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New Tubular Single-Stranded Helix of Poly-L-amino Acids Suggested by Molecular Mechanics Calculations: I. Homopolypeptides in Isolated Environments

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ABSTRACT A search was made in terms of molecular mechanics calculation for tubular, or pore-forming, single-stranded helices of poly-L-amino acids. Such a helix was found in the vicinity of $(\phi, \psi, \omega) = (81°, 98°, 170°)$ in the conformational space. It was the 6.2_{20} helix of right-handedness. The 6.2_{20} helix, here named the "μ helix," had a cylindrical pore along its helical axis. The diameter of the pore was 6.6 Å on the basis of the atom centers of carbonyl carbons and amino nitrogens. The left-handed μ helix was less stable than the right-handed counterpart. The conformation energy of the μ helix, expressed relative to that of the α helix of the same polypeptide, depended to a great extent on amino acid composition. It varied over a range of a few kilocalories per mol per residue above and below the conformation energy of the α helix of the same polypeptide. This marked diversity in the relative conformation energy of the μ helix can be ascribed primarily to the difference in the relative position of α-carbons between the μ and the α helices. The conformational entropy made only a small contribution, if any, to the relative stability of the μ helix. There was a hydrogen-bonded network of side chains in the μ helices of poly-L-glutamine and poly-L-asparagine.

INTRODUCTION

The transmembrane ion-conducting channel formed by polypeptide antibiotic gramicidin A is known to be a head-to-head (i.e., N-terminal-to-N-terminal) dimer of two gramicidin monomers, each of which is basically a single-stranded β helix consisting of 6.3 residues per turn (e.g., Urry et al., 1971, 1983; Weinstein et al., 1985; Arseniev et al., 1985; Wallace, 1986; Nicholson and Cross, 1989; Hing et al., 1990; Ketchen et al., 1993). The $\beta^{6.3}$ helix has a cylindrical pore along the longitudinal axis of the helix. The diameter of the pore was initially estimated to be ~4 Å (Urry et al., 1971) and later 3.7 Å (Busath et al., 1988; Monoi, 1993a). The hollow tubular nature of this helix is usually attributed to the unique primary structure of the polypeptide, which is composed of alternating L- and D-amino acid residues. Recently, Ghadiri et al. (1993) reported that a crystallized tubular structure is produced by the stacking of numerous molecules of an eight-residue cyclic peptide. This peptide also comprises alternating L- and D-amino acid residues.

A question thus arises: Do there occur any stable tubular, or pore-forming, single-stranded helices of all-L polypeptides? An attempt to answer this question in terms of molecular mechanics has led to this report and a subsequent one. The purposes of these studies are, first, to determine whether energy wells corresponding to such tubular helices occur in the conformational space and, if such energy wells are present, to examine the possibility of the natural existence of those helical species and on the other hand collect basic data necessary to construct them artificially. Those helices would form transmembrane channels or water-soluble nanoscale tubes, depending on the degree of hydrophobicity of the side chains. (The term "tubular" will be used below to signify that a structure has a long pore that can accommodate small ions or molecules.)

In this paper, as a first step in a study along this line, we confine ourselves to homopolymer poly-L-amino acids of infinite chain length. Isolated environments are also postulated. With infinitely long homopolypeptides in such environments, a conformational one-residue periodicity can tentatively be assumed. They are, therefore, convenient to use in determining the helix parameters, the backbone internal coordinates, and other fundamental features of hitherto undescribed helical conformations. The study of homopolypeptides is not necessarily a mere intellectual exercise, because not a few naturally occurring proteins have homopolymer segments in the primary structure (e.g., Kao et al., 1990; Laurent et al., 1990; Johnson et al., 1993).

The molecular mechanics calculations presented below formulate a new tubular single-stranded helix of poly-L-amino acids, which corresponds to the 6.2_{20} helix in the nomenclature of Bragg et al. (1950). In this helix the NH group of a residue is hydrogen-bonded to the CO group of the fifth residue behind it along the chain. Interestingly, the conformation energy of the 6.2_{20} helix depends to a great extent on amino acid species. A detailed analysis was made of the origin of the diversity in the conformation energy of this helix.

In this paper the 6.2_{20} helix will be referred to as the "μ helix" (μ is an abbreviation derived from the first letter of "microtube").

