

Energy-minimized Conformation of Gramicidin-like Channels. II. Periodicity of the Lowest Energy Conformation of an Infinitely Long Poly-(L,D)-alanine $\beta^{6.3}$ -Helix

Hiroshi Monoi

Department of Physiology, Tohoku University School of Medicine, Sendai, Japan 980

ABSTRACT If an infinitely long polymer has a primary structure characterized by an N -residue periodicity, a minimum energy conformation of the polymer under the constraint of the conformational N -residue periodicity corresponds to an equilibrium structure (energy minimal or unstable equilibrium structure) when this constraint is absent. Molecular mechanics calculations showed that with an infinitely long poly-(L,D)-alanine single-stranded $\beta^{6.3}$ -helix (which has a 2-residue periodicity with respect to the primary structure), its lowest energy conformation within the framework of the conformational 2-residue periodicity is also the lowest energy form of this $\beta^{6.3}$ -helix even when no conformational periodicity is assumed. In the course of this study, contour maps of helix parameters and conformation energies for β structures of poly-(L,D)-alanine were examined. It was also found that $\beta^{6.3}$ -, $\beta^{4.5}$ -, $\alpha_{L,D}$ -, and $\pi_{L,D}$ -helices constitute the global minima in the whole conformational space of this polypeptide. In the present calculation, an improved formulation of the conformation energy was introduced to estimate the structure and conformation energy of an infinite periodic chain from results on a chain of finite length.

INTRODUCTION

The backbone structure of the gramicidin channel is basically a single-stranded $\beta^{6.3}$ -helix (Wallace, 1986; Urry et al., 1971; Urry et al., 1983; Weinstein et al., 1985; Arseniev et al., 1985; Nicholson and Cross, 1989; Hing et al., 1990). In the preceding report (Monoi, 1993), we calculated, by the molecular mechanics method, the energy-minimized conformation of an infinitely long chain of poly-(L,D)-alanine in single-stranded $\beta^{6.3}$ -helix. In that calculation, it was assumed that the minimum energy conformation(s) of this polypeptide chain will possess a 2-residue periodicity with respect to the conformation, i.e., the minimum energy conformation(s) will consist of a sequence of dipeptide units in which corresponding internal coordinates of different dipeptide units assume the same value ("conformational 2-residue periodicity"). A criticism may arise concerning this assumption: can the minimum energy conformation that is reached under the assumption of such a conformational periodicity be also at an energy minimum even when no conformational periodicity is supposed? The main purpose of this report is to answer this question.

METHODS

Molecular mechanics calculation

Only a single-stranded poly-(L,D)-alanine chain will be considered. In the preceding work (Monoi, 1993), the original ECEPP83 force field (Momany et al., 1975; Chuman et al., 1984) was modified with respect to the hydrogen bonding. Unless otherwise stated, the same modi-

fied force field and the same calculation procedures were also used in this work, including the mode of nonbonded cutoff (residue-number-based cutoff). But the angular dependence of the Lennard-Jones-type term, E_{HB} , of the hydrogen-bond energy has further been improved by introducing a second hydrogen-bond angle θ_2 :

$$E_{HB} = \cos \theta'_1 \cos \theta'_2 (A_{HB}/r^{12} - B_{HB}/r^6) + (1 - \cos \theta'_1 \cos \theta'_2)(A/r^{12} - B/r^6), \quad (1a)$$

$$\theta'_1 = \theta_1^\alpha (\pi/2)^{1-\alpha}, \quad (1b)$$

$$\theta'_2 = \theta_2^\beta (\pi/2)^{1-\beta}, \quad (1c)$$

where r is the distance between the hydrogen and the acceptor, θ_1 and θ_2 are the supplements of the donor-H-acceptor and H-acceptor-acceptor-antecedant angles, respectively, and α and β are constants which are specific for the type of hydrogen bonds and related to the degree of angular dependence. When θ_1 and/or $\theta_2 \geq \pi/2$ radians, only the normal Lennard-Jones term is used (put θ_1 and/or $\theta_2 = \pi/2$ in Eq. 1). On the basis of a preliminary ab initio SCF result, we simply put $\alpha = 1.0$ and $\beta = 1.0$ for the hydrogen bond between the amino and carbonyl groups of the peptide backbone. Eq. 1 can thus be reduced to a simple form.

Independent variables in energy minimization

Only the dihedral angles were adopted as the variables in energy minimization: ϕ_i , ψ_i , ω_i , and χ_i ($i = L$ and D), where the IUPAC-IUB convention on nomenclature (IUPAC-IUB Commission of Biochemical Nomenclature, 1970) is used, and subscripts L and D denote L - and D -isomers; i.e., ϕ_i and ψ_i are the dihedral angles with respect to the $N-C^\alpha$ and the $C^\alpha-C'$ bond, respectively, of the peptide backbone, ω_i is the dihedral angle of the peptide

Received for publication 28 December 1992 and in final form 23 August 1993.

Address reprint requests to Dr. Hiroshi Monoi, Department of Physiology, Tohoku University School of Medicine, 2-1 Seiryochō, Sendai, Japan 980.

bond, and χ_i represents the rotation of the sidechain around the $C^\alpha-C^\beta$ bond. The bond lengths and angles were treated as constants.

In the present calculation, a conformational N -residue periodicity ($N = 2$ to 24) was assumed (except for the normal-mode calculations). Under this assumption, the number of independent variables of a polyaniline chain is equal to $4N$.

Starting conformations in energy minimization

Unless otherwise stated, starting conformations in energy minimization are set to be within a range such that $\phi_L < 0$, $\phi_D > 0$, $\psi_L > 0$, and $\psi_D < 0$. This range of dihedral angles corresponds to the "β-helix region" of the conformational space (as long as the peptide bonds are not far apart from the planar trans configuration).

