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Theoretical study of the packing of α-helices into possible transmembrane bundles. Sequences including alanines, leucines and serines

Sylvie Furois-Corbin and Alberte Pullman

Institut de Biologie Physico-Chimique, Laboratoire de Biochimie Théorique associé au C.N.R.S., 13 rue Pierre et Marie Curie, 75005 Paris (France)

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Energy optimizations are carried out on packages of two and five α-helices containing leucines on their faces of contact and made otherwise of alanines. The effect of these bulky side-chains on the optimal arrangements is analysed and compared to the results previously obtained for pure poly(L-alanine) packages; the essential pairing properties are conserved (near antiparallelism, preponderous role of the non-bonded interactions, possibility of existence of parallel pairs); five α-helices made of 8 alanines and 6 leucines (three on each interface) can pack in different stable P5L bundles including various holes, according to the tilt and relative sliding of the helices. Substitution of serines to the alanines lying on the inner wall affects very little the interhelix packing. The seryl side chains adapt their conformation at best to their surroundings. The P5L packages can be used to represent individual subunits arranged in 'superbundles' around a central pit in a channel-forming protein.

Introduction

In view of the growing amount of experimental data pointing to the possible role of α-helix bundles in the formation of ion channels in membranes [1–7], we have undertaken theoretical studies on the packing properties of α-helices and on their ability to form conducting bundles, using appropriate energy-minimization techniques recently developed in our laboratory [8]. In a preliminary step [9] we have examined the factors governing the packing of pairs of poly(L-alanine) α-helices of different lengths, more specifically the influence of the length of the helices on the structure and energy characteristics of the most stable arrangement. In a second study [10] we have performed energy optimizations of packages of N poly(L-alanine) α-helices, disposed initially along the edges of various polygonal prisms for $N = 3$ to 7, and examined the characteristics of the packings obtained. Most of the stable packages made of five helices arranged approximately along the edges of an irregular pentagonal prism, displayed a rather large hole in their interior and it was shown (by computation of an 'energy profile') that this ready-made pore could easily accommodate a sodium ion and/or a water molecule with a favorable energy all along the channel length, a conclusion which lends an explicit support to the possibility of conduction of bundles of purely hydrophobic α-helices [5–7].

In view of bringing our model closer to the α-helix bundles possibly involved in transport by intrinsic membrane proteins, we have then considered the effect of the presence of polar (non-charged) residues by introducing serines instead of

Correspondence: A. Pullman, Institut de Biologie Physico-Chimique, Laboratoire de Biochimie Théorique associé au CNRS, 13, rue Pierre et Marie Curie, 75005 Paris, France

alanines along the channel inner wall [11]. It was found, in particular, that the presence of serines deepened the energy profile of Na^+ all along the channel length, owing to the favorable interactions of their hydroxylic oxygens with the ion, interactions facilitated by the lability of the seryl side chains which adapt easily their dihedral angles to permit their oxygen to interact at best with the ion at every step of its progression, such conformational adaptation of successive serines possibly allowing a 'passing over' of the ion from one hydroxyl group to the next available one.

In order to test further the applicability of our conclusions to the situations encountered in intrinsic membrane proteins, we have now investigated the effect of the presence of bulky hydrophobic side chains on the various properties considered earlier.

Standpoint and Methodology

An examination of the sequences given in Refs. 1–3 indicates that among the purely hydrocarbon side chains of the probable α-helical segments, leucines are the most numerous and that they are scattered over the entire structure. It is thus likely that some of them are present on the faces of contact between the helices. It is these interfacing bulky side chains which are the most susceptible to affect the packing of the helices (particularly their distances), hence the formation and the properties of the bundles. To study these effects we have introduced leucines at the interface between α-helices made initially of poly(L-alanine). We present first the results of energy optimization of pairs of such helices and the effect, on the packing properties, of the growing number of leucines (related to the growing length of the helix), in comparison to the corresponding results obtained for two poly(L-alanine) helices [9]. In a second step we consider a bundle made of five such α-helices carrying leucines at all their interfaces, examining the stable structures obtained from the point of view of energy, conformation, size of the inner hole, etc. and compare these structures with the corresponding pure polyalanine packages [10]. Finally serines are introduced on the inner wall in one of the stable bundles, and energy optimization is carried out on the resulting package of five α-helices made of small amino acids (alanines), amino acids with bulky side chains (leucines), and polar residues (serines).

As in our previous studies, we start from right-handed α-helices set in the geometry given by Arnott [12]. All the energy-optimizations are done using the FLEX methodology described earlier (see Refs. 8 and 10 for details) which uses an expression of the total energy of the system including all the terms of the theory of intermolecular forces (electrostatic, polarization, repulsion, dispersion and torsion).

The relative positions of the $N-1$ helices of a package of N helices are defined by six parameters recalled for convenience in Fig. 1 [9]. The helices are located in a general (x, y, z) orthogonal coordinate system; the z axis coincides with the helical axis of h_1 which is kept fixed during all energy-optimizations. In the present study all the backbone dihedral angles are kept fixed at the values of reference [12] and the dihedral angles of the alanyl side chains are maintained fixed at their

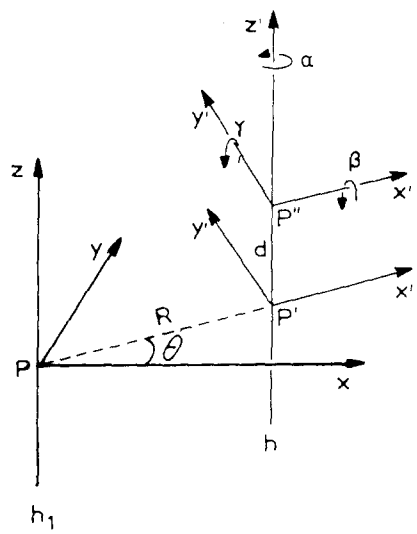

Fig. 1. The six parameters defining the position of a helix h with respect to h_1 in a complex. h_1 is the reference helix kept fixed during all energy-optimization. Its helical axis, from N-terminal to C-terminal, is defined as the z axis of the x, y, z orthogonal coordinate system. P is the pivot of h_1 (center of its length). R and θ fix the position of the projection P' of the pivot P'' of h on a circle centered at P, α, β and γ, twist, tilt and tip, respectively, define the rotations of h around the directions indicated. d is the vertical translation.

optimal value since their labilization was shown to bring very little modifications in the energy and mode of packing [9,13].

Energy minimizations have been carried out in two different ways: in procedure A, the total energy of the package is minimized in a first step labilizing the dihedral angles of the side chains (4 for leucines, 2 for serines) while freezing the 'interhelix variables', i.e. the six degrees of freedom fixing each of the $N-1$ helices with respect to h_1. In a second step the structure obtained in the first one is reoptimized by labilizing both the internal and the interhelix variables. In procedure B, the total energy of the package is minimized with respect to both internal and interhelix variables simultaneously, from the starting point. Both procedures were followed using in all cases several starting configurations. It is obvious that the greater the number of helices in a package, the more complex is the optimization procedure so that different starting configurations are likely to yield different final conformations for large packages due to the existence of many local energy minima, all of which, in general, correspond to stable structures. Let us underline that, as in our earlier work, our purpose is not the search of the most stable arrangement of all (except for pairs, vide infra), but the search of stable packages of α-helices of an appropriate shape to span a membrane lipid phase and possibly form pores.

