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CHANNEL STRUCTURES IN SYNTHETIC POLYPEPTIDES WITH ALTERNATING CONFIGURATIONS

CONFORMATIONAL ANALYSIS OF POLY(DL-PROLINE)

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Theoretical conformational analysis of L,D alternating sequences of poly α-amino acids is reported in connection with the ability of naturally occurring peptide and depsipeptide having alternating configurations to increase selectively the ion permeability across membranes. The most stable structures of poly(DL-proline), of which the conformational variability is practically limited to the choice between *cis* and *trans* conformations of the peptide bonds, were characterized. The all-*trans* conformation results in a flat helical structure possessing the main features for acting as an ion channel across membranes as actually found experimentally. Random *cis-trans* conformational sequences provide an alternative mechanism of ion transport intermediate between the ion channel and the ion carrier.

1. Introduction

Certain naturally occurring peptide and depsipeptide antibiotics are capable of selectively increasing the ion permeability of artificial and biological membranes [1,2].

This ability is the result of the presence of hydrophobic side chains which ensure their insertion in the lipidic phase of membranes as well as of the regular configurational changes along the chain.

The last feature, in fact, under conditions of conformational equivalence (or quasi-equivalence) of the monomer units results in structures capable of interacting with ions, characterized by the presence of an ion complexing cavity or of hydrophilic channels suitable for ion passage. Their dimension, rigidity and dynamics control the permeoselectivity of ion transport across membranes.

Therefore, a conformational analysis of L,D alternating peptides becomes a useful tool for investigating the conformational features, stability and dynamics of such structures as well as for designing synthetic peptides suitable for inducing ion permeability in membranes.

This paper reports the conformational analysis of the alternating L,D sequence of proline residues whose hexameric cycle was predicted to be an effective and highly selective ion carrier [3,4] where the homologous polymer is shown to be capable of forming transmembrane ion channels as well as assisting the ion crossing by providing activated pathways of ion complexing sites along the chain.

2. Conformational analysis

The regular conformations of polypeptide chains with alternating configurations can be selected assuming conformational equivalence between all

monomeric units along the chain. This corresponds to associating with any configurational change a conformational inversion of the sense of the torsional angles.

A formally simple representation emerges if cartesian frameworks are adopted with opposite handedness to represent the structure of an amino acid unit in connection with its configuration. In fact, if A represents the *trans* conformation matrix between a coordinate system to the next one in the case of a homo-configurational chain, $A\sigma$ will represent the transformation of a system in the next one when alternating configurations occur at each residue along a polypeptide chain having equivalent local structures, where σ is the reflection matrix through the xy plane.

If A is obtained following the skeleton bonds, it becomes a function of the torsional and bond angles.

In fact, it was shown in a previous paper [3] that the conformational equivalence of the amino acid residues in peptides with alternating configuration results in cyclic structures with S_n symmetry, n ranging between 6 and ∞ (in the last case the rotoreflection symmetry degenerates into the glide symmetry). In terms of the internal parameters, A is given by

$$A = \Omega\theta_N\Phi\theta c_\alpha\Psi\theta_{C'}$$

where Ω, Φ and Ψ are the pure rotation matrices around the x-axis of the angles of rotation ω, φ, and ψ, respectively, around C′-N,N-C_α and C_α-C′ skeletal bonds; $\theta_N\theta_{C_\alpha}$ and $\theta_{C'}$ are the pure rotation matrices around the z-axis of the bond angles at the skeleton atoms indicated by the respective subscript. Among such cyclic structures the family with $n = 6$ is particularly interesting because it is characterized by inner dimensions suitable for complexing or travelling of alkali metal ions. It is defined by the equation:

$$a_{11} + a_{22} - a_{33} = 0$$

Fig. 1 shows, in the φ-ψ plane, the values of n in the case of the *trans* conformation of the peptide bonds ($\omega = 180°$) within the sterically allowed regions of the L-amino acid dipeptide unit. Besides the obvious presence of centres of symmetry, the figure presents mirror symmetries corresponding