Conformation energy of the repeating unit

Unless otherwise noted, the conformation energy is represented by the energy of a repeating unit positioned in the middle of a helix that is characterized by a conformational N -residue periodicity and composed of at least $(N + 2N_{\text{cut}} - 2)$ amino acid residues, where N_{cut} is the cutoff residue number in the residue-number-based nonbonded cutoff¹; N_{cut} was taken to be 31 as in the previous work (Monoï, 1993). The "conformation energy of the repeating unit" will be defined as

$$\begin{aligned} & \sum \left(E_{\text{ES}} \text{ and } E_{\text{NB}} \begin{array}{c} \text{between any pair of atoms} \\ \text{within the unit} \end{array} \right) \\ & + \frac{1}{2} \sum \left(E_{\text{ES}} \text{ and } E_{\text{NB}} \begin{array}{c} \text{between any atom of the unit} \\ \text{and any outside it} \end{array} \right) \\ & + \sum (E_{\text{TOR}} \text{ of any bond of the unit}), \end{aligned} \quad (2)$$

where E_{ES} is the electrostatic energy, E_{NB} is the energy of nonbonded interactions, and E_{TOR} is the torsional energy of bonds. Obviously, any end groups can be used.

The conformation energy as defined above is equal to the conformation energy (per repeating unit) of an infinitely long periodic chain which has the same repeating unit with the same values of internal coordinates. In what follows, when the conformation energy in this sense is being considered, a helix will be referred to as an infinitely long helix, although the helix actually being computed is of finite length.

Helix parameters

The longitudinal axis of a helix was calculated in the same manner as described before (Monoï, 1993). The axis was

defined as a line such that (a) all the $L-C^\alpha$ atoms are at one distance from the line, and, (b) at the same time, all the $D-C^\alpha$ atoms are at another distance from it. The two distances are not necessarily equal to each other. Once the helix axis is determined, helix parameters (number of residues per turn and axial translation per turn) can easily be computed. In order to perform grid calculations of the helix parameters (and of the corresponding conformation energy), a routine was added to the original ECEPP83 software.

Normal mode analysis

The normal vibration of an infinitely long poly-(L,D)-alanine $\beta^{6,3}$ -helix around its energy minimized conformation was calculated in terms of molecular mechanics. It is, however, difficult to perform the normal mode analysis of a system with an infinite number of independently variable coordinates. In the present calculation, only the dihedral angles of n successive residues are treated as independent variables. The remaining coordinates were fixed at their values in the energy minimized structure. The conformation energy was represented by the total conformation energy of a sufficiently long portion of the infinite helix (not by the energy of the repeating unit). In the middle of that portion, were placed the residues having variable dihedral angles. The term "sufficiently long" used here means that the portion consists of at least $(n + 2N_{\text{cut}} - 2)$ amino acid residues. One can, therefore, use a helix consisting of at least $(n + 2N_{\text{cut}} - 2)$ amino acid residues instead of treating an infinite helix. The value of n examined was 2 to 24.

Subprograms for the normal mode computation were added to the ECEPP83 software. They were written on the basis of the algorithm by Noguti and Go (1983).

RESULTS AND DISCUSSION

A theoretical consideration

Suppose that an infinitely long polypeptide possesses an N -residue periodicity with respect to the primary structure, and that the repeating unit has N' variable internal coordinates. All the variable internal coordinates of the peptide will be numbered in a way such that the corresponding coordinates of successive units appear every N' coordinates. The j th coordinate of the i th unit will be denoted by q_j^i ($i = -\infty, \dots, -1, 0, 1, \dots, +\infty$ and $j = 1, 2, \dots, N'$).

The conformation energy E of the polypeptide is a function of the internal coordinates q_j^i :

$$E = E(\{q_j^i\}), \quad (3)$$

where the braces $\{ \}$ represent an ensemble of ordered elements. The conformation energy, E^P , under the constraint of the conformational N -residue periodicity is a function of N' independent variables q_j^0 . We may write

$$E^P = E^P(\{q_j^0\}) = E(\{q_j^i | q_j^i = q_j^0\}). \quad (4)$$

¹ In this mode of cutoff of nonbonded and electrostatic interactions, it is supposed that only if one residue is apart from another residue (on the primary structure) by not-more-than- N_{cut} residues (including the two residues in question), every atom in the former residue will see the field due to every atom of the other residue (for details, see Monoï (1993)).

At an energy minimum, we have, under the constraint of conformational N -residue periodicity,

$$\frac{\partial E^p}{\partial q_j^0} = \sum_i \frac{\partial E}{\partial q_j^i} \frac{\partial q_j^i}{\partial q_j^0} = \sum_i \frac{\partial E}{\partial q_j^i} = 0. \quad (5)$$

From the periodicity of the primary structure of the infinitely long chain, all the terms on the right-hand side of Eq. 5 are equal to each other, and hence

$$\frac{\partial E}{\partial q_j^i} = 0 \quad (6)$$

for any i and j .

Therefore, if an infinitely long polypeptide has a primary structure characterized by an N -residue periodicity, a minimum energy conformation of the polypeptide within the framework of the conformational N -residue periodicity corresponds to an equilibrium structure (either energy minimal or unstable equilibrium structure) when no conformational periodicity is postulated; therefore, it is not necessarily at an energy minimum.

Poly-(L,D)-alanine has a 2-residue periodicity with respect to the primary structure. With an infinitely long single-stranded $\beta^{6.3}$ -helix of this polypeptide, its lowest energy conformation within the framework of the conformational 2-residue periodicity was also the lowest-energy form of this $\beta^{6.3}$ -helix even when no conformational periodicity was supposed. That will be shown below in terms of molecular mechanics.

