (γ) OF THE HELIX AXES WITH RESPECT TO THE h_1 ONE IN TYPICAL PACKAGES

Packing name	Helix	R (Å)	d (Å)	β (degrees)	γ (degrees)
P_{2a}	h_2	7.9	1.0	−165.7	−0.2
P_{2b}	h_2	8.1	−1.0	15.9	−0.3
P_{3a}	h_2	8.8	1.1	−178.9	7.8
	h_3	8.4	0.5	−178.9	−7.6
P_{3b}	h_2	8.0	1.4	−168.3	−3.6
	h_3	8.1	1.5	−166.5	−1.3
P_{3d}	h_2	7.9	1.0	−165.6	−0.2
	h_3	7.9	0.9	−165.5	0.2
P_{4b}	h_2	8.3	1.2	−177.3	−8.2
	h_3	11.4	0.0	14.8	13.9
	h_4	8.0	1.3	−164.4	−0.1
P_{4d}	h_2	8.1	−5.1	35.8	6.6
	h_3	11.4	−1.5	−138.0	7.0
	h_4	8.0	2.7	−168.2	−2.1
P_{5b}	h_2	7.9	0.8	−166.0	−0.3
	h_3	8.0	0.0	−166.6	0.2
	h_4	13.5	−0.3	22.9	−0.4
	h_5	14.3	−1.8	−155.8	0.8
P_6	h_2	8.6	0.7	170.1	−12.8
	h_3	11.7	−2.2	11.2	26.1
	h_4	16.0	0.3	−177.3	8.4
	h_5	15.7	−0.3	−3.4	2.8
	h_6	8.6	2.2	166.1	−11.0
P_7	h_2	8.6	0.6	173.3	−12.9
	h_3	14.7	−2.4	10.5	18.3
	h_4	18.9	−2.7	−153.0	−22.0
	h_5	24.0	−2.8	16.3	3.1
	h_6	16.6	−0.3	−149.9	4.0
	h_7	8.7	1.6	15.7	−2.1

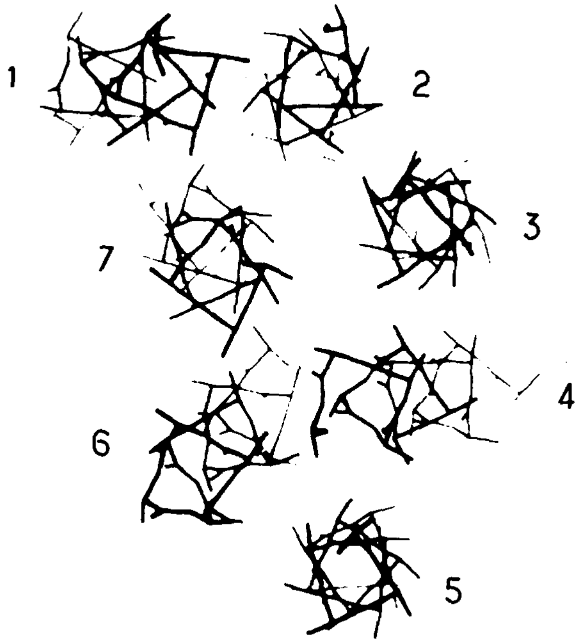

Fig. 2. (continued) (2.3)

With P_{3e} we have aimed at exploring a configuration where the 312 angle is intermediate between the starting values 60° and 100° investigated before. Thus in the starting configuration, the three helices form a isosceles triangle with an angle 312 equal to 80°. The energy optimization yields a more acute angle (312 = 66.2°) with a very favorable interaction energy of h_1 and h_2 (about 0.3 kcal less favorable than in P_{2a}). The interaction energy between h_1 and h_3 is not as favorable (3 kcal/mol less stable) but is compensated by the interaction energy of the h_2-h_3 pair where the helices, although parallel, display a favorable interaction.