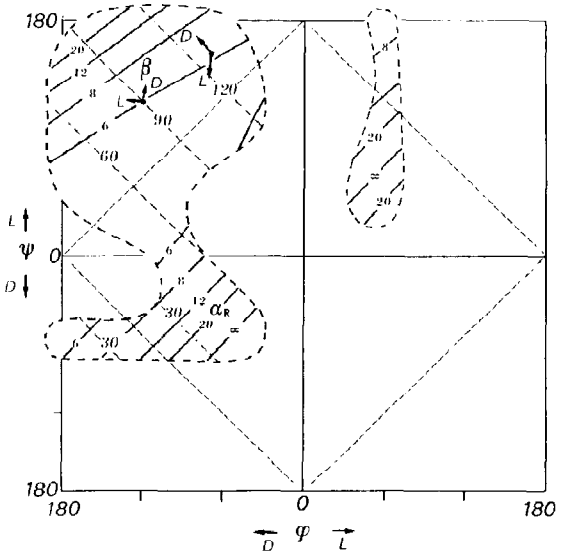


Fig. 1. Number of amino acid residues per ring in terms of the angles of rotation φ or ψ within the sterically allowed regions of the dipeptide unit. The values of φ and ψ for the L and D residues have opposite signs. The conformational splittings of the β_{LD} and poly(DL-proline) helices are indicated by arrows. Trends of the angles between the peptide group and the symmetry axis are shown.

to the planes $\varphi = \pm(180 - \psi)$.

In the same figure the values of the angle between the peptide group and the S_n axis are reported.

The cosine of this angle is in fact easily obtained as the z component of the symmetry axis S_n: $(a_{12} - a_{21})/2 \sin(360/n)$

It should be noted that the hexamers are located in the regions of the extended polypeptide chains; as a consequence the conformational stability of such DL-cyclopeptides is comparable with that of the poly L-peptides having the same local conformations. Thus, the DL-cyclohexapeptide with $\varphi = -\psi = -120°$, which is characterized by a local β-pleated conformation, has conformational energy very close to that of the all-L-polymers and virtual molecular associations through hydrogen bonds similar to those in the parallel and antiparallel β-pleated sheets. Likewise, DL-cyclohexaproline has a local conformation very similar to that of poly(L-proline) II.

