Conformational Analysis of Regular Enantiomeric Sequences

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ABSTRACT: Cylindrical coordinates and symmetries of regular enantiomeric sequences are derived on the basis of eigenvalue–eigenvectors solutions of the Eyring-transformation matrix. Since the conformational equivalence is assumed, the general structure of these systems is a ring. However, helical structures may be built, where a quasi-conformational equivalence exists between the monomeric units. In the case of L,D regular copolypeptides these structures are stabilized by both van der Waals and hydrogen-bond interactions and are found to be more stable than the α helix when configurational disorders are absent. Structural relations with naturally occurring ion-carrier antibiotics are discussed.

Recently considerable attention has been paid to and promising prospects have opened for polypeptide chains with regular sequences of L- and D-amino acid residues. These polymers appear to assume, besides α-helical conformations, new structures different from those characterizing the poly(L-amino acid) chains, as suggested by some experimental evidence. In fact Spach *et al.*[1] have recently prepared regular alternating poly(γ-benzyl D,L-glutamate) and from optical measurements concluded that it mainly assumes the left handed α-helical conformation which is dictated by the slight predominance of D units (47% L, 53% D) owing to an appreciable racemization during the polymerization. Nmr results by Bovey *et al.*[2] support this conclusion.

Similar results have been reached by Rydon *et al.*[3] on the basis of spectroscopic studies; they obtained however the additional evidence that, when racemization is negligible, a new conformation, the nature of which is uncertain, appears to be formed. It is noteworthy that some years ago Tsuboi *et al.*[4] showed that X-ray and ir measurements on fibers of random copoly(γ-benzyl D,L-glutamate) give the typical patterns of α-helical structure. Their suggestion was that a partial stereoselectivity characterizes the polymerization of mixtures of D- and L-amino acid *N*-carboxyanhydrides, which supplies some sequences of homo configurational residues required for initiating an α helix.

The question of whether sequential D,L copolymers of amino acids can exist in a stable α-helical conformation has been recently considered by Scheraga *et al.*[5] on the basis of energy calculations. They concluded that α-helical conformations of L,D copolypeptides possess conformational energies comparable to that of poly(L-peptides) and less than that of some other structures (L,D ribbon and L,D helix) previously proposed by Ramachandran *et al.*[6] However, in the case of the pentadecapeptide antibiotic gramicidin A, which may be considered as belonging to the same class of polymers, a new helical structure (π_{LD}), corresponding to enantiomorphous sequences of β-type conformers, has been recently proposed by Urry[7-9] on the basis of nmr and spectroscopic studies. A systematic conformational analysis of these types of polymers seems therefore to be useful in view of the detailed studies in this field. The main aim of the present paper is to derive for enantiomeric sequences general relations for the chain symmetries in terms of the monomer conformation. In particular a compact transformation of the internal parameters, namely, the angles of rotation which define the monomer conformation, into the external parameters, namely, the cylindrical coordinates, was obtained.

Applications to polypeptide chains are illustrated, which allow us to draw some interesting consequences and to explain some experimental results.

Symmetries in Regular Enantiomeric Sequences LD-LD··· Let us consider orthogonal coordinate systems $[\mathbf{X}_i]$ and $[\mathbf{X}_{i+1}]$ fixed equivalently at the ith and $(i+1)$th monomeric units, respectively, with the origins at equivalent atoms and therefore chosen in such a way as to make the coordinate systems right-handed and left-handed, respectively. Let $\mathbf{A}_i^{i+1}$ be the rotation matrix which related the two systems and b_i the pertinent translation, then

$$[\mathbf{X}_i] = \mathbf{A}_i^{i+1}[\mathbf{X}_{i+1}] + b_i$$

Thus $\mathbf{A}_i^{i+1}$ is an improper orthogonal transformation which has a determinant of -1. Assuming the z axis has unique character, the diagonal form of $\mathbf{A}_i^{i+1}$ is

$$\mathbf{S} = \begin{bmatrix} e^{i\alpha} & 0 & 0 \\ 0 & e^{-i\alpha} & 0 \\ 0 & 0 & -1 \end{bmatrix}$$

and its real form

$$\mathbf{S}' = \begin{bmatrix} \cos\alpha & \sin\alpha & 0 \\ -\sin\alpha & \cos\alpha & 0 \\ 0 & 0 & -1 \end{bmatrix}$$

The transformation $\mathbf{A}_i^{i+1}$ is therefore equivalent to a rotation of α around the z axis followed by a reflection across the plane perpendicular to the same axis.[10] An identical result is obtained with the matrix $\mathbf{A}_{i+1}^{i+2}$ which transforms the coordinate system $[\mathbf{X}_{i+2}]$ into $[\mathbf{X}_{i+1}]$ since the conformational equivalence is assumed between all the monomers.

Therefore the repetition of this symmetry operation ($\mathbf{S}_n$, $n = 2\pi/\alpha$) generates cyclic structures which have physical meaning only when n is an integer even number. The "glide" symmetry is the limiting case when $n \to \infty$ and represents open chains where the monomeric units are related by the operation of reflection across a plane contain-

(1) F. Heitz and G. Spach, *Macromolecules*, 4, 429 (1971).
(2) F. A. Bovey, J. J. Ryan, G. Spach, and F. Heitz, *Macromolecules*, 4, 433 (1971).
(3) P. M. Hardy, J. C. Haylock, D. I. Marlborough, H. N. Rydon, and H. T. Storey, *Macromolecules*, 4, 435 (1971).
(4) M. Tsuboi, Y. Mitsui, A. Wada, T. Miyazawa, and N. Nagashima, *Biopolymers*, 1, 297 (1963).
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(9) D. W. Urry, J. D. Glickson, D. F. Mayers, and J. Haider, *Biochemistry*, 11, 487 (1972).
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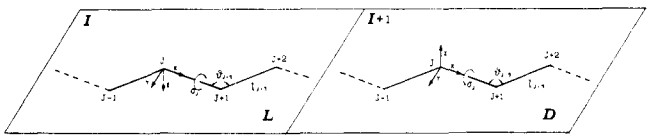

Figure 1.

ing the chain axis followed by a translation along this axis. The same conclusions hold in the case of chains characterized by sequences of enantiomers in pairs, like LLDDLLDD ··· In this case a pair of monomeric units represents the repeating units.