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MATERIALS AND METHODS

The results of molecular mechanics calculation presented in this paper are for an infinite chain of homopolymer poly-L-amino acids in isolated environments. Although polypeptide chains of relatively short length were actually computed, the results hold for infinitely long chains of the polypeptides (see below). Except for the calculation of normal vibrations, a one-residue periodicity was assumed with respect to the conformation; i.e., the optimized conformation was hypothesized to consist of a sequence of residues in which corresponding internal coordinates of different residues assume the same value.

The source program used in the present molecular mechanics calculation is ECEPP83 (Japan Chemical Program Exchange, Tokyo, program P024). But it has been modified and extended to be equipped with several additional features, as will be described below.

Molecular mechanics force field

The force field employed is basically that of ECEPP83 (Momany et al, 1975; Chuman et al., 1984), which is essentially equivalent to that of ECEPP/2 (QCPE program 454). But it has been extended to involve two additional energy terms. The hydrogen-bond force field has also been modified.

In the original form of the ECEPP83 force field the conformation energy is given by

$$E = E_{ES} + E_{NB} + E_{TOR}, \tag{1}$$

where E_{ES} is the electrostatic energy, E_{NB} is the energy of nonbonded interactions expressed by Lennard–Jones terms, and E_{TOR} is the torsional energy of bonds.

In the modified force field used in this study, two additional energy terms are also taken into consideration:

$$E_{BEND} = \Sigma K_\theta(\theta - \theta_{eq})^2, \tag{2}$$

$$E_{STR} = \Sigma K_l(l - l_{eq})^2, \tag{3}$$

where E_{BEND} is the energy of the bending of bond angles θ, and E_{STR} is the energy of the stretching of bond lengths l; the subscript eq denotes equilibrium values. (The reason for the addition of these two energy terms is discussed below.)

The values of force constants K_θ and K_l were taken from the AMBER force field (Pearlman et al., 1991). Default values of the ECEPP83 force field were adopted for the equilibrium bond lengths and angles. In the original version of the ECEPP83 force field, some of the corresponding backbone bond angles of different amino acid residues are given different values. To such bond angles, averages of the values for several amino acids were attributed. In the ECEPP83 the intrachain bond angle $\angle NC^\alpha C'$ at the α-carbon, for example, ranges from 108° to 111°, depending on amino acid species. This angle was simply put at 109.5°.

In the original ECEPP83 force field the hydrogen-bond energy is composed of an electrostatic term and a Lennard–Jones 10–12 term. This hydrogen-bond force field involves no explicit angular dependence, and it also gives considerably small energies (of the order of 2 kcal/mol at most) to the hydrogen bond between the amino and the carbonyl groups. In the present calculation the Lennard–Jones term has been modified as described below (the electrostatic term is unchanged).

The modified Lennard–Jones term, E'_{HB}, of the hydrogen bond energy involves two hydrogen-bond angles:

$$E'_{HB} = \cos\theta_1\cos\theta_2(A_{HB}/r^{12} - B_{HB}/r^{10}) + (1 - \cos\theta_1\cos\theta_2)(A/r^{12} - B/r^{10}), \tag{4}$$

where r is the distance between the hydrogen and the acceptor, and θ_1 and θ_2 are the supplements of the donor–H–acceptor and H–acceptor–acceptor-antecedant angles, respectively. When θ_1 and/or $\theta_2 \geq \pi/2$ rad, only the second term was used (put θ_1 and/or $\theta_2 = \pi/2$ in Eq. 4). From an ab initio calculation based on the 6–31G basis set, we put A_{HB} = 36,320 Å^{12} kcal/mol, B_{HB} = 13,160 Å^{10} kcal/mol, A = 96,410 Å^{12} kcal/mol, and B = 23,900 Å^{10} kcal/mol for the hydrogen bond between the amino and the carbonyl groups. (For other types of hydrogen bonds, the original ECEPP83 force field was employed, because such hydrogen bonds make no significant contribution to the conformation energy for the six amino acids studied in this paper. In the modified force field the total interaction energy between two hydrogen-bonded alanine residues in the α helix was 5.5 kcal/mol.

The 6–31G ab initio results suggest that the angular dependence of the hydrogen-bond energy is much greater than that in the original ECEPP83 force field (unpublished results). Another reason for modifying the hydrogen-bond force field arose when energy minimization of the $\beta^{6.3}$ helix of poly-(L,D)-alanine was performed under the constraints of rigid planar *trans* peptide bonds (see Monoi, 1993a). When the original ECEPP83 force field was used in the presence of the constraints, the backbone carbonyl and amino groups of the energy-minimized $\beta^{6.3}$ helix were greatly inclined inward or outward from their normal positions, despite the fact that ω deviated from 180° by only 4° even when ω was freely relaxed. A further study indicated that this anomalous situation can be attributed to the considerably weak angular dependence of the hydrogen-bond energy in the original ECEPP83 force field.