Results

I. Pairs of helices having leucines on their faces of contact

We call $h_{n/m}$ one α-helix made of m amino-acids among which n leucines and $C_{n/m}$ the complex of two $h_{n/m}$ helices.

Our studies on poly(L-alanine) couples [9] having shown that increasing the length of the chain above 14 amino acids did not affect appreciably their mode of packing, we started the present investigation with $m = 14$. The first leucine-containing sequence was deduced from a chain of 14 L-alanines by replacing alanines by leucines every fourth residue, starting with residue 4 to avoid end effects. This insures a reasonable spacing of the bulky leucines while keeping them on the same 'face' of the helix (see Fig. 2).

We then increased the length of the α-helices, increasing simultaneously the number of leucines so as to distribute them at best within the same 'face', while keeping a reasonable spacing. This imposes to place them in positions 15 and 19 as shown in Fig. 3 which indicates the projections, on a plane perpendicular to the helical axis, of the twenty-one first α carbon atoms of an α-helix. Adopting these positions for the leucines and keeping the N-terminal and C-terminal compositions similar to that of $h_{3/14}$, led to the two sequences shown below.

Since 21 amino acids in an α-helix span a length of 31 Å, roughly sufficient to cross the lipid phase of a membrane, we did not go beyond this number.

In view of a comparison with the results obtained for couples of poly(L-alanine)s, we first consider the packing of two identical $h_{n/m}$ helices disposed in an antiparallel fashion for the three above-defined sequences, then we compare the parallel and antiparallel packing of two $h_{3/14}$ helices.

The starting points for the energy optimizations were arrangements of strictly antiparallel (or strictly parallel) helices, placed sufficiently far apart to avoid too strong repulsive contacts between leucyl side chains (about 11.7 Å between the helical axes); the two helices are identical, displaying leucines only on one side, and the two rods are disposed initially in such a way that leucines on one helix face leucines on the other, in order to maximize the effect of the presence of such bulky side chains at the interface. The leucyl side chains were set initially in the conformation corresponding to an optimized isolated helix. When approaching two helices to 11.7 Å, the worst contacts were relieved by modifying the leucine dihedral angles when necessary with the aid of an interactive graphic terminal before starting the minimization process. Since in the case of

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$h_{4/17}$ = Ala Ala Ala Leu Ala Ala Ala Leu Ala Ala Ala Leu Ala Ala Leu Ala Ala

$h_{5/21}$ = Ala Ala Ala Leu Ala Ala Ala Leu Ala Ala Ala Leu Ala Ala Leu Ala Ala Ala Leu Ala Ala

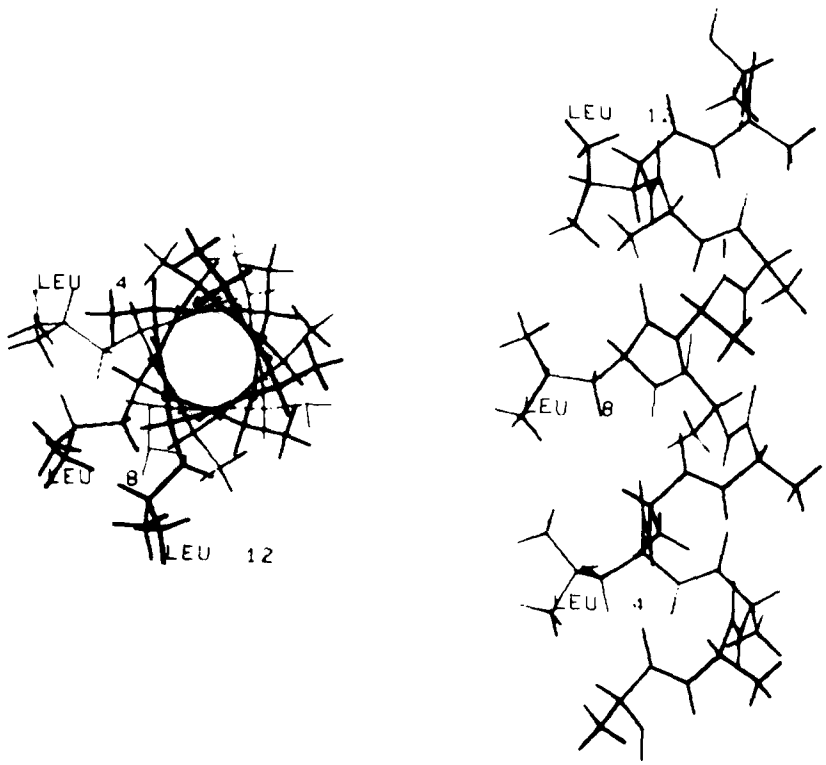

Fig. 2. Positions of the leucines in the $h_{3/14}$ helix. (a) View down the axis from the C-terminal atom. (b) View perpendicular to the axis.

couples we searched for the best possible arrangement, we utilized, besides procedures A and B, various different ways of energy minimization, in order to avoid remaining in local minima for each $C_{n/m}$ investigated.

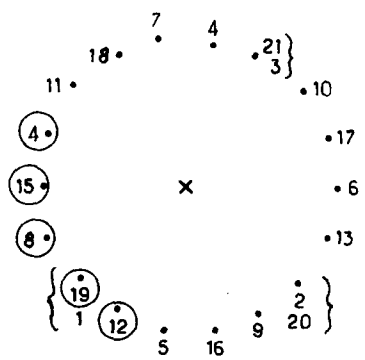

Fig. 3. Projection on the plane perpendicular to the helical axis (figured by the cross) of the α-carbons of an α-helix 21 amino acids long. Positions occupied by leucines in the computations on the pairs are indicated.

(1) The optimal energies and structures of the $C_{n/m}$ antiparallel complexes of different length

Fig. 4 gives the variation of the interaction energy of the two helices in the optimal structure and its components as a function of m, the increasing number of residues, related to n, the increasing number of leucines. Curve (a) represents the interaction energy E_i of the two helices including the total polarization of the system; (b) is the interaction energy minus this term; (c) is the Lennard-Jones (repulsion + dispersion) component of the interaction energy; (d) is the corresponding pure electrostatic component. Also given is the difference $A = E - E_0$ where E is the total energy of the optimized couple and E_0 the energy of the complex when the two helices are at an infinite distance with their side chains in their initial optimal configuration. The difference between A and E_i stems from the fact that the side chains in the final complex have modified their conformations with respect to their optimal state at infinite separation.