Transformation between the Internal Parameters and Cylindrical Parameters of the Atoms in the Monomeric Unit. In order to obtain the cylindrical coordinates and symmetry parameters of the monomeric units in terms of the angles of rotation which define its conformation let us consider the matrices $\mathbf{A}_i^{i+1}$ built as a product of simple rotation matrices, following the Eyring matrix method.[11,12] Thus the $[\mathbf{X}_i]$ system has its x axis along the bond $j \rightarrow j+1$ its y axis having a positive direction in the smaller-angle side of $j-1, \hat{j}, j+1$; the direction of the z axis is chosen in such a way as to make the coordinate system right-handed. The $[\mathbf{X}_{i+1}]$ system is fixed identically at the equivalent atom of the following enantiomorphous monomer, namely, with the x and the y axes in the quivalent direction but the z axis in opposite directions in order to make the coordinate system left-handed. This is schematically illustrated in Figure 1, where i and $i+1$ refer to the monomeric units and $j-1$, j, and $j+1$ refer to the atomic positions within these units. σ_j is the angle of rotation around the $j \rightarrow j+1$ bond related to the cis conformation of the atoms $j-1, j, j+1, j+2$; and θ_{j+1} is the bond angle $(j)\widehat{(j+1)}(j+2)$; l_{j+1} represents the bond length $(j+1)(j+2)$. In the general case where the conformation of the monomeric unit is specified by the appropriate set of $l_j\sigma_j\theta_j$ triplets of internal coordinates

$$\mathbf{A}_i^{i+1} = \mathbf{A}_{i+1}^{i+2} = \ldots = \mathbf{A} = [\Gamma_1\Delta_1 \ldots\ldots \Gamma_m\Delta_m]\mathbf{R}$$

and

$$b_i = b_{i+1} = \ldots = b = l_1 + \sum_{j=1}^{m}[\Gamma_1\Delta_1 \ldots\ldots \Gamma_{j-1}\Delta_{j-1}]l_j$$

since the conformational equivalence is assumed.

$$l_j = \begin{bmatrix} l_j \\ 0 \\ 0 \end{bmatrix}$$

m is the number of the skeletal bonds in the monomeric unit

$$\Gamma_j = \begin{bmatrix} 1 & 0 & 0 \\ 0 & \cos\sigma_j & -\sin\sigma_j \\ 0 & \sin\sigma_j & \cos\sigma_j \end{bmatrix}$$

$$\Delta_j = \begin{bmatrix} -\cos\theta_j & -\sin\theta_j & 0 \\ \sin\theta_j & -\cos\theta_j & 0 \\ 0 & 0 & 1 \end{bmatrix}$$

and

$$\mathbf{R} = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & -1 \end{bmatrix}$$

(11) H. Eyring, *Phys. Rev.*, 39, 746 (1932).
(12) A. Damiani and P. De Santis, *J. Chem. Phys.*, 48, 4071 (1968).

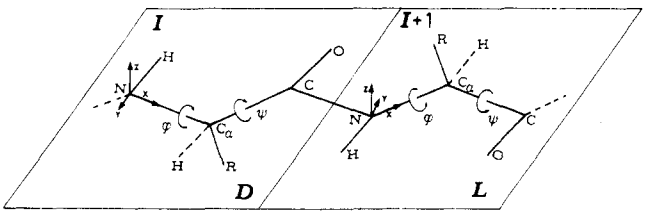

Figure 2.

which transform the right-handed to the left-handed system by an inversion of the z-axis direction. The equivalence of matrices $\mathbf{A}$ and $\mathbf{S}$ (as already discussed) requires that trace $\mathbf{A}$ = trace $\mathbf{S}$ = $2\cos\alpha - 1$, thus the number of monomers in the ring

$$n = 2\pi/\alpha = 2/\cos^{-1}\left(\frac{\text{trace } \mathbf{A} + 1}{2}\right)$$

and have physical meaning only when n is an integer and even. Furthermore the eigenvector of $\mathbf{A}$ of the eigenvalue -1 represents the axis of symmetry s.[12] Thus

$$\mathbf{A}s = -1s \qquad (\text{and } \mathbf{A}\tilde{\mathbf{A}}s = -1\tilde{\mathbf{A}}s)$$

then

$$\tilde{\mathbf{A}}s = -1s$$

$$(\mathbf{A} - \tilde{\mathbf{A}})s = 0s$$

where $\tilde{\mathbf{A}}$ represents the transpose of the matrix $\mathbf{A}$. Thus

$$s = \begin{bmatrix} a_{32} - a_{23} \\ a_{13} - a_{31} \\ a_{21} - a_{12} \end{bmatrix}/N$$

where a_{ij} are the matrix elements of $\mathbf{A}$ and N $(= 2\sin\alpha)$ is the normalization factor.[13]