It may be noted that, in P_{3c} and P_{3d}, the 312 angle has not significantly changed during the energy optimization, being unable to reach the value of the configuration P_{3e}, although this one is energetically more favorable. This is due to the complexity of the system and the number of variables to treat by the minimization procedure. Clearly, the P_{3c} and P_{3d} conformations are the manifestation of a very strong local minimum of energy of an assembly of three $(\text{L-Ala})_{14}$ helices. These two packages, which have very similar conformations and energies, are two quite stable structures (see E_{tot}).

Overall, the values of the respective energies obtained for all the P_3 packings investigated show a great stability, even when the two parallel helices are at a short distance and even when their interaction energy is unfavorable. This is due to the fact that the unfavorable electrostatic interaction energy of two parallel helices is **never** larger than the absolute value of the sum of the two favorable electrostatic interactions between the antiparallel helices present in the package. We shall see that this situation is quite general.

Let us turn to the P4 packages. Among them, P4a, P4b and P4c contain no pairs of parallel adjacent helices. It is observed here that the pairs of antiparallel helices are globally less favorable than in the P_2 and P_3 packages (compare the values of E_{anti} for four pairs of antiparallel helices with E_{anti} in P_{2a} (one such pair) and E_{anti} in the P_3 (two such pairs) bundles). This is a result of the greater constraint felt by each such pair due to the fact that, in a P_4 assembly, each helix interacts not only with one or two but with three others.

P_{4a} results from optimization with a starting structure in which the adjacent helices, alternately oriented, are 9 Å apart in a square arrangement. In the final packing, one of the four helices is rather shifted upwards with respect to the other ones. Unscrewing it (as in Ref. 25) and reoptimizing the structure yields the structure P_{4b}, very similar in shape and energy to P_{4a} but without vertical shift of h_1. In P_{4a} and in P_{4b} the parallel pairs, h_1-h_3 and h_2-h_4, placed at the ends of the diagonals of the square are relatively distant (11.4 Å). As a consequence, their Lennard-Jones interactions are weak and the unfavorable long-range electrostatic term governs their interaction energy. Interestingly these two P_4 are quite reminiscent of arrangements proposed in proteins that incorporate a 4-helical pattern where the four nearly antiparallel helices are packed in an array of approximately square cross section [32,33].

P_{4c} results from the energy optimization of a starting arrangement constructed on the basis of the most stable P_3 complex: h_1, h_2 and h_4 are positionned as h_1, h_2 and h_3 in P_{3e} and h_3 is set so as to keep the regularity of a rhomb as well as the alternate orientation of the adjacent helices and so as to avoid repulsive contacts with the other helices. It is interesting to observe that after energy optimization the total energy is about 10 kcal/mol more stable than that of the two more regular quadrilaters P_{4a} and P_{4b}, this stabilization coming essentially from the pairs of parallel helices: indeed the large distance between h_1 and h_3 (13.7 Å) makes their Lennard-Jones favorable interaction energy weak, but simultaneously it has also a weakening effect on the unfavorable electrostatic component with respect to the value of this quantity in the other pairs of parallel helices seen earlier. In the other parallel pair, h_2 and h_4 are at a shorter distance and the Lennard-Jones component is strong enough to be predominant over the electrostatic repulsion. As a result, the sum of the interactions for these two pairs is much less unfavorable (by about 9 kcal/mol) than in P_{4a} and P_{4b}.

To investigate in more details the consequences of the presence of parallel helices on the stability of the packages we have finally built a P_4 complex similar to the starting P_{4a} but with two pairs of adjacent parallel helices. The optimized structure is schematically represented in Fig. 1 by P_{4d}. As shown in Table I, its resulting interaction energy E_{tot} is much less favorable than in the other P_4 structures investigated. Furthermore in the optimal structure the helices are significantly shifted in z with respect to one another (see Fig. 2(2)). The destabilization of P_{4d} compared to P_{4a}, P_{4b} and P_{4c} comes from the antiparallel helices for two reasons: situated at the extremities of the diagonals, they are not close enough to have strong favorable Lennard-Jones interactions; the important relative shifts observed for the two helices making these pairs (due to the presence for each of them of a parallel adjacent helix) disfavors the energy of the pair. Nevertheless the global structure is still quite stable. Again it is observed that in spite of adjacent parallel helices, the structure can be stable since the unfavorable electrostatic interaction of the parallel helices $E_{\mathrm{par}}^{\mathrm{el}}$ does not reach, and is even much smaller than the absolute value of the favorable electrostatic interactions, $E_{\mathrm{anti}}^{\mathrm{el}}$, of the antiparallel ones. It must however be stressed that in this package the tilt of the helices is somewhat larger than in the others due to the presence of two pairs of adjacent quasi-parallel helices.