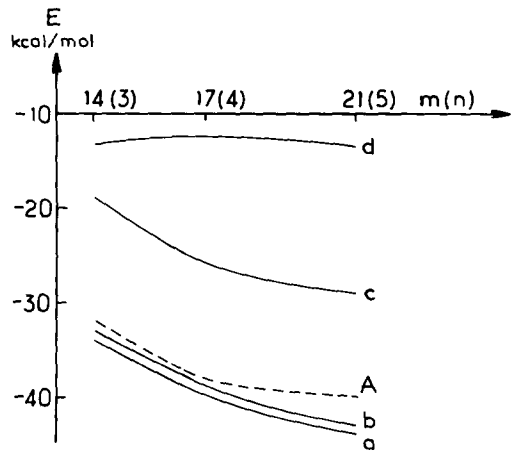

Fig. 4. (———) Plot of the optimal interaction energy E_i and of its components in the optimal pairs of antiparallel helices with increasing number of residues (m) and increasing number of leucines (n). (a) Total interaction-energy E_i including polarization. (b) E_i minus the polarization terms. (c) Lennard-Jones component of E_i. (d) pure electrostatic component of E_i. (— — —) $A = E - E_0$ (E total energy of the optimized couple, E_0 optimal energy of the complex when the two helices are at an infinite distance).

Examination of the curves of Fig. 4 leads to the following conclusions:

(i) The total interaction energy increases globally with the number of amino acids per helix, thus with the number of leucines in the contact area. The energy gain is somewhat less regular than in the homopolymers of alanine [9], a difference due in part to the slight irregularity in the distribution of the amino acids (vide supra) and in part to the large number of constraints provided by the number of leucines facing each other.

(ii) The mutual polarization energy is constant and very small (about 1 kcal/mol) as shown by comparison of curves (b) and (a), a result similar to that found for poly(L-alanine) helices [9] and due to the same reason, namely that the closest approach groups are aliphatic side chains. (It is true that such chains contain numerous polarizable bonds, but at the same time the polarizing field of their facing counterparts is small).

(iii) The electrostatic and Lennard-Jones components are both negative but while the first term is roughly constant, the second deepens with the increasing length. Both results are typically those found [9] for $(\text{L-Ala})_m$ helices with increasing m: they reflect the fact that the increase in the dispersion attraction due to the accumulation of methyl groups is more advantageous than the increase in the electrostatic attraction due to the accumulation of peptide bonds, which furthermore has an asymptotic behaviour. In the two cases (Ref. 9 and present work) the general shape of the curve of the total interaction energy is imposed by the evolution of the Lennard-Jones term. It may be noted that whereas the electrostatic component has essentially the same value in the $(\text{L-Ala})_m$ couples and the $C_{n/m}$ ones considered here, the Lennard-Jones term is more advantageous in the complexes with leucines in the contact region than in the pure alanine couples, another consequence of the greater dispersion (London) attraction due to the greater number of methyl groups. Note also that in the region of length considered the electrostatic component has practically reached its asymptotic value.

(iv) The evolution of curve A indicates that the growing number of the leucyl side chains brings about some destabilization due to an increased deviation of these side chains away from their conformation in the isolated optimized helices, in order to ensure a better aggregation with their partner in the couple. In $C_{3/14}$ and $C_{4/17}$ the resulting loss of intrachain energy is largely compensated by a large gain in interchain energy. When passing to the $C_{5/21}$ complex, the loss of conformational intrachain energy is somewhat more important and the accompanying increase in interaction energy is not, proportionally, as good as in the two preceding complexes, owing to the complex interplay of the inter- and intra-chain Van der Waals interactions. Table I shows, on the examples of the $C_{5/21}$ optimal complex, that the bulky interfacing side chains are quite flexible. In particular χ_1 or χ_2 can reach values appreciably different from their values in the isolated optimized helix. Such is apparently not the case when a $(\text{L-Leu})_{10}$ helix packs with a $(\text{L-Ala})_{10}$ helix [14] where the dihedral angles show only small variations with respect to their values in the optimized $(\text{L-Leu})_{10}$ isolated helix. The situation in this case is appreciably different: in a $(\text{L-Leu})_{10}$ helix, leucines are on every α-carbon, a feature which restricts their possible conformational variations;

TABLE I

DIHEDRAL ANGLES DEFINING THE CONFORMATION OF THE LEUCYL SIDE CHAINS

Conformation in: (a) $h_{5/21}$ isolated; (b) h_1 in $C_{5/21}$; (c) h_2 in $C_{5/21}$. Angles in degrees (1, N-C_α-C_β-C_γ; 2, C_α-C_β-C_γ-D_1; 3, C_β-C_γ-D_1-H_1D_1; 4, C_β-C_γ-D_2-H_1D_2)

		1	2	3	4
Leu 4	a	−77.9	159.6	55.9	62.5
	b	−79.2	156.3	−66.4	−60.2
	c	−77.9	159.7	55.9	62.5
Leu 8	a	−78.1	159.7	56.0	62.5
	b	−77.7	159.4	56.3	69.3
	c	−73.4	168.0	63.1	74.9
Leu 12	a	−78.0	159.8	56.0	62.5
	b	−77.9	161.0	56.5	64.1
	c	−176.0	81.4	61.4	−58.6
Leu 15	a	−171.6	77.8	54.3	60.8
	b	−170.6	77.5	54.1	59.2
	c	−79.1	160.2	57.0	63.8
Leu 19	a	−77.5	159.5	55.8	62.3
	b	−77.4	159.6	55.8	62.4
	c	−175.0	137.7	−62.9	−58.4

furthermore, there are no leucines on the packing partner, but instead a regular array of much less bulky groups.

TABLE II

DISTANCE (R), SHIFT (d), TILT (β) AND TIP (γ) OF THE TWO $h_{n/m}$ HELICES IN EACH OPTIMAL COMPLEX WITH INCREASING n AND m

Distances are in Angströms, angles in degrees.

$C_{n/m}$	R	d	β	γ
$C_{3/14}$	9.4	−0.2	−167.9	10.1
$C_{4/17}$	9.5	−0.6	−163.8	3.1
$C_{5/21}$	9.3	−0.3	−163.8	2.8

The second part of the results concerns the conformation of the optimal arrangements of the two $h_{n/m}$ α-helices. Fig. 5 gives the image of the three complexes as visualized on a graphic terminal and Table II gives the parameters positioning the second helix with respect to the first one in the three complexes. It is immediately apparent that the three structures are very similar as was the case for the poly(L-alanine) couples for $m \geqslant 14$.