The lowest energy conformation of $\beta^{6.3}$ -helix within the framework of the conformational 2-residue periodicity

First we suppose a conformational 2-residue periodicity for an infinitely long single-stranded chain of poly-(L,D)-alanine. When energy minimization was started from various initial conformations that belong to the β -helix region of the conformational space, six optimized structures were reached (also see Fig. 1 of Monoi (1993)). They are the right- and left-handed counterparts of three conformational species: $\beta^{4.5}$ -, $\beta^{6.3}$ -, and $\beta^{8.2}$ -helices. The eight independent dihedral angles of the right-handed $\beta^{6.3}$ -helix are given in Table 1 (line 1). This structure, which was calculated in terms of

TABLE 2 Helix parameters of minimum energy conformations of an infinitely long poly-(L,D)-alanine

Helix	Number of residues per turn	Axial translation per turn	Distance from the axis*			Radius of pore†
			C α	C'	N	
	/turn	Å/turn		Å		Å
$\beta^{6.3}$ -helix	6.31	5.03	3.87	3.33	3.33	1.8
$\beta^{4.5}$ -helix	4.48	5.37	2.76	2.25	2.24	0.7
$\alpha_{L,D}$ -helix	3.77	5.59	2.35	1.69	1.63	0.2
$\pi_{L,D}$ -helix	4.57	5.29	2.83	2.17	2.13	0.7

The values in this table are for both the right- and left-handed helices.

* Average of the distances for L- and D-amino acids.

† Represented as the radius of the inner cylindrical envelope for the van der Waals surfaces of C' and N atoms of the backbone (the van der Waals "closest-approach" radii of C' and N atoms are taken to be 1.5 Å).

the newly modified force field, was essentially the same as that reported before (Monoi, 1993); the helix consists of 6.3 residues per turn, and the axial translation is 5.0 Å per turn (Table 2).

It can be concluded that this structure is the *lowest* energy conformation of the $\beta^{6.3}$ -helix within the framework of the conformational 2-residue periodicity. A basis of this conclusion is that as state above, only a single species of $\beta^{6.3}$ -helix was obtained even though energy minimization was started from various initial conformations.

Similar but more definitive evidence supports this conclusion. When a grid search was executed over a sufficiently wide range of conformational space (which includes the range: ϕ_L and $\psi_D = -70^\circ$ to -170° , ϕ_D and $\psi_L = 80^\circ$ to 180° , ω_L and $\omega_D = 165^\circ$ to 195°), six energy wells were detected. They were found to correspond to the right- and left-handed counterparts of $\beta^{4.5}$ -, $\beta^{6.3}$ -, and $\beta^{8.2}$ -helices. This observation is illustrated by Figs. 1 and 2, where the following constraint are placed in order to plot energy contours and helix parameters in a two-dimensional plane:

$$\psi_L = \psi_L^{\text{opt}} - (\phi_L - \phi_L^{\text{opt}}), \quad (7a)$$

$$\psi_D = \psi_D^{\text{opt}} - (\phi_D - \phi_D^{\text{opt}}), \quad (7b)$$

$$\omega_i = \omega_i^{\text{opt}}, \quad (7c)$$

$$\chi_i = \chi_i^{\text{opt}}, \quad (7d)$$

TABLE 1 Dihedral angles and conformation energies of minimum energy conformations of an infinitely long poly-(L,D)-alanine

Helix	ϕ_L	ψ_L	ϕ_D	ψ_D	ω_L	ω_D	χ_L	χ_D	Conformation energy*
									kcal/mol
$\beta^{6.3}$ -helix	-112	123	127	-108	170	-172	-180	-179	0.0
$\beta^{4.5}$ -helix	-90	114	120	-87	-169	173	-177	-179	0.2
$\alpha_{L,D}$ -helix	-60	-48	-50	-60	-179	-174	-179	179	-0.4
$\pi_{L,D}$ -helix	-68	-64	-59	-71	-168	-170	177	175	-0.3
$\beta^{6.3}$ -helix†	-126	132	135	-112	180	180	-180	-179	1.5
$\alpha_{L,D}$ -helix†	-62	-37	-52	-56	180	180	-178	177	1.6

All the dihedral angles are for right-handed helices. Dihedral angles of corresponding left-handed helices can be obtained by applying simple symmetry rules, hence not listed here. The energy values were the same for both helical senses.

* Conformation energy per dipeptide unit, expressed relative to that of the $\beta^{6.3}$ -helix.

† Peptide bonds are fixed at planar trans configuration: ω_L and $\omega_D = 180^\circ$.