With the P_5 packages we have again at least two adjacent parallel helices. The P_{5a} arrangement was obtained by optimization from a starting configuration where the five helices form a regular pentagonal prism with adjacent helices as close as in P_{2a} and where the adjacent parallel helices are h_2 and h_3. P_{5b} comes from the reoptimization of P_{5a} after a screwing upwards of h_3 which is very shifted with respect to the four other helices in P_{5a}. P_{5c} is optimized from a starting regular pentagonal prism with equal edges 9 Å long, the parallel helices being h_1 and h_5.

In these three complexes the presence of a pair of adjacent parallel α-helices affects the interaction energy of the antiparallel helices (compare E_{anti} for six such interactions with E_{anti} in the three first P_4 complexes for four such interactions) but the resulting packages are still quite stable structures. The optimized P_{5a}, P_{5b} and P_{5c} configurations correlate interestingly with the arrangement suggested for the five subunits of the acetylcholine receptor, each of them containing α-helical segments which may be arranged regularly so as to form an approximate pentagonal symmetry about the central axis of the receptor [10].

Finally the P_{5d} package for which the starting configuration is based on the most stable square and triangle we have obtained, is more stable by about 6 kcal/mol than the other P_5 explored.

In all the P_5 studied the interaction energy summed over all the pairs of parallel helices is positive but there again the total interaction energy of the adjacent parallel pairs is always less unfavorable than that of the other couples of parallel helices. Again the value of the unfavorable electrostatic interaction energy summed over all the pairs of parallel helices ($E_{\mathrm{par}}^{\mathrm{el}}$) does never reach, and in fact is much smaller than the absolute value of the favorable electrostatic interaction energy summed over all the pairs of antiparallel helices ($E_{\mathrm{anti}}^{\mathrm{el}}$).

Due to the large computing time required, we have investigated only one P_6 and one P_7 packing. For these two investigations we have chosen a starting configuration where the helices are distributed on the vertices of a regular hexagonal prism and a regular heptagonal prism, respectively, starting with adjacent helices at a distance of 9 Å. One striking result seen in Fig. 1 is that the optimized configurations P_6 and P_7 are both quite distorted with respect to the regular starting prisms. In P_6 the orientation of the helices is regularly alternated, so that there are nine pairs of antiparallel helices. The value of E_{anti} shows that the large number of helices affects the interaction energy of each pair since each helix interacts now with five others. Nevertheless this deformed hexagonal structure is very stable and could perhaps be related to a model proposed for a lipoprotein in the outer membrane of *Escherichia coli* based on a cylindrical arrangement of six α-helices inserted hexagonally into the membrane [34].

As concerns P_7, its distortion with respect to the regular starting heptagonal prism, is still more important than that in the case of P_6. It contains twelve pairs of antiparallel helices and their interaction energy is still more affected than in P_6 because of the presence of one pair of adjacent parallel helices. Nevertheless this complex is very stable as seen by E_{tot}. It is quite interesting that optimization of a package of seven helices starting from a **regular** heptagonal arrangement leads to the final very distorted shape obtained which is reminiscent of that proposed for the seven α-helical segments of the bacteriorhodopsin transmembrane protein [11].