A conspicuous feature of the three structures is the proximity of the two helices as can be seen by the value of R (around 9.4 Å) which, although larger than in the pairs of poly(L-alanine) because of the presence of the bulky side chains, is still appreciably smaller than twice the size of the maximal peripheral radius of one $h_{n/m}$ helix

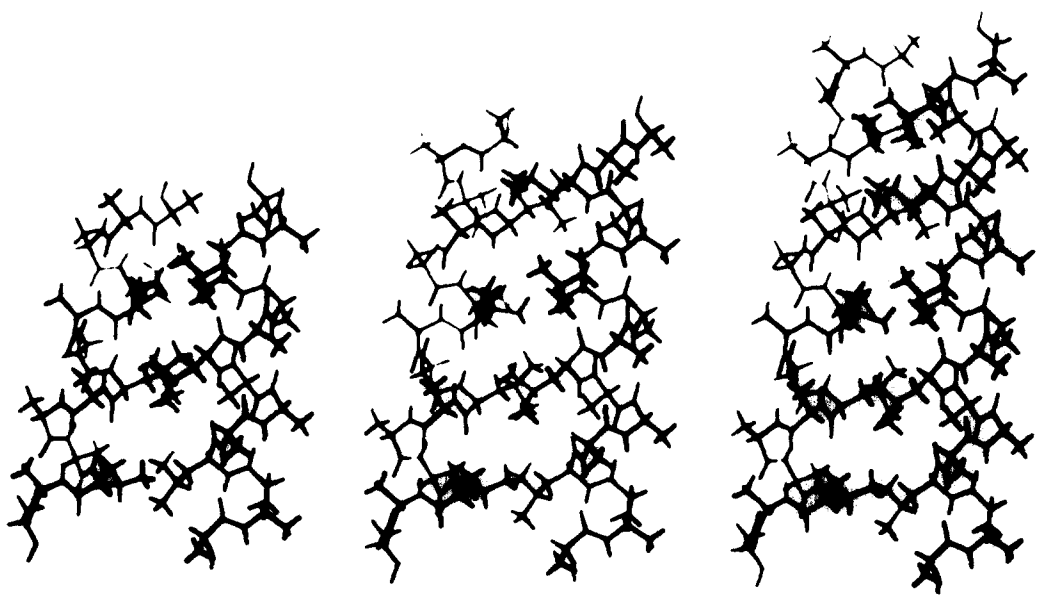

Fig. 5. View perpendicular to z of the $C_{3/14}$ (a), $C_{4/17}$ (b) and $C_{5/21}$ complexes, h_1 on the right; h_2 on the left.

(about 7.43 Å, given by the sum of r and r_H, r being the distance to the axis of the most external H atom of a leucine methyl group in an optimised isolated $h_{n/m}$ helix and r_H the van der Waals radius of H). The tilt (β) is about -165 degrees, as in the optimal pairs of poly(L-alanine) helices. The small differences observed in the values of the tip (γ) affect very little the overall structure of the pair, as can be seen in Fig. 5.

(2) Relative stabilities of antiparallel and parallel arrangements

This study was carried out on the pairs of $h_{3/14}$. Table III contains the parameters positioning the two helices in the parallel and antiparallel optimized structures (a), in comparison to the corresponding ones in the case of the pure alanine couples (b). Also given are the values of the interaction energy E_i which governs the stability and of its components ($E_{rep/disp}$: Lennard-Jones term, E_{elec}: electrostatic term, E_{pol}: polarization term). The values indicate that, in the leucine-containing couple like in the pure alanine pair, the antiparallel structure is much more stable than the parallel one, but that the parallel pair presents nevertheless a favorable, albeit small, interaction (negative E_i). In this arrangement the electrostatic component of the interaction energy is positive, as expected for interaction of two parallel helix macrodipoles [15,9], but this is overcompensated by the negative Lennard-Jones term, the dispersion attractive energy being, as in the antiparallel arrangement, very favorable. In the leucine-containing packages the imbrication of the $h_{3/14}$ helices in the parallel arrangement is not as closely realized as in the antiparallel one: the Lennard-Jones term is less negative by 6.7 kcal/mol and the helices are about 1 Å farther from each other (see R in Table III). This situation is related to the distribution of the leucines along the chains, which results in more van der Waals contacts between the interfacial groups in the parallel than in the antiparallel structure. This does not occur in the completely regular polyalanine couples where the optimal distances can thus be nearly the same.

II. Packages of five $h_{3/14}$ helices

Having found that a fair constancy in the packing of pairs of helices is obtained above a length of 13 amino acids we have built our model bundles of five helices containing 14 amino acids, as was done for the polyalanine bundles. The starting configurations were constructed with the same principle as for the couples, but this time placing three leucines on each side of contact of one helix with its two neighbours. This condition and the shape of the starting polygonal prism on which we align initially the helix axes impose the location of the leucines along the sequence: we use a regular pentagonal prism with parallel edges distant by 11 Å, along which five identical α-helices are placed alternately up and down (from their N to C terminal). In view of a comparison with the pure polyalanine package P5b of Ref. 10 we kept the same reference system and the same value of θ (Fig. 1) placing h_2 with respect to h_1. This, together with the internal angle (108°) of a regular pentagon dictates the sequence

Ala	Ala	Ala	Leu	Ala	Leu	Ala	Leu	Ala	Leu	Leu	Ala	Leu	Ala
1	2	3	4	5	6	7	8	9	10	11	12	13	14

As can be seen in Fig. 3, Leu 8, Leu 4 and Leu 11 lie on one side of the helix and Leu 6, Leu 10, Leu 13 on the other side at the appropriate angle for interfacing.

TABLE III

CHARACTERISTICS OF THE OPTIMIZED ANTIPARALLEL AND PARALLEL COMPLEXES OF TWO $h_{3/14}$ α-HELICES (a) AND OF THE CORRESPONDING (L-Ala)$_{14}$ COMPLEXES (b)

Distances are in Angströms, angles in degrees and energies in kcal/mol.

	R	d	β	γ	E_i	E_{elec}	$E_{rep/disp}$	E_{pol}
a ↑↓	9.4	−0.2	−167.9	10.1	−33.7	−13.3	−19.3	−1.2
↑↑	10.7	−1.3	5.9	3.2	−3.4	9.9	−12.6	−0.7
b ↑↓	7.9	0.2	−166.6	−0.04	−33.9	−15.9	−16.8	−1.3
↑↑	8.1	−0.9	16.5	0.03	−0.8	14.3	−14.0	−1.2

The helices are numbered 2 to 5 in a counterclockwise order from h_1 around the prism. (For practical reasons the parallel helices in the bundle are h_2 and h_3 as in Ref. 10.)

Another starting point was provided by keeping the same initial pentagonal configuration of the helices but tilting h_2, h_3, h_4, h_5 by about 15 degrees with respect to h_1.