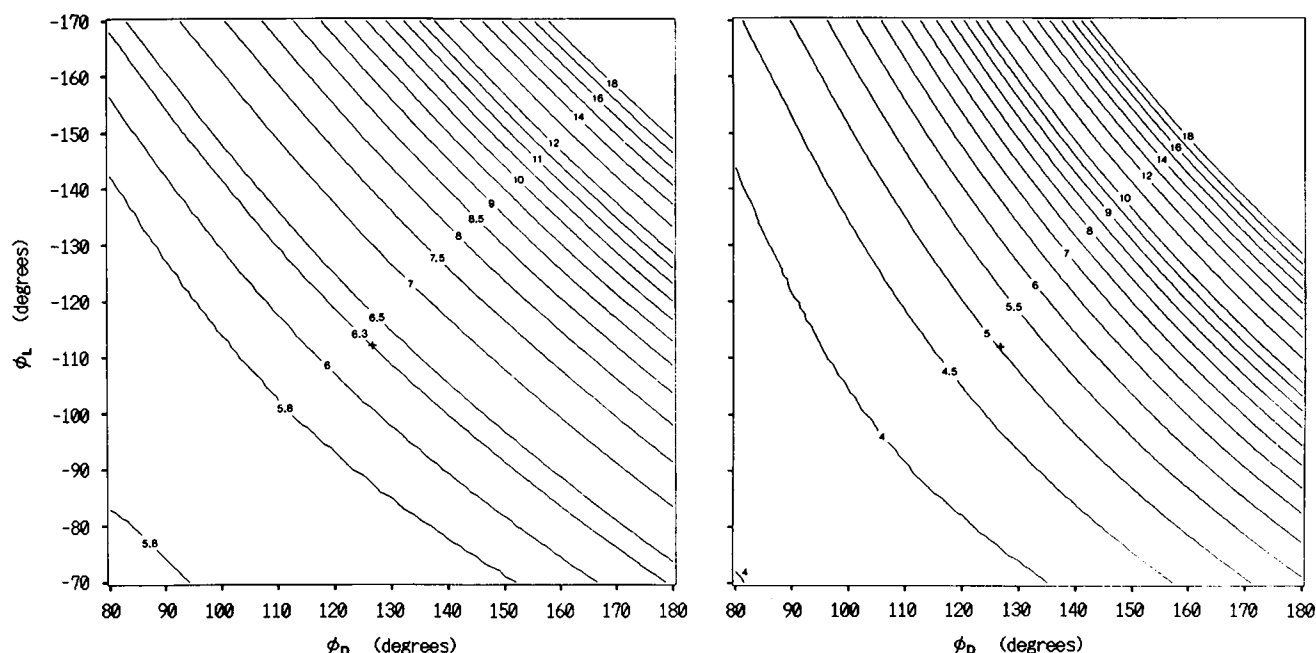


FIGURE 1 Contour maps of helix parameters as functions of ϕ_L and ϕ_D for an infinitely long poly-(L,D)-alanine on a β -helix region of the conformational space. A conformational 2-residue periodicity is postulated. Other six dihedral angles are constrained by Eq. 7. (*Left*) Number of residues per turn. (*Right*) Axial translation per turn. Numerals in the figure represent contour levels for the helix parameters. Distance is expressed in angstroms. The cross sign (+) denotes the position of the optimized $\beta^{6.3}$ -helix.

where symbols with superscript opt designate optimized values in right-handed $\beta^{6.3}$ -helix (Table 1), and i denotes L and D. All the points in those figures represent right-handed helices. The corresponding plots for left-handed helices are the mirror mirages with respect to the line $\phi_L = -\phi_D$ (in this case, symbols with superscript opt in Eq. 7 take optimized values in left-handed $\beta^{6.3}$ -helix).

In Fig. 1, the helix parameters (number of residues per helical turn n_{hel} (*left*) and axial translation per helical turn l_{hel} (*right*)) are expressed as a function of ϕ_L and ϕ_D within the frame of the above constraint (Eq. 7). In this part of the conformational space, n_{hel} took values from ~ 5.6 to ~ 30 residues per turn, and l_{hel} , ~ 3.8 to ~ 100 Å per turn. As seen from this figure, when ϕ_L and ϕ_D are simultaneously varied by approximately the same magnitude from their optimized values in the $\beta^{6.3}$ -helix, the helix parameters remain approximately unchanged, but the amino and carbonyl groups of the peptide backbone are reoriented toward the inside, or the outside, of the helix (data not shown). On the other hand, when ϕ_L and $-\phi_D$ are simultaneously increased (or decreased) from their optimized $\beta^{6.3}$ values, both of the helix parameters decrease (or increase) greatly (Fig. 1), and various orientations of the amino and the carbonyl groups appear depending on the relative magnitudes of the increments in ϕ_L and ϕ_D (data not shown). Accordingly, various aspects of the β configuration appear in this conformational space even though the dihedral angles are constrained by Eq. 7.

Fig. 2 represents the energy contours in the same part of the conformational space as in Fig. 1. Near the center of this figure, there occur an energy minimum (denoted by a cross sign), which corresponds to the optimized $\beta^{6.3}$ -helix given in

Table 1. The minima that correspond to optimized $\beta^{4.5}$ - and $\beta^{8.2}$ -helices are not found in this figure; they appear in other regions of the conformational space with different values of ω_i .

A shallow local energy well can also be seen in the left upper part of Fig. 2. It is a $\beta^{6.3}$ -helix as well (compare Figs. 1 and 2). This local minimum, however, vanished when the eight dihedral angles were independently relaxed.

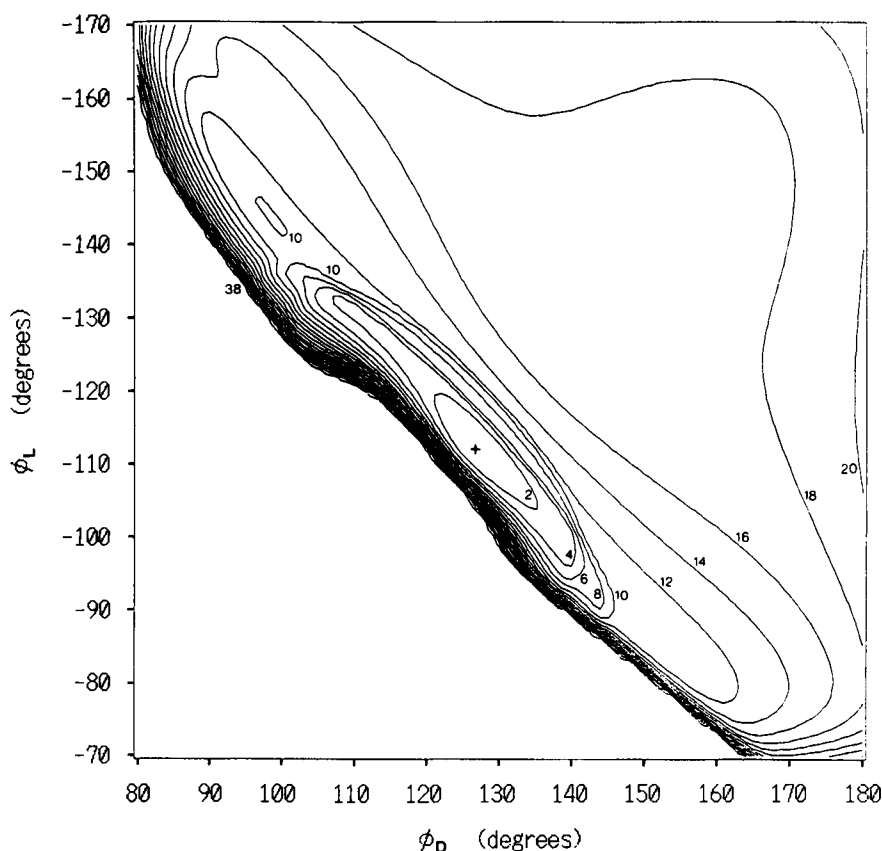
Only one minimum energy $\beta^{6.3}$ -helix (for each of the right- and left-handed counterparts) was thus found in the whole conformational space, and therefore it represents the lowest-energy conformation of $\beta^{6.3}$ -helix within the framework of the conformational 2-residue periodicity.