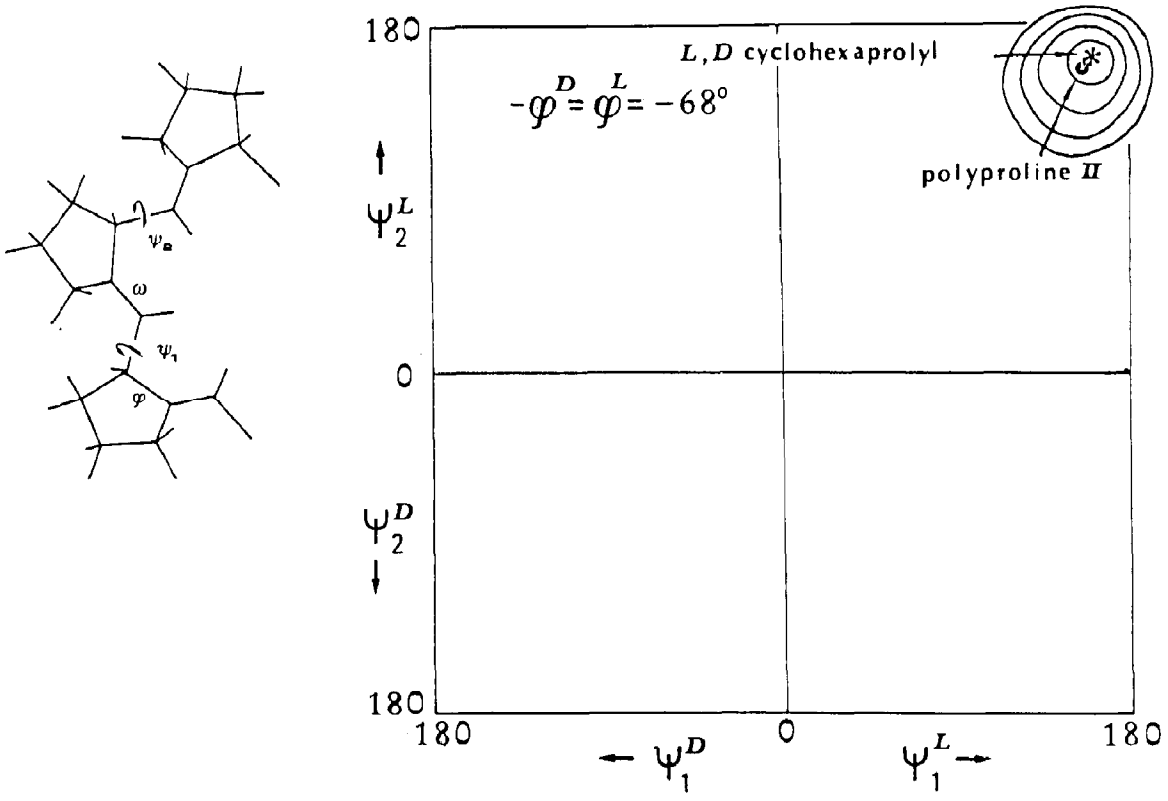


Fig. 2. Energy conformational map of the three prolyl fragment (inset) in terms of the angles of rotation around the skeleton bonds. Contour lines drawn at intervals of 1 kcal/mol.

This is illustrated in fig. 2 where the general conformational energy map of the three prolyl fragments for all the possible configurational sequences is shown in terms of the angles of rotation ψ_1 and ψ_2 indicated in the inset.

A peculiar feature of such cyclic conformations is the formal possibility to degenerate into helical structures, both left- and right-handed, practically preserving their local conformations, by relaxing the strict conformational equivalence between the enantiomeric units, which we called 'quasi-equivalence' [3]. These structures are characterized by flat helices and represent the most regular structures of poly DL-peptides. This operation leaves the conformational energy practically unchanged because of its low gradient in the extended chain region, as well as (when possible) the potential network of hydrogen bonds and the main structural features.

Two examples, pertinent to the object of the present paper, are reported in figs. 3 and 4 in the axial stereoscopic projection.

Fig. 3 illustrates the relaxing of the strict equivalence in the DL-cyclohexapeptide on the diagonal $\varphi = -\psi$ to give rise to a turn of the β_{LD}-helix as proposed for gramicidin A [5], with only slightly increased periodicity (6.2 peptides per turn); likewise, fig. 4 shows the case of DL-cyclohexaproline, characterised by a valinomycin-like ion cage [6], where a similar increase in the period (6.1) is observed. Figs. 5 and 6 illustrate the complementary stereo projections in a plane containing the helical axes. Finally, fig. 1 shows the splittings of the conformational parameters of both structures