The resulting systems (970 atoms, 120 internal variables defining the conformations of the leucine side chains and 24 interhelix variables) were optimized by the procedures A and B defined above. This led to four stable bundles: P5L1 and P5L2 derived from the non-tilted initial configuration, P5L3 and P5L4 from the tilted configuration. The characteristics of these P5L bundles are summarized in Table IV and Fig. 6. For each package the upper part of Fig. 6 indicates, in the order of decreasing stability, the total interaction energy and the relative positions of the five helical axes viewed perpendicularly to h_1. Since the results show that the relative stabilities of the four packages are governed by the values of their respective total interaction energy E' summed over all pairs of helices in the bundle, only E' is given. Nevertheless the variations ΔE of E and $\Delta E'$ of E', from each structure to the next, is indicated. In the lower part of Fig. 6 are given for each package three polygons obtained, respectively, by projecting on a plane perpendicular to the Z axis (axis of h_1) three points for each helix defined as follows: (a) the projection on its helical axis of the N-terminal atom for h_1 and h_4, initially parallel, and of the C-terminal atom of h_2, h_3 and h_5 initially antiparallel to h_1; (b) the projection, on the helical axis, of the C-terminal atom of h_1 and h_4 and of the N-terminal atom of h_2, h_3 and h_5; (c) the pivot of each helix (middle height of its axis). The polygons joining, respectively, the projections of points (a), (b), (c) allow a visualiza-

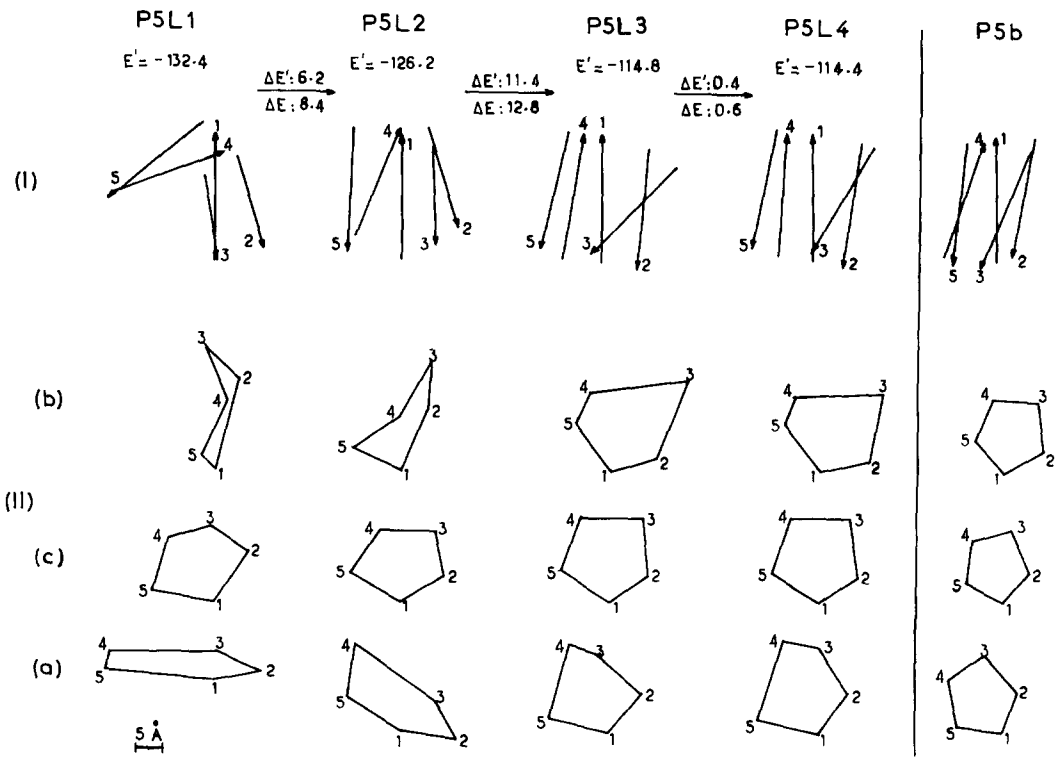

Fig. 6. Schematic representations of the relative positions and orientations of the helices in the four P5L optimized structures and in the pure poly(L-alanine) bundle P5b. (I) The five helical axes viewed perpendicularly to h_1. (II) Projections on the xy plane of the lower point (a), upper point (b), and pivot (c) of the five helical axes.

TABLE IV

DISTANCE (R), SHIFT (d), TILT (β) AND TIP (γ) OF THE HELICES RELATIVE TO h_1 IN THE OPTIMIZED PACKAGES

Distances are in Angströms, angles in degrees.

Packing	Helix	R	d	β	γ
P5L1	h_1	11.6	−0.8	143.8	−24.8
	h_3	14.5	−3.9	173.6	−48.4
	h_4	14.5	4.8	65.3	−27.5
	h_5	11.9	6.7	−168.1	48.9
P5L2	h_2	9.6	3.9	141.0	−5.5
	h_3	14.7	0.1	158.9	−33.6
	h_4	14.2	2.8	15.9	−23.2
	h_5	10.8	1.2	175.0	5.8
P5L3	h_2	9.2	−2.1	−165.8	0.9
	h_3	17.2	−2.3	−138.6	−20.8
	h_4	16.9	0.7	7.5	−8.9
	h_5	10.8	1.4	−160.2	1.4
P5L4	h_2	9.1	−1.9	−162.3	0.1
	h_3	16.9	−0.6	−149.1	−6.7
	h_4	17.1	0.7	2.2	−10.9
	h_5	10.8	1.2	−160.1	0.7
P5b (Ala)	h_2	7.9	0.8	−166.0	−0.3
	h_3	14.3	−1.8	−155.8	0.8
	h_4	13.5	−0.3	22.9	−0.4
	h_5	8.0	0.0	−166.6	0.2

tion of the variation of the size and shape of the cavity enclosed by the five helices from one to the other extremity of the various packages.

Comparison with the similar representation of the pure polyalanine bundle P5b (Fig. 6) shows that the shape of the hole enclosed by the five leucine-containing helices is much more distorted all along the height of the optimized packages than in P5b, most particularly in P5L1 and P5L2. This is due to the fact that in the pure alanyl package the helices can easily rotate around their helical axis; these rotations are sufficient to suppress the repulsive contacts between the small side chains so that no tip is necessary to reach the optimal energy (see the small values of γ in Table IV). When bulky side chains like leucines are present at the interface, with three leucines per helix at the contact area on each side, the rotation of an helix around its axis generates quasi-immediately repulsive contacts between the leucyl side-chains of neighbouring helices, making such rotations more difficult. Hence, the weight of the conformational adjustements of the helices is born by the tilt (β) and the tip (γ), leading to appreciable inclinations of the axes.

All complexes are stable, the most stable one being P5L1 which is also the structure where the relative inclinations of the helices are the most important (see Fig. 6 and Table IV). Due to these large inclinations the bundle is funnel-shaped with a relatively small hole on the C-terminal side of h_1 (about 6 Å from atom center to atom center in its wider dimension), becoming larger all along the length of the package.

In P5L2, the tip of the helices is much smaller but the inner void is still funnel-shaped. Due essentially to the different inclinations of h_4 and h_5 with respect to their inclinations in P5L1, the size of the bottom hole is smaller than the corresponding one in P5L1. As shown by the parameters in Table IV, the relative positions of the helices (see particularly the distance (R) between the pivots of h_1 and h_2, h_1 and h_5) and their inclinations are rather different in the two bundles. Nevertheless the total energies differ by only 8.4 kcal/mol.