It is interesting to note that the same relations hold in the case of sequences of identical configurations of monomeric units but in this case the matrix $\mathbf{R}$ is replaced by the identity and accordingly the $\mathbf{S}'$ matrix by the equivalent simple rotation matrix

$$\mathbf{H} = \begin{bmatrix} \cos\beta & \sin\beta & 0 \\ -\sin\beta & \cos\beta & 0 \\ 0 & 0 & 1 \end{bmatrix}$$

It is well known that in this case the chain symmetry is represented by a screw axis characterized by $n = 2\pi/\beta$ (not necessarily integer) monomeric units per turn and a monomeric repeat on the helical axis equal to $d = 3b$.[12] It

(13) When α is equal to 0 $(n = \infty)$ $\mathbf{AA} = \mathbf{U}$ (the identity matrix) and $\mathbf{A} = \tilde{\mathbf{A}}$. One of the solutions of

$$s = \begin{bmatrix} a_{11} - 1 \\ a_{21} \\ a_{31} \end{bmatrix}(2 - 2a_{11})^{-1/2}$$

$$s = \begin{bmatrix} a_{12} \\ a_{22} - 1 \\ a_{32} \end{bmatrix}(2 - 2a_{22})^{-1/2}$$

or

$$s = \begin{bmatrix} a_{13} \\ a_{23} \\ a_{33} - 1 \end{bmatrix}(2 - 2a_{33})^{-1/2}$$

is then more conveniently used. In the case $\alpha = \pi$ $(n = 2)$ and $\mathbf{A} = \mathbf{I}$ (the inversion matrix), the structure contains only an inversion center at $b/2$ and the transformation is indefinite.

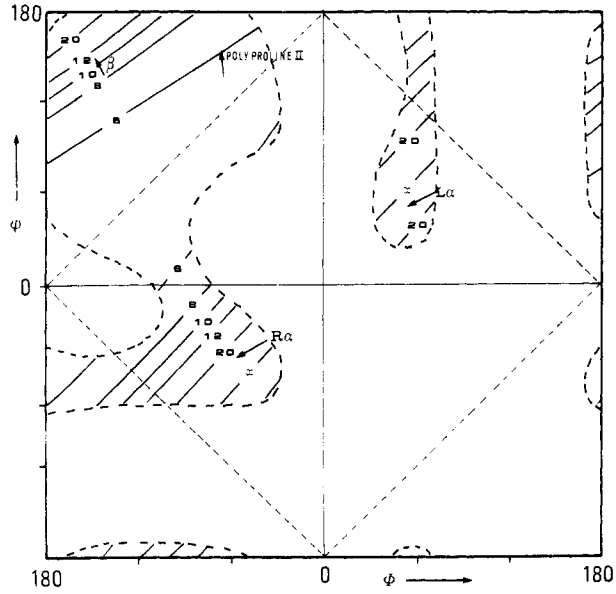

Figure 3. Number of amino acid residues in the ring in terms of the angles of rotation φ and ψ referred to the L configuration, within the sterically allowed regions.

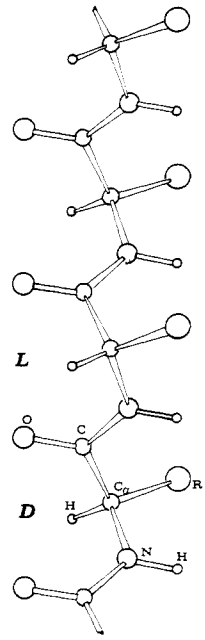

Figure 4. Three-dimensional projection on the glide plane of the right-handed α-helix-type conformation.

is now possible on the basis of geometrical considerations to obtain the transformation between the coordinates (xyz) in the [X] system to those in an orthogonal system $[\Xi](\xi\eta\zeta)$ with the ζ axis coincident with the axis of symmetry s and the ξ and η axes lying in the mirror plane. Thus

$$\begin{bmatrix}\xi\\ \eta\\ \zeta\end{bmatrix} = \mathbf{T}\begin{bmatrix}x\\ y\\ z\end{bmatrix} - \begin{bmatrix}\rho\\ 0\\ 0\end{bmatrix} - \frac{1}{2}\begin{bmatrix}0\\ 0\\ \tilde{s}b\end{bmatrix} \tag{1}$$

where

$$\tilde{\mathbf{T}} = (-r \quad r \times s \quad s)$$

is the transpose of $\mathbf{T}$

$$\rho = \mathrm{mod}(b - \tilde{s}bs)/2 \sin(\alpha/2)$$

is the radial coordinate of the system origin and

$$r = \frac{1}{N}[(\mathbf{U} - \tilde{\mathbf{A}})b - 2\tilde{s}bs] \tag{2}$$

(N is the normalization factor) represents the unit vector projection on the mirror plane of the translation vector referring to the origin of the $[\Xi]$ system in the [X] system.[14]