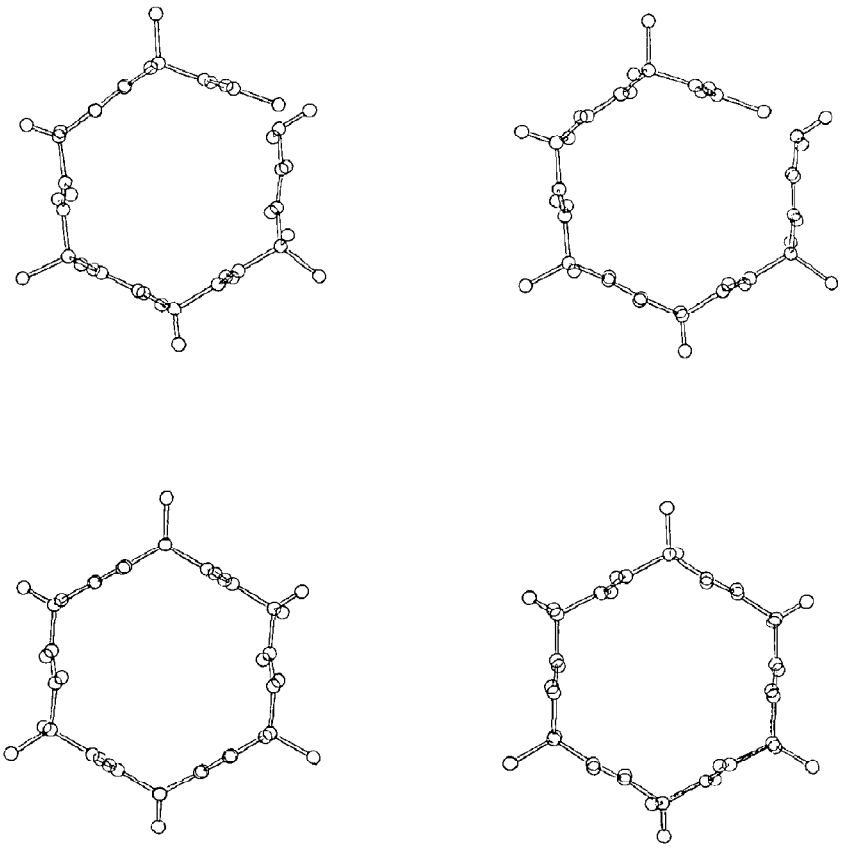


Fig. 3. Comparison of the structures of the cyclo DL-hexapeptide with local β-conformation and a turn of the corresponding β_{LD}-helix in the axial stereoviews.

after minimization of the van der Waals and hydrogen bond (in the first case) energies.

Both structures are conformationally rather rigid: the first on account of the network of hydrogen bonds and the other because of the severe conformational and steric restraints of the pyrrolidine rings; therefore, only limited changes in the libration of peptide groups are allowed. It is easy to realize that the first structure is the most stable for all poly DL-amino acids because of the further hydrogen bond stabilization, except for poly(DL-proline) which assumes in contrast, the polyproline II type local conformation of fig. 6.

As can be argued from the conformational parameters of the homologous cyclohexapeptide structures reported in fig. 1 and from the comparison of the helical models of figs. 5 and 7, the two conformations of the poly DL-peptides have very similar dimensions of the virtual bond schemes but different orientation of the peptide groups ranging from 90 to about 125° tilting with respect to the symmetry axes.

A basic feature is that both are suitable for passage of alkali metal ions by slightly relaxing the orientation of peptide groups in opposite directions while leaving almost unaltered the positions of the side chains and the pyrrolidinic rings.

The energy cost of the relaxation in the orientation of hydrogen-bonded peptide groups is paid by the dipole-ion interactions which tend to orient the

C = O amide groups toward the travelling ion; the change in the C = O orientation can be estimated to about 10°, which also accounts for the relative flexibility of bending the hydrogen bond at the oxygen atom.

On the other hand, steric effects with travelling ions tend to decrease the angle between C = O groups and the helical axis in order to increase the ion-dipole contributions when the ion penetrates into the channel, in the case of poly(DL-proline).

It is noteworthy that this mechanism corresponds to that proposed for cation capture by ion carriers like valinomycin [6,7].

Thus, the structures 'active' as ion channels are probably more similar than the 'inactive' molecular structures represented in figs. 3–6.

As in the case of all-*trans* polymers it is possible, by relaxing the conformational equivalence, to build up the all-*cis* polypeptide: the model obtained after energy refinement corresponds to a low-pitch helix with seven proline residues per turn; the structure is practically 'inverted' with respect to the all-*trans* polypeptide in that the channel is hydrophobic whereas the CO groups point toward the outside.

Besides the helical conformations of poly(DL-proline) where the monomeric units are quasi-equivalent, other structures of the polypeptide chain are allowed sterically: a regular helical structure where the conformational equivalence is re-