P5L3 and P5L4 have practically the same stability, 21–22 kcal/mol; they are less stable than P5L1, but still very stable. Their conformations (which differ essentially by the inclination of h_3) differ appreciably from those of the two others, in particular by the size and shape of the inner hole, wider and much more regular all along the length of the bundle. An illustration of these differences is provided by Fig. 7 which reproduces the visualization in three dimensions, on an interactive graphics terminal, of the two halves of P5L1 and P5L4 (each half defined as including 7 amino acids per helix) surrounded by their van der Waals envelope: (a) looking towards the N-terminal atom of h_1: (b) looking towards the C-terminal of h_1. The corresponding representation of the P5b polyalanine bundle is given in Fig. 8 for comparison. The considerable difference in the width of the hole at the two extremities of P5L1 and its more conserved size in the P5L4 structure are clearly visible. Furthermore, while the hole is the largest on the N-terminal side of h_1 in P5L1, it is the largest at the other extremity in P5L4. The pore in P5L4 does not appear as a regular cylinder. Its

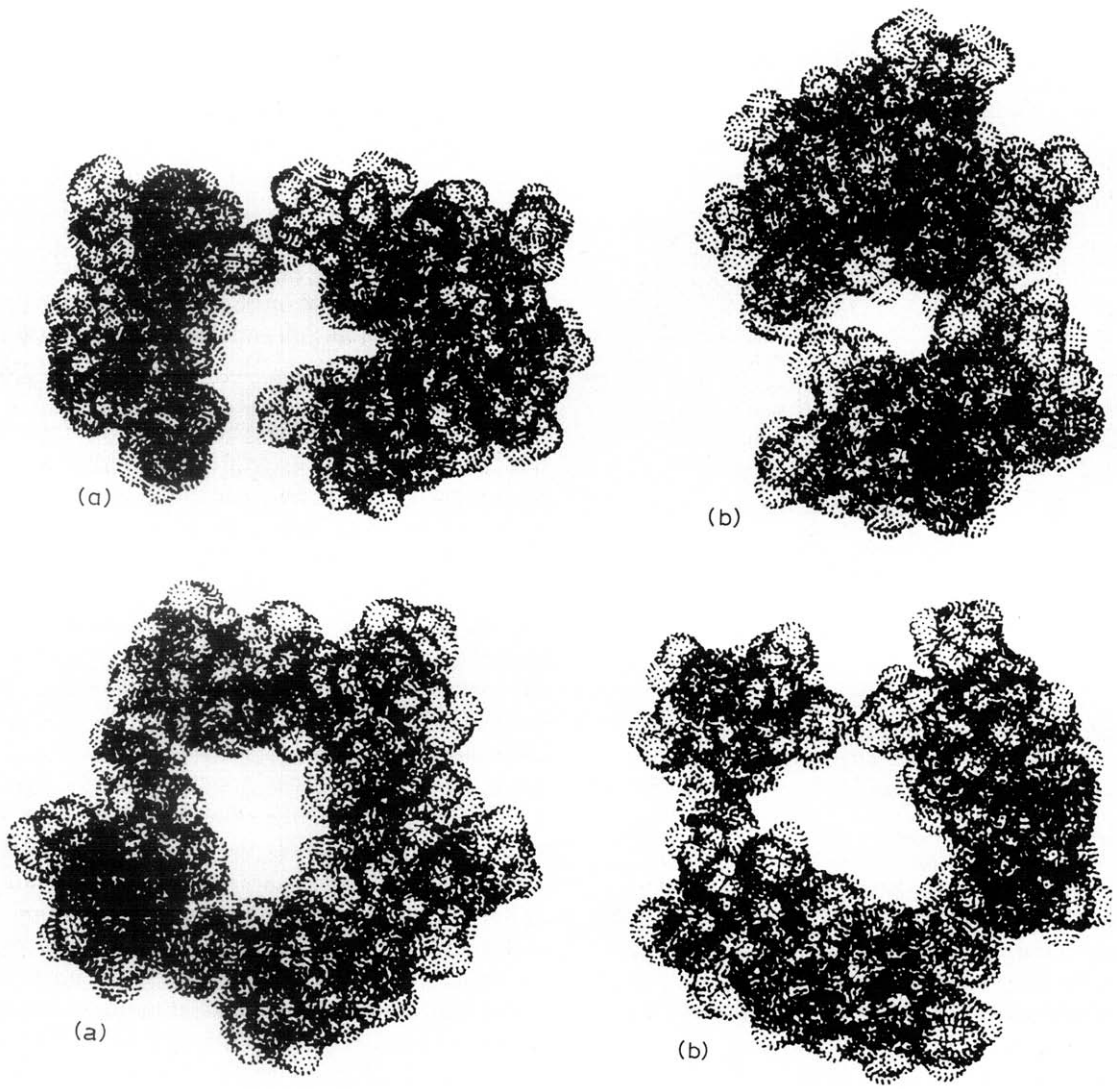

Fig. 7. View in three-dimensions of the two halves (each half including seven amino acids) of P5L1 (up) and P5L4 (bottom) surrounded by their van der Waals envelope. (a) Looking towards the N-terminal atom of h_1. (b) Looking towards the C-terminal atom of h_1. (In a) the helices 1 to 5 are seen counterclockwise; In (b) they are seen clockwise).

section is rather akin to a smooth rectangle of dimensions 6 and 12 Å (from atom center to atom center). This is not sufficient to accomodate a sodium ion surrounded by a regular octahedron of water molecules (about 8 Å diameter), but only a partially desolvated ion. This partial desolvation can be facilitated by the presence along the wall of the peptide carbonyl oxygens, which, although less accessible than in a channel like gramicidin A, provide nevertheless a favorable attraction towards a cation (see Ref. 10) all along its transit.

The results of this section offer an illustration of the variety of possible stable configurations attainable by a bundle of five α-helices under the conditions imposed. Although the packages found (particularly P5L1) represent extreme cases, the differences observed, in particular in the dimension and shape of the internal hole limited by the five helices, show explicitely how a modulation of the size and shape of a pore can be associated with