In the case of sequences of monomeric units of identical configuration the same formulas may be used but in eq 1 the third term is missing as well as the second one in the eq 2 in order to transform the internal coordinates to cylindrical axes. Thus eq 1 and 2 become

$$\begin{bmatrix}\xi\\ \eta\\ \zeta\end{bmatrix} = \mathbf{T}\begin{bmatrix}x\\ y\\ z\end{bmatrix} - \begin{bmatrix}\rho\\ 0\\ 0\end{bmatrix}$$

$$r = \frac{1}{N}[(\mathbf{U} - \tilde{\mathbf{A}})b]$$

Applications: Polypeptide Chain. In the case of a polypeptide chain the coordinate systems [X] may be arbitrarily fixed as in Figure 2. Thus, the atomic coordinate of the D-amino acid residue in the left-handed system are the same as those of the L-amino acid residue in the right-handed system. Accordingly, the angles of rotation around the chemical bonds φ, ψ, and ω,[15] and the bond angles θ_{C_α}, θ_C, and θ_N are opposite in sign in the two enantiomers but the corresponding rotation matrices are the same, since they are performing transformations in systems of opposite hand. Then the sequence of the rotation angles along the polypeptide skeleton is

$$|\varphi\theta_{C_\alpha}\psi\theta_C\omega\theta_N| - \varphi - \theta_{C_\alpha} - \psi - \theta_C - \omega - \theta_N|$$

whereas the sequence of the corresponding rotation matrices is

$$|\Phi\Theta_{C_\alpha}\Psi\Theta_C\Omega\Theta_N|\mathbf{R}|\Phi\Theta_{C_\alpha}\Psi\Theta_C\Omega\Theta_N|\mathbf{R}$$

where

$$\Phi = \begin{bmatrix}1 & 0 & 0\\ 0 & \cos\varphi & -\sin\varphi\\ 0 & \sin\varphi & \cos\varphi\end{bmatrix}$$

$$\Theta_{C_\alpha} = \begin{bmatrix}-\cos\theta_{C_\alpha} & \sin\theta_{C_\alpha} & 0\\ -\sin\theta_{C_\alpha} & -\cos\theta_{C_\alpha} & 0\\ 0 & 0 & 1\end{bmatrix}$$

$\mathbf{R}$ being the matrix which transforms the right-handed to the left-handed system by an inversion of the z axis. It results, therefore, that

$$\mathbf{A}_i^{i+1} = \mathbf{A}_{i+1}^{i+2} = \cdots = \mathbf{A} = |\Phi\Theta_{C_\alpha}\Psi\Theta_C\Omega\Theta_N|\mathbf{R}$$

Also

$$b_i = b_{i+1} = \cdots = b = l_{N\text{-}C} + \Phi\Theta_{C_\alpha}l_{C_\alpha\text{-}C} + \Psi\Theta_C l_{C\text{-}N}$$

The invariants of the A matrix are then calculated as already described.

In Figure 3 values of the number of monomeric units (peptide unit) n in the ring are shown in terms of the angles of rotation φ and ψ referred to the L-amino acid residue within the pertinent sterically allowed regions of the

(14) In the case when $n = \infty$ $-r$ is replaced by $(1/N)[(\mathbf{A} + \mathbf{U})b]$ (the glide axis) and $\rho = 0$ in eq 1.

(15) The nomenclature used for describing conformation is that recommended by IUPAC-IUB commisson. (IUPAC-IUB commission on Biochemical Nomenclature, Tentative Rules, *Biochemistry*, 9, 3471 (1970)).

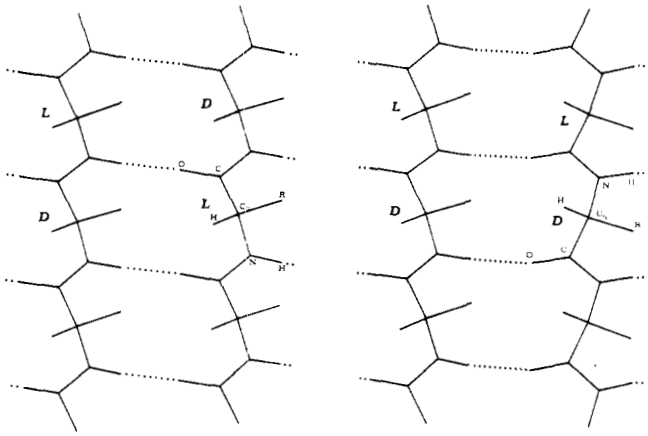

Figure 5. Schemes of parallel and antiparallel α-pleated sheets.

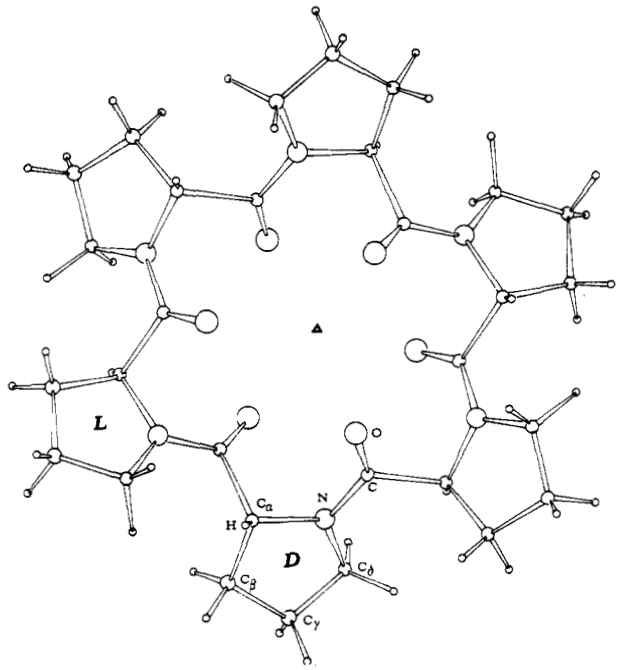

Figure 6. Three-dimensional projection along the S_6 axis of the poly(proline II)-type conformation.

peptide energy map. As can be seen from Figure 3 the diagram presents mirror planes at $\varphi \pm \psi = \pi$ and is centrosymmetric.