Overall, the energy optimization computations conducted on different kinds of packages of polyalanine α-helices have led to the obtention of various stable structures. In view of the possibility that some bundles of α-helices form membrane channels for cations we went further in our study by a more detailed examination of the packages obtained. Visualisation in three dimensions, with the aid of an interactive graphics terminal, of the structures surrounded by their van der Waals envelope has shown that there is no space for a cation in the P_3 packages except for local cavities in P_{3c} and P_{3d}. Among the P_4 arrangements only P_{4a} shows the possibility of a channel but relatively narrow: P_{4b} presents some local cavities, of small size, at different heights and there is practically no free space at all inside the two other P_4. With the P_5 structures, an appreciable free inner space is present in P_{5a}, P_{5b} and P_{5c}. (See in Fig. 4 the relative size of an α-helix interior hole and this free space). Since the inner wall of the 'channel' does not appear as a cylinder, it is not quite appropriate to talk about a 'diameter': an idea of the lumen size is given by the fact that the shortest distance, in a given plane, between two atoms of two non-adjacent helices is about 6 Å. As seen above, the complex P_{5d} is the most favorable in terms of energy among the P_5 explored, but there is no space sufficient for an ion in this structure. The optimized configuration obtained for six packed polyalanine helices does not display a real channel but only some local cavities. Finally there appears to be no space inside the P_7 package. For a given number *N* of helices per package, the most stable structures are not those which could act as channels since, in general, the larger stability of a package results from a tighter packing together of the helices. In view of the large stabilization energies found it appears very possible, however, that the arrangements we have obtained can be expanded somewhat so as to create a pore, while keeping a reasonably stabilizing energy.

(B) The P_{5b} package as a possible Na^+ channel

We have seen that among all the packings investigated, the first three P_5 arrangements present a relatively large pore, P_{5b} being the most stable of the three structures. We have therefore chosen P_{5b} to explore the possibility of a sodium cation to lie and possibly to pass through such a 'channel' formed by a system of entirely hydrophobic α-helices. It is clear, of course, that such an investigation is a model study aiming essentially at the exploration of a feasibility. As such, it must be considered as a first step towards more realistic studies. As already mentioned because of the relative shortness of the α-helical segments considered, the package spans only a length of about 22 Å and this must be kept in mind in the interpretation of the 'profiles'.

The upper curve of Fig. 3 gives the plot of the interaction energy of Na^+ with the global complex at each *z* investigated from the bottom (left-hand side) to the top (right-hand side) of the channel maintained rigid. The lower curve is the energy profile obtained in allowing, for each *z* value, the ion to optimize similarly its position in the plane, while, at the same time, letting the whole structure of the package free to reoptimize its structure under the influence of the ion. Three essential observations can be made upon examination of Fig. 3:

(1) The remarkable fact that the energy profile of Na^+ inside the bundle of five (L-Ala)$_{14}$ is favorable all along the channel length and this in

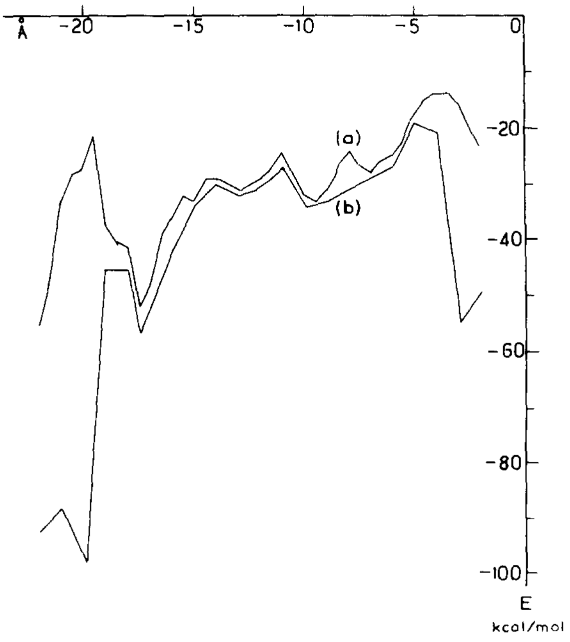

Fig. 3. Energy 'profile' of Na^+ in the P_{5b} package; (a) in the frozen structure of the package; (b) letting the package reoptimize its conformation.

spite of the protruding methyl groups on the internal surface.

(2) The similarity in the overall shape of the two 'profiles' with a more favorable energy for that obtained in the labilized channel.