The lowest energy conformation of $\beta^{6.3}$ -helix when no conformational periodicity is assumed

In this section, it will be shown that the lowest energy conformation of an infinitely long $\beta^{6.3}$ -helix of poly-(L,D)-alanine obtained above within the framework of the conformational 2-residue periodicity (which will be referred to as conformation P₂ in what follows) is also the lowest energy form of $\beta^{6.3}$ -helices of this polypeptide even when no conformational periodicity is assumed.

For that purpose, we first demonstrate, by means of normal mode analysis, that conformation P₂ is also at an energy minimum even when no conformational periodicity is assumed. Next we perform energy minimization which is started from a wide range of initial structures belonging to the β -helix region of the conformational space, and show that conformation P₂ is the only energy-minimized $\beta^{6.3}$ -helix of

FIGURE 2 Contour map of conformation energies as a function of ϕ_L and ϕ_D for an infinitely long poly-(L,D)-alanine on the same region of the conformational space as in Fig. 1. Numerals in the figure represent contour levels relative to the energy of the optimized $\beta^{6.3}$ -helix, whose position is denoted by a cross sign (+). Energies are expressed in kcal/mol per dipeptide unit.



poly-(L,D)-alanine even when conformational periodicity N is sufficiently large.

Normal mode analysis

In order to know whether or not conformation P_2 is at an energy minimum even in the absence of the constraint of conformational periodicity, its normal mode of vibration was calculated. For convenience, the dihedral angles of n successive residues alone were treated as independent variables, the remaining coordinates being fixed at their values in conformation P_2 . The range of n examined was 2 to 24, which corresponds to approximately 0.3 to 4 helical turns of the $\beta^{6.3}$ -helix. The total degrees of freedom was hence $8-96 (= 4n)$.

Normal-mode frequencies thus obtained are diagrammed in Fig. 3 for each level of n . As shown in this figure, the normal mode frequencies increased with an increase in n , except for the modes with frequencies of $220-230\text{ cm}^{-1}$, which were virtually independent of n . The number of those high-frequency modes was equal to n . Further analysis indicates that those modes correspond to the rotational vibrations of the alanine sidechains around the $C^\alpha-C^\beta$ bonds. The rotational vibrations of these bonds were practically independent of other degrees of freedom.

The total number of the normal modes that appear in this figure is equal to the number of the independently variable internal coordinates, or the internal degrees of freedom. All the normal mode frequencies are positive

definite. It can therefore be concluded that conformation P_2 is at an energy minimum even when no conformational periodicity is assumed.

Energy minimization starting from various initial structures

When no conformational periodicity is postulated, or when conformational periodicity N is sufficiently large, it is not practicable to conduct a grid search of minimum energy conformations of a long polymer. Instead we examined the correlation between starting structures and resultant optimized conformations when N is sufficiently large.

As mentioned in a preceding section, only a single species of optimized $\beta^{6.3}$ -helix was obtained from various starting structures under the constraint of the conformational 2-residue periodicity: $N = 2$. We now show that it is also the case when N is sufficiently large (N was taken to be 12 and 24).

A representative result is presented in Fig. 4, where the starting values of dihedral angles ϕ_L and ϕ_D are plotted on the same portion of the conformational space as in Figs. 1 and 2, and N was taken to be 12 residues, which corresponds to approximately two helical turns of the $\beta^{6.3}$ -helix. In order to plot starting structures on a two-dimensional ϕ_L - ϕ_D plane, a conformational 2-residue periodicity was hypothesized for the starting structures (but not for structures during minimization), and the remaining six dihedral angles (ψ_i , ω_i , and χ_i ; $i = L$ and D) were again constrained by Eq. 7. The range of the starting structures examined includes almost the whole