The "glide" symmetry is restricted to the curve $n = \infty$ which lies about the line $\varphi = \psi$ and crosses only the right-handed and left-handed α-helical regions within the sterically allowed conformations. Therefore only α-helix-type conformations represent an indefinite chain in L,D alternating polypeptides, if the conformational equivalence is assumed.

In Figure 4 the right-handed α-helix-type chain conformation of conformationally equivalent L,D alternating polypeptides is schematically shown in the three-dimensional projection on the glide plane. As it may be seen the C=O groups lie closely on a plane parallel to the glide plane as well as the N—H groups but in opposite directions. Therefore, this conformation is able to form regular hydrogen bonds with adjacent polymer chains of the parallel and antiparallel type, as it is shown schematically in Figure 5. It is also possible that the same polypeptide chain may be folded back to give an antiparallel sheet of hydrogen bonds. Two type of bends become sterically allowed—one is a turn of right-handed α helix and the other is similar to the γ turn proposed by Némethy.[16]

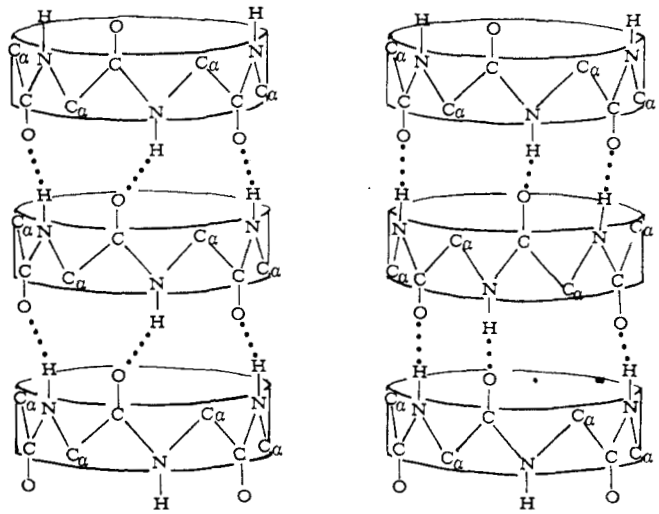

Figure 7. Schemes of parallel and antiparallel interanular association of β-type rings.

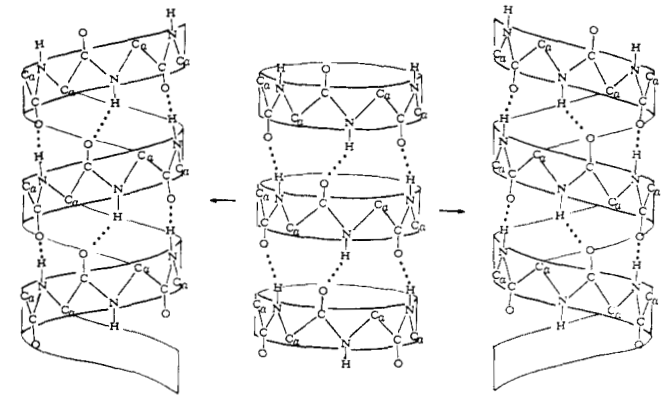

Figure 8. Scheme of geometrical transformation of parallel interanular association of β-type rings into equivalent right-handed and left-handed helical structures.

It is worth noting that this situation is very similar to that of the β-pleated sheet and cross-β structures in the case of poly(L-peptides). For this reason we called the structures presented above as α-pleated sheet and cross-α, respectively.

In the other regions of the dipeptide map only the curves with n = even integer have physical meaning and represent rings with n ranging between ∞ (glide) and 6. It may be of interest that the poly(proline II)-type conformation corresponds exactly to a six-member ring. This structure is represented in Figure 6 in the three-dimensional projection along the S_6 axis. It must be noted that this structure shows features similar to cyclodepsipeptide antibiotics enniatin A, B, and C[17] and valinomycin[17,18] in the distribution of C=O groups which form an octahedral-type cavity. This structure however presents a higher degree of rigidity because the rotation around Cα—N bonds are restricted by the pyrrolidine ring geometry; pre-

(16) M. P. Printz, G. Némethy, and H. Bleich, *Nature (London), New Biol.*, 237, 135 (1972).
(17) Yu. A. Ovchinnikov, V. T. Inanov, and A. M. Shrob in "Molecular Mechanism of Antibiotic Action on Protein Biosynthesis and Membranes," Proceedings of a Symposium, Granada, June 1-4, 1971, E. Munoz, F. Garcia-Fernandiz, and D. Vasquez, Ed., Elsevier Scientific Publishing Co., Amsterdam, 1972.
(18) W. L. Duax, H. Hauptman, C. M. Weeks, and D. A. Norton, *Science*, 176, 911 (1972).