(3) A clearcut distinction between the end regions with deep interaction energies and a more internal region with less favorable (although still largely negative energies.

These three characteristic features are due to the nature of the interaction between Na^+ and the atoms forming the pore and in particular with the oxygens of the peptide carbonyl groups lining up the inner wall of the package. When the sodium ion is placed at the bottom of the packing (planes $z = -22, -21, -20$ Å), its interaction energy with the five helices is strongly negative due to 2 or even 3 stabilizing electrostatic interactions with carbonyl oxygens and a relatively small 'hindrance' by methyl groups. A similar behaviour is found at the top of the packing ($z = -3, -2$ Å) due to two favorable carbonyl attractions. When Na^+ is really 'inside' the channel, the carbonyl oxygens are less accessible because of the accumulation of methyl groups and the interaction energy is less strongly negative. Nevertheless at least one Na^+-O favorable interaction is found all along the channel and, when the flexibility of P_5 is allowed, the helices move slightly to favor such an interaction; in some cases even (for example at $z = -10$ Å) two such interactions participate but at longer distances. In each case the energy loss due to the deformation of the package is overcompensated by the gain in Na^+-P_5 interaction, so that the profile is deeper in energy than with a rigid channel. The fact that, in the upper part of the channel

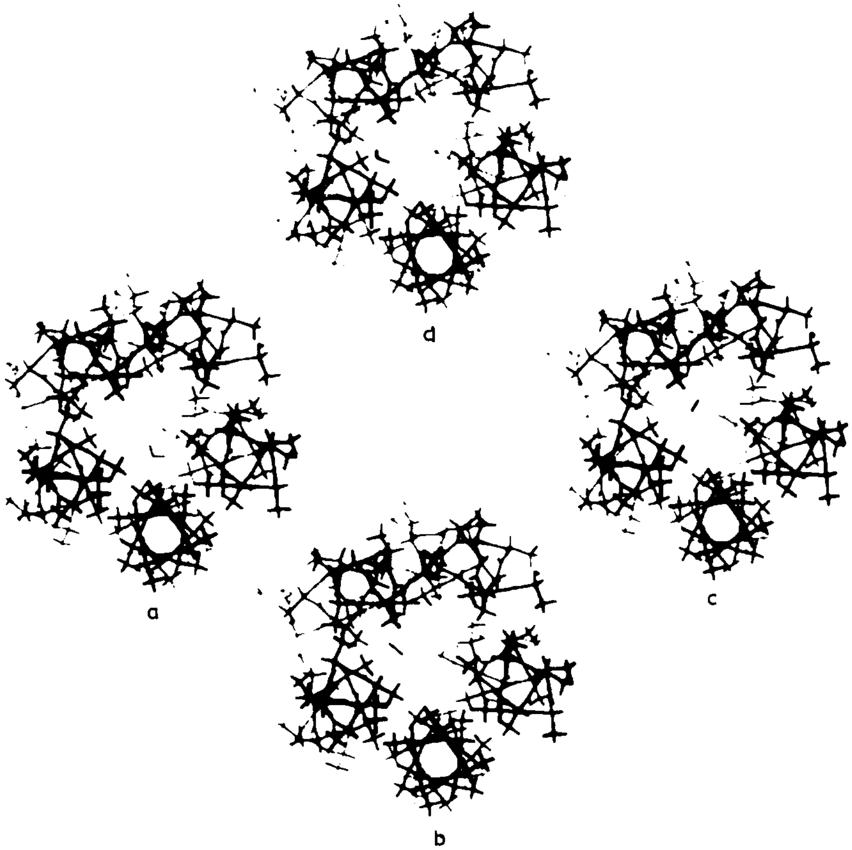

Fig. 4.Position of the water molecule at various heights in the P_{5b} 'channel' (the z coordinate is that of the oxygen atom of the water molecule and the associate E is the interaction energy of the water molecule with P_{5b}). (a) $z = -15.99$ Å, $E = -6.7$ kcal/mol; (b) $z = -9.10$ Å, $E = -4.9$ kcal/mol; (c) $z = -6.65$ Å, $E = -4.4$ kcal/mol; (d) $z = -3.69$ Å, $E = -7.5$ zkcal/mol.

the interaction energy of the cation with the helices is globally less stabilizing than in the lower part is due to a greater hindrance of the methyl groups leading to a greater number of unfavorable interactions.