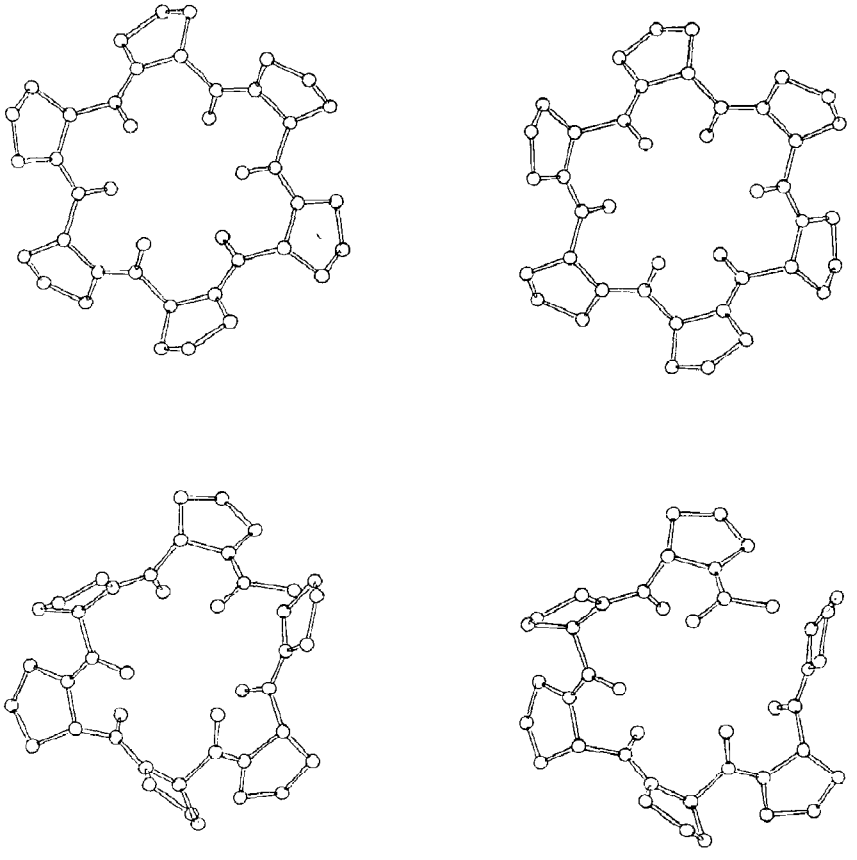


Fig. 4. Comparison of the structures of the cyclo DL-hexaproline with a turn of the corresponding DL-helix in the axial stereoviews.

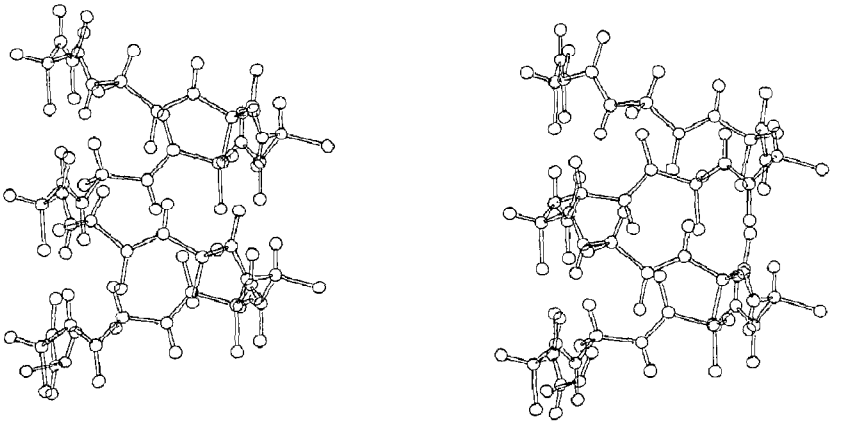


Fig. 5. Stereoview in a plane containing the helical axis of the most stable β_{LD}-helix.

duced to a pair of DL-peptides and non-regular structures where the different proline residues have non-uniform conformations. In the first case the only possibility is related to a regular alternation of *cis* and *trans* conformations of the peptide bonds with practically equivalent ϕ, ψ pairs.

The stereo projection of such a structure after energy refinement is shown in fig. 7, perpendicular