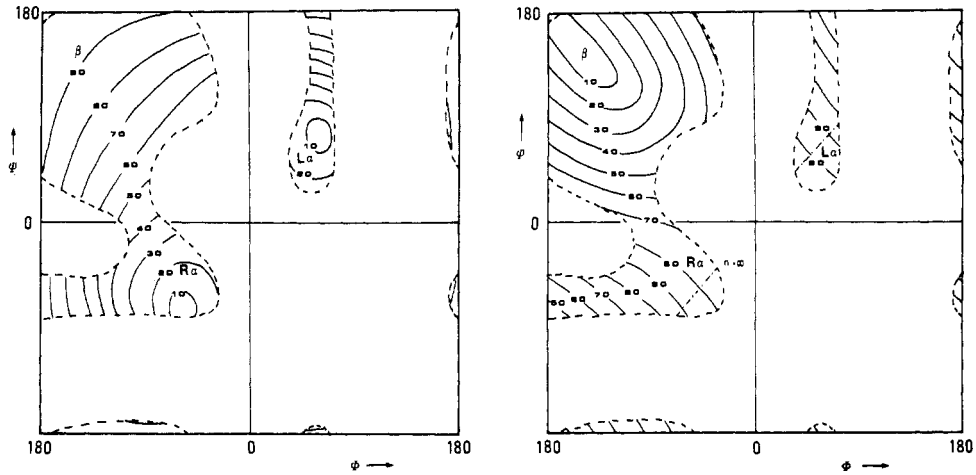

Figure 9. Diagrams of the angle between the N—H bond and the cylindrical axis in terms of the angles of rotation φ and ψ referred to the L configuration within the sterically allowed regions: (a, left) poly(L-peptide); (b, right) poly(L,D-peptide).

sumably it may reflect an enhanced selectivity in ion binding and transport and possibly antibiotic activity. The synthesis of (L-Pro-D-Pro)$_3$ cyclopeptide is now in progress in our laboratory.

β-Type conformations give rise to rings containing 6–20 residues. In these structures CO and NH bonds point in opposite directions but nearly parallel to the symmetry axis. Therefore they are able to stabilize interanular associations with cylindrical symmetry; two situations arise where the hydrogen bonds are of the parallel or antiparallel type, as illustrated in Figure 7.

As discussed in the next section it may be useful to consider the cylindrical structures of the parallel type as degenerate helical conformations, which may be considered to be related as represented in Figure 8. In this connection it may be interesting to note a sort of complementarity between poly(L,D-peptides) and poly(L-peptides) in that α conformations give rise to pleated sheets whereas β conformations to (degenerate) helices. This is conveniently illustrated in Figure 9a,b where values of the angles between the helical (cylindrical) axis and the direction of N—H groups are represented in terms of the angles of rotation φ and ψ in the case of poly(L-peptides) and poly(L,D-peptides). As may be noted angles at about 90° which favor interchain associations of pleated-sheet type are located on the β and α regions for poly(L-peptides) and poly(L,D-peptides), respectively, whereas the lowest values of the angles which characterize intrachain hydrogen bonds are located in the α and β regions, respectively.

Quasi-equivalent Helical Conformations of L,D Alternating Polypeptides. From the above it results that conformational equivalence of monomeric units excludes the possibility that it is obtained in a polymeric sequence of enantiomeric conformers (but the case of glide symmetry which, on the other hand, imposes a rigid limitation to the monomer conformation). Thus the ordered conformations of a polymer chain are built with two different monomeric conformers corresponding to different energy minima in the conformational map, or alternatively slightly perturbing equivalent anular structures to obtain a quasi-equivalence between the enantiomeric units. This is the only possibility when one of the conformers is by far the most stable.

In the case of polypeptide chains the relatively large number of monomers in the ring suggests that small deviations from the conformational equivalence are required in order to obtain helical structures from rings. In fact, starting from β-type rings and using a "refining" programm which minimizes the overall conformational energy by changing the angles of rotations, we obtained the helical conformations reported in Table I; they are characterized by the conformational equivalence of pairs of monomeric units and by a quasi-equivalence with respect to the monomeric unit. These helical conformations are designated as Lβn or Rβn in which L and R represent the handedness of the helix, β the local conformation, and n is the number of residues per turn. In Figure 10a the three-dimensional projection of the Lβ4.8 and Lβ6.2 helices are illustrated in a plane containing the helical axis. The projections in a plane perpendicular to the helical axis are schematically shown in Figure 10b. As it may be observed these structures present similar features in that the CO and NH groups are quasi-parallel to the helical axis but in alternating opposite directions and form intrachain hydrogen bonds of the parallel pleated-sheet type, equivalent in pairs, as reported in Table I. These features give rise to compact channel structures with pore sizes increasing proportionally with the number of monomers per turn.

Table I
Characteristics of the Lβn Helices[a]

Helix	Pore Size (Å)	H-Bonded Rings	Dihedral Angles (deg)			
			φ_L	ψ_L	φ_D	ψ_D
Lβ4.8	2.3	16, 14	−120	82	92	−110
Lβ6.2	3.3	22, 20	−133	117	118	−130
Lβ8.2	4.7	28, 26	−145	140	132	−145

[a] The enantiomeric Rβn helices are obtained by interchanging the angles of rotation $\varphi_L\psi_L$ with $-\varphi_D-\psi_D$.

The conformational energy evaluated using our best set of potential functions[19,20] give comparable values for all these structures in the case of poly(L,D-alanine) and of the same order (but higher) with respect to the right-handed α helix of poly(L-alanine). However it should be noted that while β6.2 and β8.2 structures may be stabilized by forming clathrates of ions or solvents β4.8 does not. It is possible, however, that transitions may occur between these structures when changing solvent.

Finally it is interesting to note that these structures are strongly destabilized if configurational inversions occur along the chain because of both local (within the dipep-

(19) P. De Santis, and A. M. Liquori, *Biopolymers* **10**, 699 (1971).
(20) P. DeSantis, R. Rizzo, and G. Ughetto, *Biopolymers*, **11**, 279 (1972).