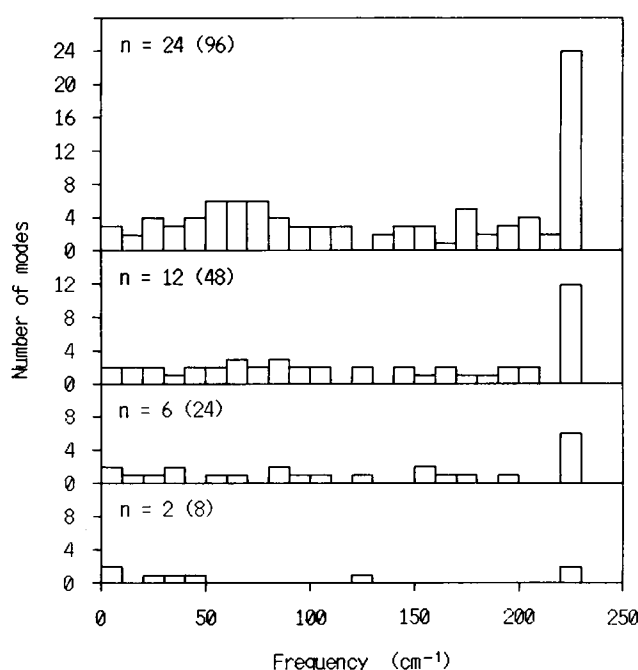


FIGURE 3 Normal mode frequencies of an infinitely long poly-(L,D)-alanine $\beta^{6.3}$ -helix at various levels of the degrees of freedom. The dihedral angles of n successive residues alone are assumed to be independent variables; the total degrees of freedom is $4n$. Columns represent the number of normal modes per 10 cm^{-1} . The numerals in the parentheses indicate the total number of modes with positive definite frequencies.

domain surrounded by the contour at 10 kcal/mol (per dipeptide unit) above the energy of conformation P_2 . (When minimization was started from outside this range, convergence was very slow so that the computation was interrupted in the course of minimization.)

In Fig. 4, two species of optimized conformations can be seen. Both of them have a conformational 2-residue periodicity in spite of the fact that a conformational 12-residue periodicity was assumed during minimization. One is a $\beta^{6.3}$ -helix, and their dihedral angles agree with those of conformation P_2 (Table 1, line 1). The other corresponds to $\beta^{8.2}$ -helix, and its geometry is essentially the same as that report before (Monoï, 1993). It is considerably unstable; its conformation energy is higher than that of the optimized $\beta^{6.3}$ -helix by 4 kcal/mol dipeptide unit.

In the above minimization, conformations were initially constrained by the conformational 2-residue periodicity, but they no longer had any conformational periodicity immediately after minimization started; and, during minimization, corresponding dihedral angles of different residues (including ω_i , but not χ_i) often differ from each other by more than 10° , and sometimes by more than 20° . (Nevertheless the optimized conformations finally reached possess a conformational 2-residue periodicity.) It can therefore be considered that starting structures implicitly involved in this figure will include starting structures with a conformational 12-residue periodicity.

Conformation P_2 was also the only optimized $\beta^{6.3}$ -helix even when conformational periodicity N was as great as 24

residues, which corresponds to approximately four helical turns of $\beta^{6.3}$ -helix. It was also the case when a conformational 12-residue periodicity were postulated not only for structures during minimization but also for starting conformations. In those cases, however, the starting conformations that could be examined were limited to a narrower range of conformational space (because energy minimization usually demands much computer time on those occasions).

It may be concluded, therefore, that conformation P_2 is the lowest energy structure of infinitely long poly-(L,D)-alanine $\beta^{6.3}$ -helices even in the absence of the constraint of conformational periodicity.

Global minima in the whole conformational space

It is known that $\alpha_{L,D}$ -helix is the global minimum structure of poly-(L,D)-alanine (Hesslink and Scheraga, 1972). To re-examine this point, a grid search was conducted over the whole conformational space within the framework of the conformational 2-residue periodicity; and the search was further followed by energy minimization within the same framework. The result is summarized in Table 1. The first four helical species in this table were found to constitute a group of global minima: they were $\beta^{6.3}$ -, $\beta^{4.5}$ -, $\alpha_{L,D}$ -, and $\pi_{L,D}$ -helices² (Fig. 5; their helix parameters are listed in Table 2). The lowest is nominally the $\alpha_{L,D}$ -helix, but the differences in energy between those helical species are small and insignificant.

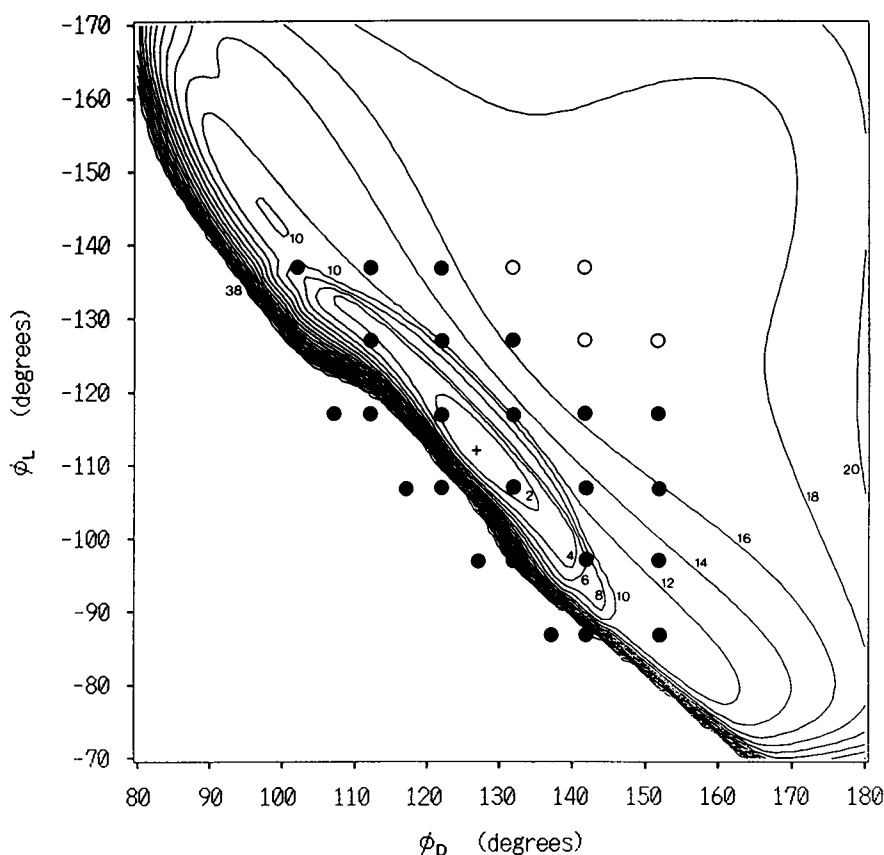
In evaluating relative stabilities of the helices, their free energies should be compared with each other. It is laborious, however, to calculate the conformational entropy of an infinite polypeptide chain. A preliminary computation on the basis of the newly modified ECEPP83 force field indicates that the entropy term of the conformational free energy is more favourable for $\beta^{6.3}$ -helix than for $\alpha_{L,D}$ -helix by several hundred calories per mol of dipeptide unit, and that it is also more favourable for $\beta^{4.5}$ - and $\pi_{L,D}$ -helices than for $\alpha_{L,D}$ -helix by less magnitudes. Consequently, the differences in stability between the four helical species are small and insignificant.