With poly-L-alanine the conformation energy of the μ helix (expressed relative to that of the α helix) calculated in terms of the modified hydrogen-bond force field was not significantly different (<0.1 kcal/mol per residue) from that in terms of the original hydrogen-bond force field of ECEPP83. With structures in which greater hydrogen-bond angles are involved, however, differential effects of the two force fields may be more prominent.

The present hydrogen-bond force field is considerably simplified compared with that used for the study of β helices in the preceding papers (Monoi, 1993a,b). The two force fields give practically the same minimum-energy conformations and relative conformation energies when hydrogen-bond angles θ_1 and θ_2 are not greater than 20° (as is the case for optimized β helices).

Independent variables in energy minimization

In the original ECEPP83 software, only the dihedral angles ϕ, ψ, ω, and χ_i (i = 1, 2, . . .) are variables in energy minimization, where the IUPAC-IUB convention on nomenclature (IUPAC-IUB Commission of Biochemical Nomenclature, 1970) is used; i.e., ϕ and ψ are the dihedral angles with respect to the N—C$^\alpha$ and the C$^\alpha$—C′ bonds, respectively, of the peptide backbone, ω is the dihedral angle of the peptide bond, and χ_i represents the rotation of the rotatable side-chain bonds.

In the present computation the bond angles and the bond lengths were also treated as variables in energy minimization unless otherwise stated; but the side-chain bond angles whose pivot atoms are behind the C$^\beta$ atom and the side-chain bond lengths behind the C$^\gamma$ atom were fixed at their equilibrium values. In this treatment the alanyl side chain is fully relaxed, and the aromatic rings of phenylalanine, tyrosine, and tryptophan are kept rigid. In some of the calculations on poly-L-glutamine all the bond angles and bond lengths were relaxed for the purpose of comparison.

A conformational one-residue periodicity was assumed in energy minimization. Consequently, unless constraints are applied to the bond angles and/or the bond lengths, the number of independent variables is 27 + m, where m is the number of rotatable bonds in the side chain concerned; but it is 47 for poly-L-glutamine when all the bond angles and bond lengths are relaxed.

Constraints of bond angles and bond lengths

In some of the present calculations the bond angles and/or the bond lengths were constrained in energy minimization. Two methods were used for this purpose. In the first method, bond angles and/or bond lengths are not taken as variables and are fixed at their equilibrium values. In the second, they

are treated as variables, but sufficiently large values are assigned to the force constants for bond angles and/or bond lengths. Both methods gave the same results.

Conformation energy of the repeating unit

For the purpose of estimating the conformation energy of an infinite periodic peptide chain from results on a chain of finite length, the notion of the conformation energy of the repeating unit was introduced in a previous paper (Monoi, 1993a). This 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 $N + 2N_{cut} - 2$ amino acid residues, where N_{cut} is the cutoff residue number in the residue-number-based cutoff of nonbonded interactions (see below); N_{cut} was taken to be 31 as in the previous paper. The conformation energy of the repeating unit is defined as

$$\begin{aligned}&\Sigma(E_{ES} \text{ and } E_{NB} \text{ between any pair of atoms within the unit})\\&+ (1/2)\Sigma\,(E_{ES} \text{ and } E_{NB} \text{ between any atom of the unit and any outside it})\\&+ \Sigma(E_{TOR}, E_{BEND}, \text{ and } E_{STR} \text{ within the unit}).\end{aligned} \quad (5)$$

Obviously, any end groups can be used because the energy as it is defined above involves no contribution of the end groups.

The conformation energy as defined above is equal to the conformation energy (per repeating unit) of an infinitely long periodic chain that possesses the same repeating unit with the same values of internal coordinates. By using this definition of conformation energy, one can use a relatively short peptide chain to estimate the energy and conformation of a polypeptide of infinite length. 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 computed is of finite length.

Residue-number-based nonbonded cutoff

The mode of nonbonded cutoff employed is the same as that introduced in an earlier paper (Monoi, 1993a). It is a residue-based nonbonded cutoff. In this mode of nonbonded cutoff, it is assumed that only if one residue is separated from another residue (along the primary structure) by not more than N_{cut} residues (including the two residues in question), every atom in the former residue sees the field produced by every atom of the latter residue (residue-number-based cutoff or cutoff at the N_{cut}-th residue). Parameter N_{cut} was taken to be 31.