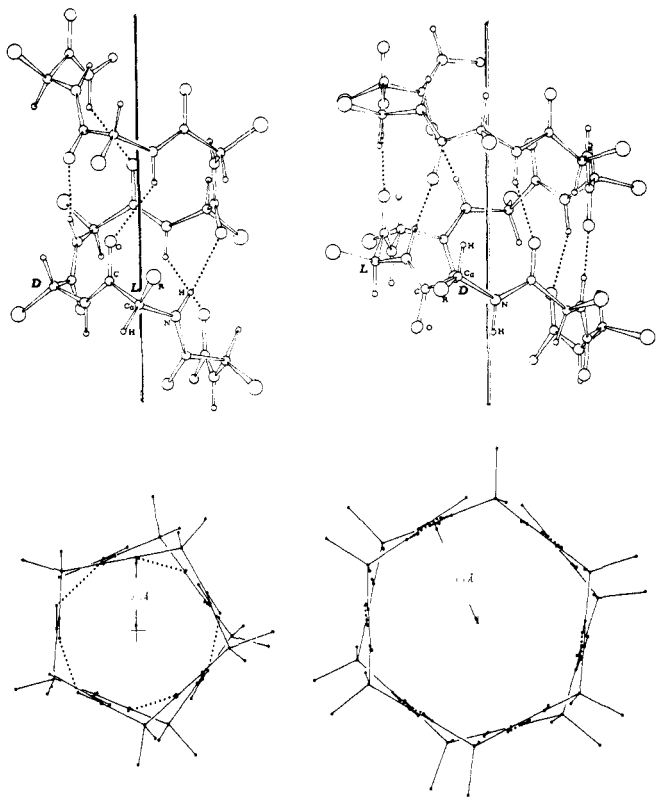

Figure 10. (a, top) Three-dimensional projection of Lβ4.8 and Lβ6.2 structures parallel to helical axis. (b, bottom) Projection of Lβ4.8 and Lβ6.2 structures on the plane normal to the helical axis.

tide unit) and long-range steric hindrance (between adjacent helical turns). Preliminary results obtained on L-D alternating copolypeptides (L-Ala-D-Val)$_n$ as well as oligopeptides (n = 4) where the racemization seems to be prevented, indicate that a β6.2-type helical structure is assumed by these compounds.[21] It is very interesting that the pentadecapeptide antibiotic gramicidin A, which may be considered as an L,D alternating copolypeptide (glycine residue replaces D-amino acid) seems to adopt these type of helical conformations as proposed by Urry[7-9] on the basis of spectroscopic studies. Moreover the π_{LD} structures of Urry are very similar to the Lβn helices presented in this paper. Moreover it should be mentioned that the LD helix proposed by Ramachandran and Chandrasekharan[6] is practically identical to our β6.2 structure.

Nonequivalent Helical Conformations of LD Alternating Polypeptides. Helical conformations of a polypeptide chain may be built up as sequences of different monomeric conformers selected in the correspondence to the minima of the conformational energy diagrams, equivalent in pairs. Within these, three types of conformations can be stabilized by recurrent intra- or intermolecular hydrogen bonds, as may be seen from the analysis of the map in Figure 11, where values of the angles between the NH bonds in the L-dipeptide unit are represented in terms of φ and ψ. These conformations are diagrammatically indicated in the Figure 11 as segments between pairs of α- or β-type conformers. In fact only for these structures the directions of the NH (and CO) bonds are of the parallel or antiparallel type, and hence they may be involved in hydrogen bonds.

β-Rα and β-Lα conformations have similar skeletal

(21) F. Ascoli, G. De Angelis, F. Del Bianco, and P. De Santis, Presented in part at The Stockholm Symposium on The Structure of Biological Molecules, Stockholm, Sweden, July 9-11, 1973.

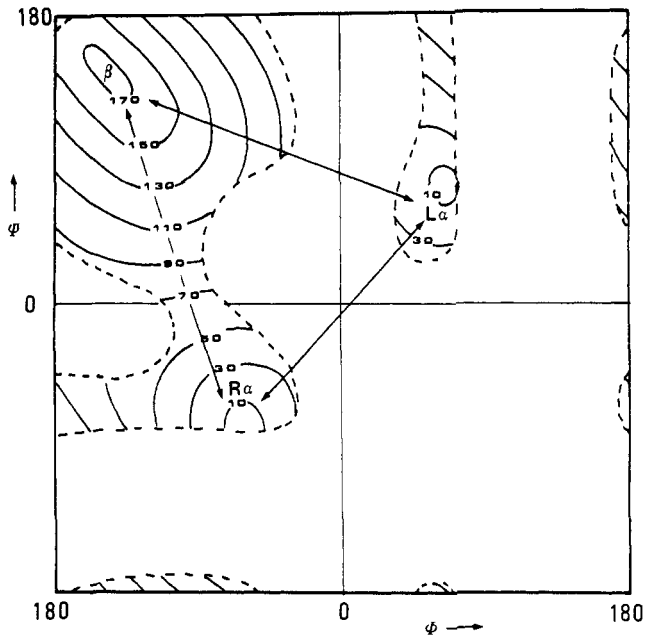

Figure 11. Diagram of the angle between the N—H bonds of the dipeptide unit in terms of the angles of rotation φ and ψ referred to the L configuration, within the sterically allowed regions.