The above result is incompatible with that of Hesslink and Scheraga (1972). According to them, the $\alpha_{L,D}$ -helix of an infinite poly-(L,D)-alanine chain has by far the lowest conformation energy among various structures of this polypeptide, and the conformation energy of $\alpha_{L,D}$ -helix is lower than that of $\beta^{6.3}$ -helix by 5.0 kcal/mol per dipeptide unit. In their calculation, a conformational 2-residue periodicity was assumed to make the result on short helices applicable to the infinite chain, as is also the case with the present calculation. What is the origin of this discrepancy?

In their calculation, the peptide bond was assumed to have a rigid planar trans configuration ($\omega_i = 180^\circ$), whereas it was freely relaxed in the present calculation. Under the assumption of the rigid planar peptide bond, the conformation energy of $\beta^{6.3}$ -helix was still comparable to that for $\alpha_{L,D}$ -helix

² The last two helices are tentatively named after α - and π -helices of all-L, or all-D, polypeptides.

FIGURE 4 Starting structures and energy minimized conformations of an infinitely long poly-(L,D)-alanine β -helix when a conformational 12-residue periodicity is assumed during minimization. For the starting structures, a conformational 2-residue periodicity is supposed. The ordinate and abscissa represent starting values of ϕ_L and ϕ_D , respectively, which are plotted on the same conformational space as in Figs. 1 and 2; starting values of other dihedral angles are constrained by Eq. 7. The same marks denote convergence to a common optimized conformation, which corresponds to a $\beta^{6,3}$ -helix (●) or a $\beta^{8,2}$ -helix (○). The cross sign (+) stands for the optimized $\beta^{6,3}$ -helix. Energy contours are superimposed; numerals in the figure indicate energy levels relative to the energy of the optimized $\beta^{6,3}$ -helix, expressed in kcal/mol per dipeptide unit.



(Table 1, lines 5–6), which cannot explain the discrepancy. (Interestingly, small deviations of the peptide bonds from the planar configuration caused a considerable lowering of the conformation energy of $\beta^{6,3}$ -helix (Monoi, 1993) and of $\alpha_{L,D}$ -helix as well, the lowerings being 1.5 and 2.0 kcal/mol per dipeptide unit, respectively (Table 1).)

In their calculation, the term conformation energy has the conventional sense, representing the conformation energy of the whole polypeptide molecule. In the present study, it is

defined by Eq. 2 (conformation energy of the repeating unit). We thus examined the effect of the chain length of poly-(L,D)-alanine upon its optimized structure and conformation energy when the conformation energy is defined in the former sense. It was found that the optimized structure converges rapidly with an increase in chain length (with $\beta^{6,3}$ -helices, the ϕ_L and ϕ_D values for the 36-residue chain were different by 0.2–0.4° from the value extrapolated to the infinite length), whereas the conformation energy (per dipep-

FIGURE 5 Space-filling representations of the minimum-energy conformations of $\alpha_{L,D}$ -helix (top) and $\pi_{L,D}$ -helix (bottom) of poly-(L,D)-alanine. Only their backbones are shown. The amino-terminal ends are to the left. C' denotes carbonyl C; C_L and C_D are α -C in L- and D-configuration, respectively. Atoms are rendered as spheres with approximate van der Waals "closest-approach" radii: H, 1.00 Å; C, 1.50 Å; N, 1.45 Å; O, 1.35 Å.