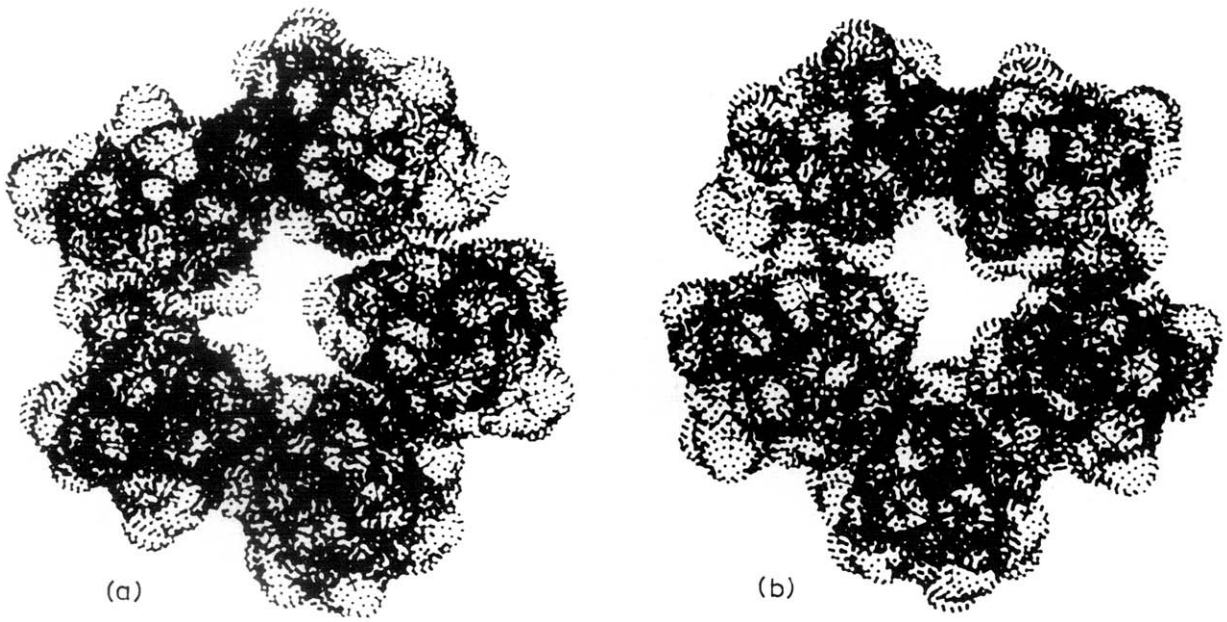

Fig. 8. Same as in Fig. 7 for the pure poly(L-alanine) P5b structure.

sliding or/and tilting of the helices. Such modulations are at the basis of various models for opening/closing of channels [16,2,17] (see also Ref. 18) but, to our knowledge, have not yet been supported by explicit energy calculations. It must be added, however, that while the present study demonstrates the possibility of existence of bundles of different, but relatively close, stabilities, suggesting the feasibility of interconversion, the energy path which can lead from one to the other remains to be explored.

III. Effect of serines on the shape and properties of the P5L bundle

In the same spirit as in Ref. 11, we have chosen the most regular 'channel', P5L4, to investigate the effect of serines replacing all the alanines situated on the inner wall of the hole. The resulting structure contains thus 15 serine residues (three per helix) pointing inside. The five helices are identical, with the sequence

Ala	Ala	Ser	Leu	Ala	Leu	Ser	Leu	Ala	Leu	Leu	Ala	Leu	Ser
1	2	3	4	5	6	7	8	9	10	11	12	13	14

Starting from the P5L4 structure, optimization by procedures A and B led to the same final structure of the serine-containing bundle, called P5LS in what follows.

As can be seen in Table V, the overall shape of the structure has not been significantly modified by the presence of the serines. The largest changes observed are the vertical displacement of h_3 and h_4 (respectively, 0.9 and 1 Å) and the tilt of h_4 (4.8 degrees). These modifications occur in order to permit the hydroxyl groups of some serines to achieve strong interactions, either with another serine OH group or with a carbonyl oxygen of another residue on another helix. The main interactions of this type are indicated in Table VI. In

TABLE V

DISTANCE (R), SHIFT (d), TILT (β) AND TIP (γ) OF THE HELICES RELATIVES TO h_1 IN THE OPTIMIZED STRUCTURE INCLUDING SERINES, P5LS

Distances are in Angströms, angles in degrees. See Table IV for comparison with the purely hydrophobic structure P5L4.

Helix number	R	d	β	γ
h_2	9.1	−2.1	−164.8	0.05
h_3	17.2	−1.5	−152.2	−7.7
h_4	16.9	−0.3	6.7	−9.5
h_5	10.8	1.0	−158.9	0.4

TABLE VI

THE STRONG INTERACTIONS FORMED BY SERINE RESIDUES OF DIFFERENT HELICES IN THE P5LS OPTIMAL STRUCTURE

d(O...H) in Angströms, θ is in degrees. O(H) and H(O) are, respectively, the oxygen and the hydrogen atom of the serine hydroxyl group. O(C) is a carbonyl oxygen. θ = (AH, HB) for A–H...B.

Helix	Residue	Atom	Helix	Residue	Atom	d(O...H)	θ
h_3	Ser 14	O(H)	h_4	Ser 3	H(O)	2.43	77.5
h_3	Ser 14	H(O)	h_4	Ser 3	O(H)	2.46	79.1
h_4	Ser 10	O(C)	h_5	Ser 3	H(O)	2.95	66.8
h_4	Ser 14	O(H)	h_5	Ser 3	H(O)	1.96	11.0

view of the large values of the AH, HB angle θ for the interactions involved in the first three lines of the table these are not 'real' hydrogen bonds; only the interaction shown in the last line can be considered as such. This situation differs from that observed [11] in the polyalanine package substituted by serines on the inner wall (P5S) where 5 'real' hydrogen bonds (with d(O...H) about 2 Å and θ below 10 degrees) were formed between the serine residues of the different helices. In the present P5LS package, the leucyl side chains lying at the surface of contact between the helices cause an expansion of the package, so that the seryl side chains of different helices are farther away from each other than in P5S. Furthermore, as noted earlier, the interfacial leucines prevent rotation of the helices around their axis which could facilitate interactions between serines belonging to different helices. Nevertheless enough favorable interactions remain to deepen the total interaction energy by about 23 kcal/mol with respect to the corresponding quantity in P5L4.

The conformations of the seryl side chains, given by the values of their two dihedral angles (Table VII) confirm their considerable flexibility (see Ref. 11) and the variety of the optimal conformations adopted according to their surroundings: thus, the oxygen atom of the hydroxyl group, O(H), can be directed towards the hydrogen on the N atom, H(N), of the same serine residue, with d(O...H) about 2.5 Å (e.g. Ser 3 of h_1), or the hydrogen atom of the hydroxyl group, H(O), can be hydrogen bonded to the carbonyl oxygen of a serine residue in a preceding turn of the same helix (e.g. Ser 7 and Ser 3 of h_3 (with d(O...H) = 2. 16 Å and θ = 16.1 degrees) (The existence of such a conformation has been noted [19] in α-helical segments of globular proteins); in a third possibility (realized also in the Ala-Ser package), the serine can be involved in hydrogen bonding with a carbonyl group of a serine on another helix or in strong favorable interaction with a residue (serine or leucine) of another helix. Clearly one cannot speak of a single strongly prefered conformation of seryl side chains in a package of α-helices but of several possible conformations according to the surroundings of these polar groups which can adapt themselves in different ways so as

TABLE VII

THE VARIOUS CONFORMATIONS DEFINED BY THEIR DIHEDRAL ANGLES OF THE SERYL SIDE CHAINS IN THE P5LS PACKAGE

Angles in degrees (1, N-C_α-C_β-O; 2, C_α-C_β-O-H).