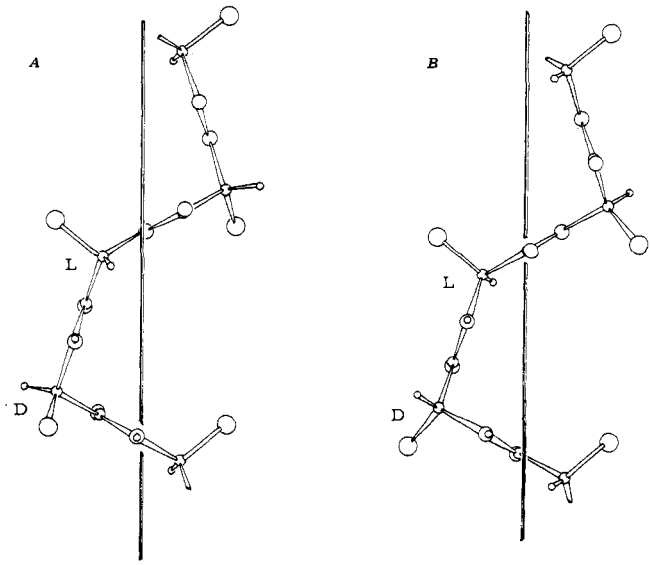

Figure 12. Three-dimensional projections parallel to the dyad axis of β-Rα (A) and β-Lα (B) structures. Interchain hydrogen bonds between parallel and identical conformations can be formed along the perpendicular to the plane of the figure.

features, corresponding to a "crankshaft" structure in which two consecutive pairs of amino acid residues are related by a dyad axis. In this way, the formation of hydrogen bonds between identical and parallel chains, where pairs of consecutive NH (and CO) groups point in alternating directions, occurs. These two structures are shown in Figure 12 as three-dimensional projections parallel to the dyad axis. It is worth noting that both these structures were recently found in poly(L-Ala-Gly) (Gly may be replaced by a D-amino acid residue) by Lotz and Keith and a similar structure was proposed by the same authors for silk I on the basis of X-ray and electron diffraction data.[22] Rα-Lα conformation gives rise to an α helix, its handedness depending on the corresponding sequence of

(22) B. Lotz and H. D. Keith, *J. Mol. Biol.*, 61, 201 (1971).

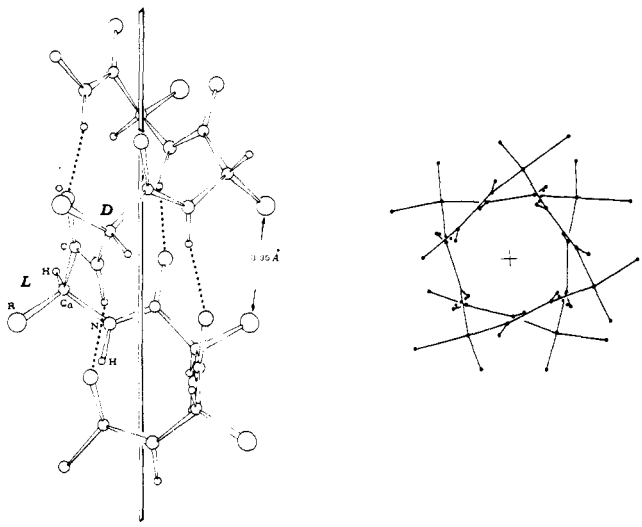

Figure 13. Fragment of an α helix with alternating configurational sequence in both the cylindrical projections.

configurations: L-D → right-handed and D-L → left-handed.

This structure is conformationally equivalent with respect to the skeleton, which is, on the other hand, strongly stabilized by van der Waals energy and hydrogen bonding, but not equivalent with respect to the interactions in which the side chains are involved. Figure 13 represents a fragment of the right-handed α helix in both the cylindrical projections.

It is noteworthy that in a fragment of seven peptides the alternation of configurations introduces only one contact (indicated in the Figure 13) closer than van der Waals distance between β carbon atoms in position 1–4; however half of the dipeptide is in a left-handed α-helical-type conformation.

We have refined this structure and found, according to Scheraga,[5] that small deviations from the conformational equivalence of skeleton are required in order to stabilize this type of structure; using our set of potential functions the conformational energy of a right-handed α helix of L,D copolymers is higher than that of a poly(L-peptide) in the case (examined) of polyalanine, and comparable, although with a lower stability, than βn conformations.

On the basis of theoretical considerations presented here it is now possible to explain the experimental findings which demonstrate the occurrence of α helix[1-3] in L,D alternating copolypeptides where configurational defects may be tolerated, as well as the presence of a new structure, which we predict to be of the βn-type, where racemization is negligible. The latter conformation, in fact, is more stable than the α helix in a "perfect" and suitably long L,D alternating sequence, whereas, on the other hand, it cooperatively breaks where configurational inversion occurs.

Finally it is worth noting that a chain having the sequence ···LDLDLD··· can have a straight ribbon structure corresponding to a series of β bends similar to that found in gramicidin S[19] and characterized by recurrent hydrogen bonds in direction parallel to the chain axis. However, only one-half of possible hydrogen bonds is formed intramolecularly whereas the other half can be involved in interchains hydrogen bonding in the direction perpendicular to the LD ribbon. It should be mentioned that the LD ribbon conformation has been recently described by Ramachandran and Chandrasekharan.[6]

Conclusion. On the basis of the present theoretical analysis it has been demonstrated that the general structure of conformationally equivalent enantiomeric sequences is a ring. In the case of L,D alternating polypeptides, however, helical structures, where the monomeric units are quasi-equivalent, are stabilized by van der Waals energy and intrachain hydrogen bonding. The structure proposed for gramicidin A belongs to this class of conformations and it is reasonable to expect that the same conformations can be adopted by any "perfect" regular-sequence D,L copolypeptide. Finally, it must be stressed that both rings and helical structures having β-type conformations are closely related to the structures found in naturally occurring cyclodepsipeptide and polypeptide antibiotics capable of transporting ions. This property is related to the ability of L,D sequences to form folded structures which provide suitable cavities for ion binding.

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