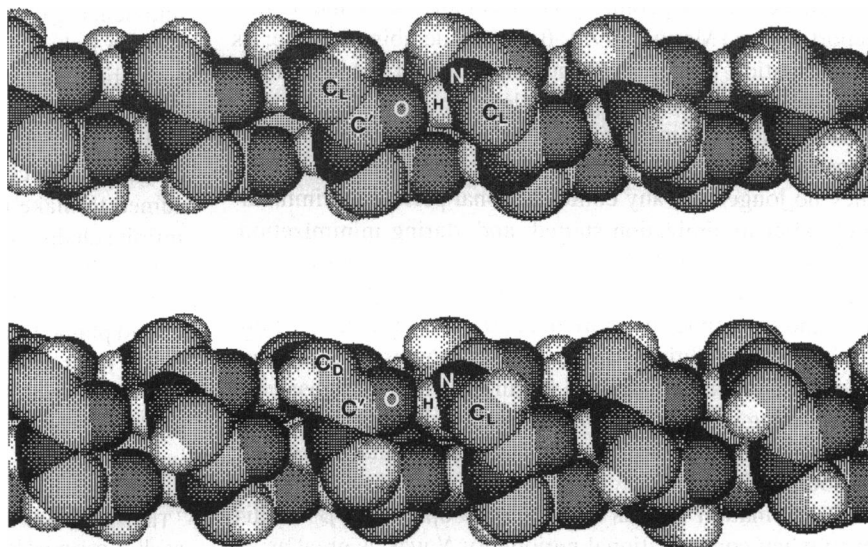
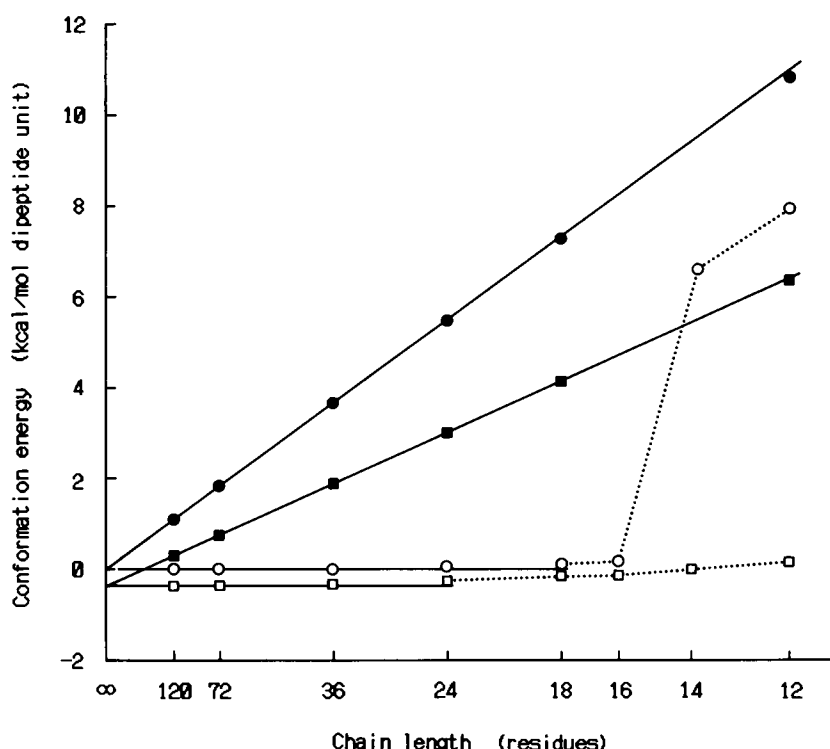


FIGURE 6 Conformation energies as functions of chain length for the optimized $\beta^{6,3}$ - and $\alpha_{L,D}$ -helices of a poly-(L,D)-alanine chain of finite length within the framework of the conformational 2-residue periodicity. \circ and \bullet , $\beta^{6,3}$ -helix; \square and \blacksquare , $\alpha_{L,D}$ -helix. The coordinate is the conformation energy, which represents the total conformation energy divided by the number of dipeptide units (closed symbols) and the conformation energy of the repeating unit defined by Eq. 2 (open symbols). The abscissa is the reciprocal of the number of residues in the chain. The amino-terminal group is always an L-residue. Solid lines are fitted to points for longer chain lengths.



tide unit) converges much more slowly (Fig. 6, *closed symbols*). As shown in Fig. 6, there is a linear relationship between the conformation energy (per dipeptide unit) and the reciprocal of the chain length, and that the rate of convergence of the energy depends on helical species. The rate for $\beta^{6,3}$ -helix is slower than that for $\alpha_{L,D}$ -helix. As a result, $\beta^{6,3}$ -helix is considerably unstable compared with $\alpha_{L,D}$ -helix at shorter chain lengths, although both have comparable energies in the limit of infinite length. When peptides are composed of 12 alanine residues, the difference in conformation energy between the two helices is as great as 4.5 kcal/dipeptide, approximately equal to the value (5.0 kcal/dipeptide) reported by Hesselink and Scheraga (1972) for a helix consisting of 13 alternating L- and D-alanines.

On the other hand, when the conformation energy is defined by Eq. 2 (no nonbonded cutoff being employed here), the convergence is very fast for both of the energy (Fig. 5, *open symbols*) and the structure (with $\beta^{6,3}$ -helices, the ϕ_L and ϕ_D values for the 36-residue chain were different by less than 0.02° from the value in the limit of infinite length). The present values for the conformation energies of infinite chains were obtained by assuming an N_{cut} value of 31 (see Methods), which corresponds to a chain length of approximately 61 residues. The origin of the discrepancy is now clear; the discrepancy is attributable to the differences in (a) the chain length employed and (b) the definition of conformation energy used to estimate the energy of the infinite chain from results on chains of finite length.

The normal modes were also calculated of the energy-minimized conformations of the $\beta^{6,3}$ -, $\beta^{4,5}$ -, $\alpha_{L,D}$ -, and $\pi_{L,D}$ -helices obtained above, which indicates that those confor-

mations are at an energy minimum as well even when no conformational periodicity is assumed.

The conformations and conformation energies of structural motifs of polypeptides such as α - and β -helix, β -sheet, and β -barrel are usually investigated on the basis of polypeptide chains of finite length, and the energy minimization of those structures is often performed with respect to the total conformation energy of the finite chains (e.g., Chou et al., 1982, 1983, 1990). As is clearly indicated in Fig. 6, however, without proper care such approaches might lead to erroneous conclusion, especially, about the relative stabilities of different conformational species of the same polypeptide.

The author is grateful to Dr. H. Chuman (Kureha Chemical Industry Co. Ltd., Tokyo, Japan) for offering him the original ECEPP83 software and the subprograms for normal mode calculations. The former was provided through Japan Chemical Program Exchange (JCPE), Tokyo, Japan (JCPE program P024). He is also indebted to the Computer Center, Institute for Molecular Science, Okazaki National Research institutes, for the use of the HITAC M-680H computer (Hitachi, Ltd., Tokyo, Japan), which was employed in a dominant part of the present study.

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