Normal-mode analysis

The normal-mode analysis of an infinitely long helix was made in terms of molecular mechanics. It is laborious to calculate the normal modes of a system with an infinite number of independent variables. In the present computation, only the dihedral angles of n successive residues were treated as independent variables. The internal coordinates of the other residues and the bond angles and bond lengths of the n residues were fixed at the optimized values. 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 this portion were placed the residues having variable internal coordinates. The term "sufficiently long" used here means that the portion consists of at least $n + 2N_{cut} - 2$ amino acid residues. One can thus employ chains of finite lengths instead of treating an infinite helix. The values of n examined were 2 to 48.

The entropy term (relative value) of the conformational free energy can be put as (Go and Scheraga, 1969, 1976)

$$-TS = (1/2)RT \ln(\det F), \quad (6)$$

where F is the second-derivative matrix of the conformation energy with respect to the coordinates at an energy-minimum point, R is the gas constant, and T is the absolute temperature.

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

Axis of a helix

The longitudinal axis of a helix was calculated by the least-squares method. The helix axis was defined as a line such that all the C^{α} atoms are at the same distance from the line (see Monoi, 1993a).

RESULTS

A preliminary consideration

Regular single-stranded helices of poly-L-amino acids can be classified into two categories: the α-series and the γ-series helices. In the former category, to which the α helix belongs, the NH group of a residue is hydrogen-bonded to the CO group of the Nth residue before it along the chain ($N = 2, 3, 4, \ldots$); hence there are $3N + 1$ atoms in the hydrogen-bonded loop thus formed:

$$\underbrace{\text{—CO—(NH—CHR—CO)}_{N-1}\text{—NH—}}, \quad (7)$$

where R represents a side chain. In the latter category, to which the γ helix belongs, the NH group of a residue is hydrogen-bonded to the CO group of the Nth residue behind it; there are $3N + 5$ atoms in the hydrogen-bonded ring so formed:

$$\text{—}\underbrace{\text{NH—CHR—CO—(NH—CHR—CO)}_{N-1}\text{—NH—CHR—CO}}\text{—}. \quad (8)$$

A fundamental conformational difference between the two categories is that the CO groups of an α-series helix point toward the C-terminal end, whereas those of a γ-series helix face toward the N-terminal end.

Table 1 lists the helix parameters, n and d, of typical single-stranded helical structures (of poly-L-amino acids) that have been proposed, where n is the number of residues per turn, and d is the axial translation per residue. In this table each helix is labeled the corresponding value of N to show the hierarchy of these helices. Designations of the type n_R are based on the nomenclature of Bragg et al. (1950), where n denotes the screw symmetry (or the number of residues per turn), and R is the number of atoms in the hydrogen-bonded loop. The helices with R greater than 17 have not hitherto been described. Evidently, tubular, or pore-possessing, single-stranded helices must possess n greater than 6, the corresponding values of N being not less than 7 and 5 for α- and γ-series helices, respectively.

When a contour map of the helix parameter n for regular single-stranded polypeptides is plotted on the ϕ–ψ plane

TABLE 1 Typical single-stranded helices of poly-L-amino acids

N*	Designation	R‡	n§	d¶	References
α Series ($R = 3N + 1$)					
2	2.2_7 helix	7	2.2	2.75	Donohue (1953)
3	3_{10} helix	10	3.0	2.00	Donohue (1953)
4	α helix	13	3.7	1.47	Pauling et al. (1951)
5	π helix	16	4.4	1.15	Low and Grenville-Wells (1953)
γ Series ($R = 3N + 5$)					
2	No possible structure‖				Donohue (1953)
3	4.3_{14} helix	14	4.3	1.20	Donohue (1953)
4	γ helix	17	5.1	0.99	Pauling et al. (1951)
5	6.2_{20} helix	20	6.2	0.81	This study

* See text.
‡ Number of atoms in the hydrogen-bonded loop.
§ Number of residues per turn.
¶ Axial translation per residue (in Å).
‖ Less stable then the α helix by more than 5 kcal/mol per residue (Donohue, 1953).

under the constraints of the rigid planar *trans* configuration of peptide bonds ($\omega = 180°$, where ω is the dihedral angle of the peptide bond), n takes only values ranging from 2 to ~5 (see, e.g., Fig. 12 *B* in Ramachandran and Sasisekharan, 1968); accordingly, no helices on this map have hollow pores that can accommodate small molecules or ions. An immediate consequence of this is that hollow tubular single-stranded helices would possess nonplanar peptide bonds ($\omega \neq 180°$).