Residue	1	2
Ser 3, h_1	−56.4	−147.5
Ser 7, h_1	−68.8	177.4
Ser 14, h_1	−67.2	−179.4
Ser 3, h_2	−54.2	−98.4
Ser 7, h_2	−178.4	175.1
Ser 14, h_2	−66.2	−179.4
Ser 3, h_3	176.9	179.8
Ser 7, h_3	−64.4	79.3
Ser 14, h_3	−173.8	−179.4
Ser 3, h_4	−178.6	−172.7
Ser 7, h_4	−166.6	164.9
Ser 14, h_4	−63.9	−179.4
Ser 3, h_5	175.1	155.4
Ser 7, h_5	−173.3	165.5
Ser 14, h_5	−174.7	173.5

to ensure, through the best possible utilization of their 2-fold hydrogen bonding capability together with their intrinsic lability, the best possible global energy balance in the bundle. We have shown earlier [11] that these properties can also be utilized by serines for interactions with cations and/or water molecules, indicating a possible role of such side chains in the channeling of ions.

Concluding remarks

As concerns the effect of the presence of bulky hydrocarbon side chains at the interfaces of α-helices in couples and bundles, this study has led to the following principal conclusions.

(1) The essential pairing properties found previously for pairs of poly(L-alanine) are conserved, namely: (a) the regularity in the most stable packing configuration (near-antiparallelism) observed upon increasing the number of residues from 14 to 21; (b) the role of the non-bonded interactions dominating over the pure electrostatic one; (c) the possibility of existence of stable parallel pairs.

(2) A variety of stable arrangements of five α-helices made of eight alanines and six leucines (three on each interface) can be obtained, starting from a regular up-down-up-down-up disposition along the edges of a pentagonal prism (without or with initial tilt). These stable structures enclose holes of variable shapes (going from funnel-like to

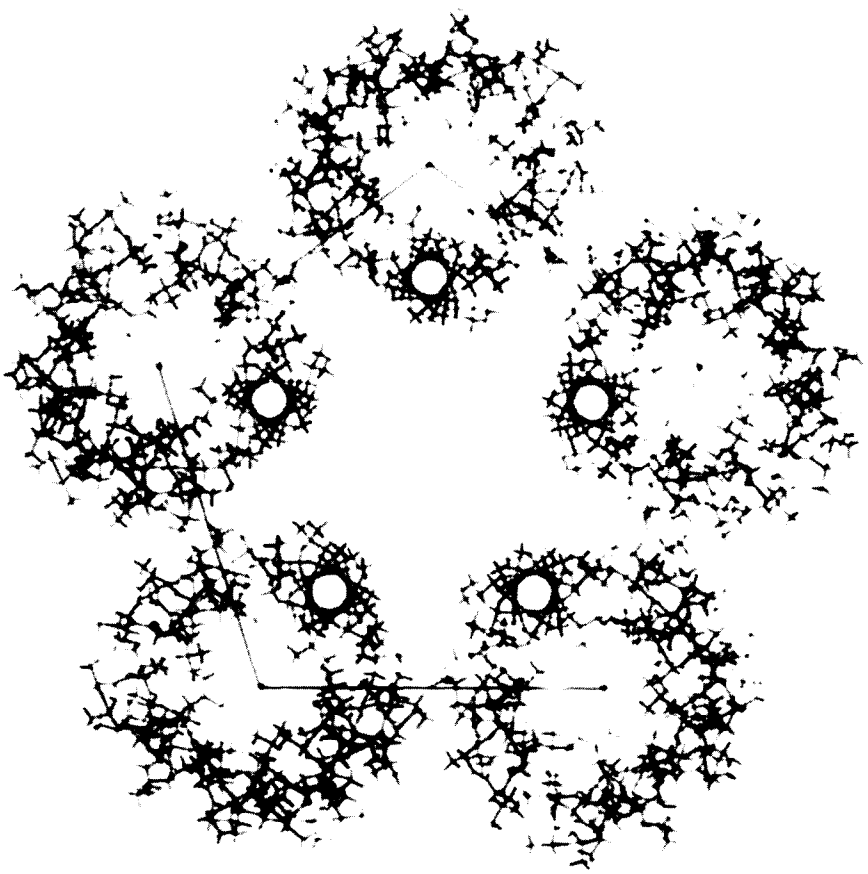

Fig. 9. A possible arrangement of five P5L4 bundles, following a pentagonal symmetry around a central pit. Using the numbering of section II, the innermost helix is h_3 in each P5L4. All h_3's are placed perpendicularly to the plane of the figure and viewed from the N-terminal along their parallel axes. In this disposition, the interfacing between two bundles is made by two antiparallel helices. The shortest interatomic distance (atom-center to atom-center) between two non-adjacent helices lining the hole is about 18 Å.

more regular tunnels) according to the tilt and relative sliding of the helices.

(3) The substitution of serines for the alanines protruding on the inner wall of one of the P5L bundles affects very little the interhelix packing, a result already observed upon similar substitution in the polyalanine packages. The different seryl side chains show no strong preference for a unique conformation and adapt themselves to interact in different ways with the available neighbouring groups.

This set of results concern the intrinsic packing properties of the α-helices considered. Although these features may be modulated in membranes by the presence of the surrounding lipid phase, there is presently no basis for expecting a complete upsetting of these conclusions. An explicit study of this problem as well as of the possible role of water is underway.

Concerning the relevance of our bundles to the structure of channel-forming proteins in membranes, let us add the following remark: in the present study, as in our previous one on packages of three to seven α-helices, we have oriented the helices successively up, down, up, down, etc. around a polygonal prism. This arrangement mimicks the situation realized by a single protein chain crossing the membrane a number of times, as appears to be the case in bacteriorhodopsin [1,20,21,22]. (An analogous situation seems to occur in the sodium-channel of *Electrophorus electricus* which, although made of different subunits α, β_1 and β_2, apparently contains the ion-channel in the α subunit, a long polypeptide chain which displays four homologous domains containing a number of transmembrane segments thought to be α-helices [23,24].

In other channel-forming membrane proteins for which the role of α-helix bundles has been suggested, the situation appears different in the sense that the helices forming the inner channel wall belong to different subunits, each subunit forming itself a bundle of alternate up, down, up, down... helices by threading a number of times through the membrane, the best documented example being the AcCh receptor channel where five subunits, each crossing the membrane four or five times, contribute one helix each to the inner wall of a pentagonal pit (see Ref. 2 and the models of Refs. 16, 25, 26). In that case the pore is limited by parallel helices: our bundles are thus not representative of the pore itself, but rather of the possible arrangements of the individual subunits. To build the pore, these may be associated together into appropriate pentagonal 'superbundles'. An illustration of one of these possible associations is given in Fig. 9, using five identical P5L4 packages. The dimensions of the inner hole obtained in this way is reasonable (see caption of Fig. 9). This derives from the fact that the average diameter of P5L4 is about 26 Å leading to a corresponding sectional area about 530 $Å^2$ over the extent of the bundle, a value very close to that observed [27] for the cross-sectional area per subunit in the AcCh receptor channel. A study of this and other superbundles is presently underway and will be reported separately.

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