The energy profiles obtained show an appreciable barrier at the channel mouths essentially because of the strong negative value of the interaction energy of Na^+ with the helices at the ends of the packing. Although present in both profiles these barriers appear higher in curve b relatively to the external minima. In fact this is due to a strong lowering of the external minima because, when the ion is in these regions, the structure of the package changes very significantly to insure a better attraction by the end carbonyls. The significance of this conformational change, however, cannot be assessed until a study of the situation is performed taking into account the hydration of the ion and the presence of the lipid medium. It may be expected that the intensity of the favorable Na^+-O interactions would be reduced by both effects.

(C) Interaction of P_{5b} with water

The computations of the optimized interaction energy of a water molecule with the P_{5b} bundle show that this interaction is favorable all along the pore. In the lower part of the channel the water molecule is hydrogen-bonded to a carbonyl oxygen of one of the α-helices with an interaction energy varying, according to the binding site, between −6.7 and −7.6 kcal/mol. In the upper part of the channel, the carbonyl oxygens of the helices are less accessible, but nevertheless, attractive, so that favorable sites (with no real hydrogen bonds, however) are found with an interaction energy varying between −4.3 and −4.9 kcal/mol. At the very top of the channel, hydrogen bonds can again be made. Fig. 4 gives an illustration of the positions found for the water molecule at various heights in the channel. The situation observed here is interesting to compare with the situation found (unpublished results and Ref. 28) in the Gramicidin A channel: although the order of magnitude of the binding energies of water to the channel is similar in the two cases, the values are overall more uniform in Gramicidin A due to a regular formation of hydrogen bonds to the carbonyl oxygens. Here, although the space available is always sufficient for a water molecule to lie in the pore, the formation of hydrogen bonds with the carbonyls is, at some spots, hindered by the protruding methyl groups.

Conclusion

The present set of optimum-energy computations carried out on systems formed by N equivalent α-helices $(\text{L-Ala})_{14}$ with N increasing from 2 to 7, has brought into evidence the factors involved in the stability of the bundles. One of the main striking features resulting from these calculations is the fact that the existence of a pair of adjacent parallel polyalanine helices is not detrimental to the stability of a bundle: the packages in which the helices are not oriented alternately upwards and downwards are all quite stable structures. As shown in details on the example of the systems formed by three α-helices, the electrostatic interaction between the two parallel helices is unfavorable but its absolute value is never greater than that of the sum of the two other favorable electrostatic interactions. The stability of the packages formed by an odd number of α-helices is not due to the predominance of the Lennard-Jones interactions but is intrinsically due to the favorable total electrostatic component of the energy of the packing. This could thus be extrapolated to helices other than polyalanines. This finding concurs with the fact that apparently stable structures involving the folding of a continuous polypeptide chain into a package with an odd number of α-helices do exist, as shown in particular by the structure of the bacteriorhodopsin transmembrane protein.

Concerning the possibility of pore-forming bundles, it was found that some of the structures obtained by energy optimization could behave as a channel to transport ions through membranes in terms of the width of the pore existing inside the package. Others present only some local cavities and in others the helices are so tightly packed that there is no pore inside the optimal package. But the large number of possible structures formed and the interplay of the energy components observed indicate a very wide lability so that the structures formed could be expanded to form a pore, while remaining stable.

In the case of one of the optimized bundles made of five (L-Ala)$_{14}$ α-helices which displayed the existence of an internal hole it was found that the interaction energy of Na^+ with the 'channel' is favorable all the way through, in spite of the presence of the internal methyl groups, and that the small internal barriers along the profile are appreciably lowered when the pore is allowed to deform upon passage of the ion. It was equally shown that the same P_5 bundle can easily accommodate one water molecule in its internal pore with a favorable interaction energy at every height.

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