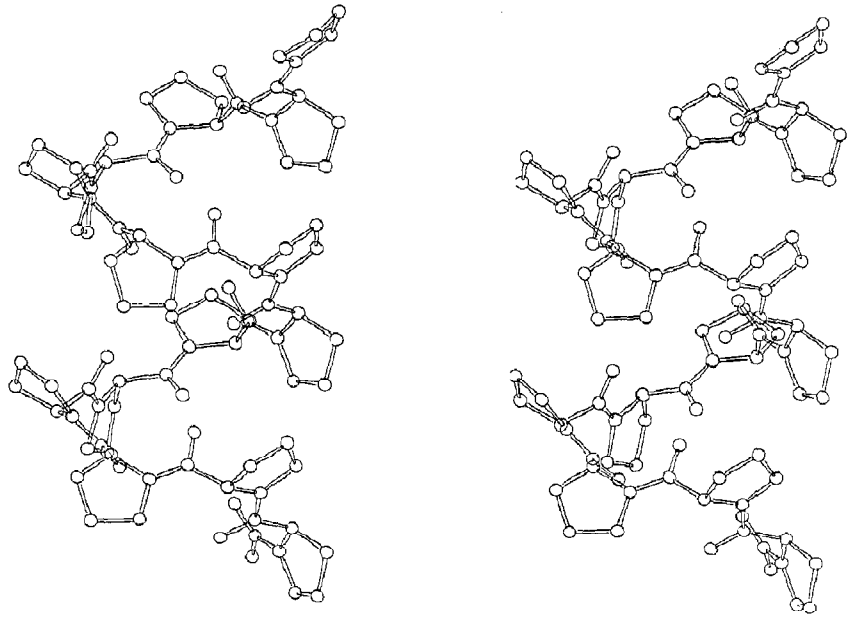


Fig. 6. Stereoview in a plane containing the helical axis of the most stable poly(DL-proline) helix.

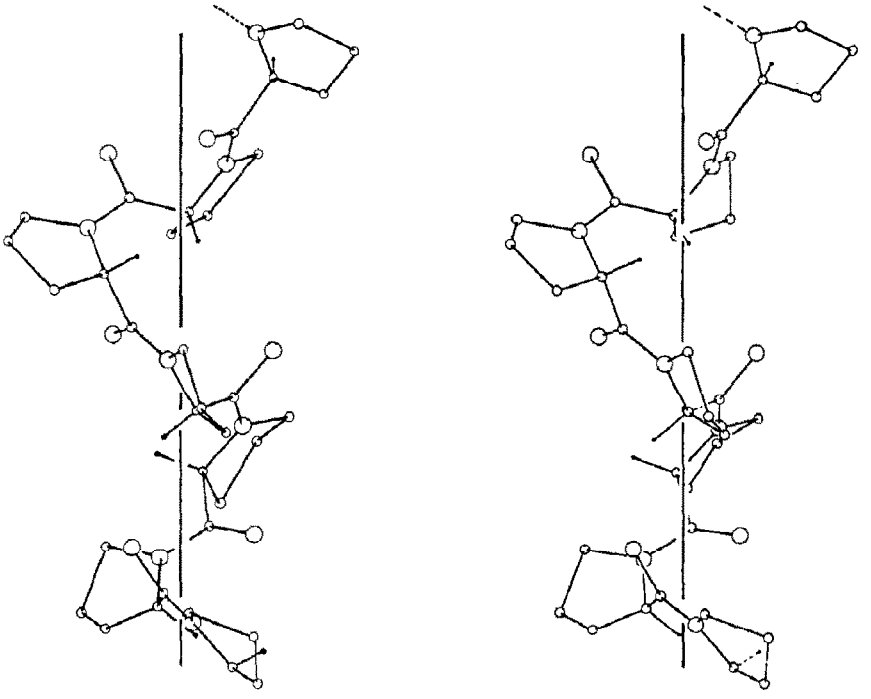
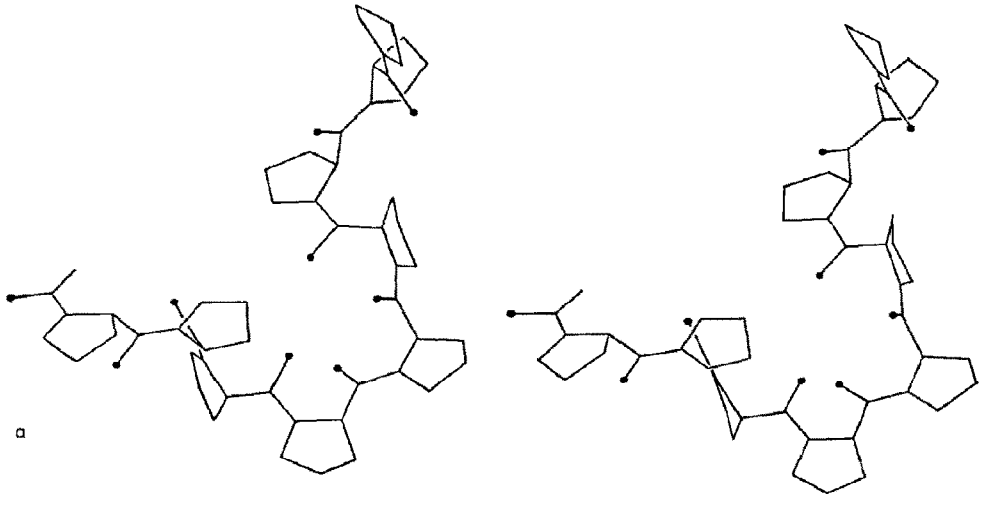


Fig. 7. Stereoview of the most stable *cis-trans* conformation of poly(DL-proline).

to the helical axis. It is characterized by 1.3 monomeric units (corresponding to a pair of proline residues) per turn and by 4.9 Å of monomer repeat on the helical axis.

This conformation characterizes the crystal structure of the homologous oligomer Boc(L-Pro,D-Pro)$_2$OH, recently analyzed in our laboratory [8].



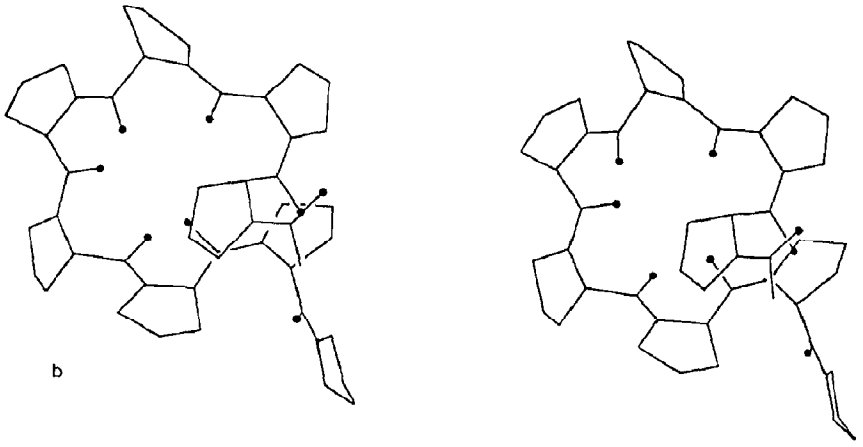


Fig. 8. Stereoviews of two non-regular sequences of *cis* and *trans* conformers in poly(DL-proline): (a) ctctttctc, (b) tcttttct (c, *cis*; t, *trans*).

Non regular structures of poly(DL-proline) arise from non-uniform sequences of *cis* and *trans* conformers owing to the fact that all the pairs of torsional angle ϕ and ψ are equivalent along the chain (see fig. 2).

Fig. 8a and b shows schematically two examples of such structures in stereoscopic views.

It should be noted that where few '*trans*' conformations occur in sequence along the peptide chain, ion complexing sites are obtained toward which the CO groups point as in DL-cyclohexaproline; in the case of more than four all-*trans* peptide sequences a complete turn of the channel structure is generated (see fig. 4).

Such non-regular structures have in vacuo conformational energies comparable with that of the regular *cis-trans* helical structure. Solvents, however, as in the case of the homo-configurational poly(L-proline) I (all-*cis*) and II (all-*trans*), could favour the regular structures (all-*trans* or all-*cis* channels and *cis-trans* helix).

The first structure is expected to be further stabilized in a lipidic phase by travelling ions.

3. Concluding remarks

Generally, DL-polypeptides can give rise to several conformations such as regular β-helices, α-pleated sheets and α-helices stabilized by recurrent hydrogen bonds [3], except poly(DL-proline) which has possible regular conformations characterized by similar φ and ψ values but different peptide conformations: the all-*trans* channel structure with 6.1 proline residues per turn, the *cis-trans* helical structure with 2.6 proline residues per turn, and the all-*cis* helix with 7 residues per turn.

The all-*trans* conformation is particularly interesting in connection with its expected property to behave as an ion channel across membranes.

As a matter of fact, we have recently shown the presence of characteristic regular current fluctuations in synthetic membranes doped with poly(DL-proline) which are generally considered as a manifestation of ion channels [4].

It is possible, however, that in spite of the similarity of the structures and the membrane phenomena with gramicidin A, a different mechanism of ion transport can be advanced for poly(DL-proline) based on the possible occurrence in membranes of non-regular sequences of *cis* and *trans* conformations.

In this case the distribution of ion complexing sites along the chain while the polymer crossing the membranes should provide activated pathways for the ion into the membranes. This mechanism of ion transport seems to be intermediate between that of ion channels and ion carriers, and plausibly closer to what occurs in membrane proteins.

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