Thus we made a geometrical analysis of the relationship between helix parameters and backbone dihedral angles under the restriction that favorable hydrogen bonds are to be formed. The results indicate that ω for helices with higher levels of N will be greater than 180° for right-handed α-series helices and less than 180° for right-handed γ-series helices. This is because when ω is less (or greater) than 180° in the former (or latter) helices, the intrachain bond angle $\angle NC^{\alpha}C'$ at the α carbon is at least ~120° (under the above restriction), which is too large compared with the standard bond angle (~109.5°) of the "tetrahedral" carbon and hence energetically very unfavorable. It is also inferred that ω and backbone bond angles of tubular α-series helices would be more distorted than those of the corresponding helices of the γ-series, and, consequently, the occurrence of the former helices seems less favored. It is implied as well that right-handed γ-series helices are more stable than their left-handed counterparts.

On the basis of such results of geometrical consideration, a search was made, in terms of molecular mechanics, for tubular γ-series helices of right handedness. The domain of the conformational space surveyed was $\phi = 60–100°$, $\psi = 80–120°$, and $\omega = 160–180°$. For this domain, repeated energy minimization was performed, starting from each point of intersection of the grid lines that were placed at intervals of 10° for ϕ and ψ and 5° for ω. After this grid search, three energy-minimized structures of the γ series were found. They were right-handed helices with different values of n. All of them were in the neighborhood of $(\phi, \psi) = (80°, 100°)$, but they were characterized by different values of ω, which were approximately 180°, 170°, and 165° in increasing order of n. The first one was found to be the γ helix (Pauling et al., 1951). The second and the third have not hitherto been described. They were 20-atom-ring and 23-atom-ring helices of the γ series ($N = 5, 6$), composed of 6.2 and 7.2 residues per turn, respectively (with axial translations of 0.81 and 0.70 Å/residue, respectively). Therefore they correspond to the 6.2_{20} and 7.2_{23} helices by the Bragg–Kendrew–Perutz nomenclature. The 7.2_{20} helix was less stable than the 6.2_{20} helix.

In the subsections below, several characteristics of the 6.2_{20} helix are described. This helix will be referred to as the μ helix.

Basic features of the μ helix

Table 2 lists the dihedral angles of the minimum-energy μ helices of the infinitely long homopolymers of several L-amino acids. This table also gives, for the purpose of comparison, the optimized dihedral angles of α and γ helices calculated by the same force field. As shown in this table, the backbone dihedral angles ϕ, ψ, and ω of the minimum-energy μ helices were approximately 81°, 98°, and 170°, respectively, depending only slightly on amino acid species. The helices in Table 2 are right-handed in the helical sense. Left-handed μ helices were considerably unstable. This is due to the steric hindrance between the side chains and the backbone in left-handed μ helices.

As indicated in Table 2, the peptide bonds of μ helices deviate from the planar *trans* configuration by approximately 10°. This is in contrast with α and γ helices, whose peptide bonds exhibit only slight deviations from the planar structure. Nonplanar peptide bonds are essential for the formation of μ helices, which are never reached in energy minimization when the peptide bonds are constrained at the planar *trans* configuration ($\omega = 180°$). This is the reason that the energy well corresponding to the μ helix cannot be found on contour maps in which ω is constrained at 180°.

TABLE 2 Dihedral angles, bond angles and lengths, and relative conformation energies of the minimum-energy μ helices of homopolymer poly-L-amino acids

Polypeptide	Dihedral Angle (deg)							rms Deviation*		Relative Conformation Energy§ (kcal/mol/res)
	ϕ	ψ	ω	χ_1	χ_2	χ_3	χ_4	Bond Angles‡ (deg)	Bond Lengths (Å)	
Poly-L-alanine										
α helix	−53	−54	179	179	–	–	–	0.86 (111.4)	0.0035	0.0
γ helix	80	97	179	−171	–	–	–	1.99 (110.3)	0.0041	2.3
μ helix	81	98	170	−173	–	–	–	2.18 (113.2)	0.0040	2.5
Poly-L-tyrosine										
α helix	−53	−52	178	−52	147	0.5	–	1.70 (111.4)	0.0049	0.0
α helix¶	−53	−52	178	−52	−33	0.6	–	1.68 (111.4)	0.0050	0.01
μ helix	81	98	169	−44	112	0.9	–	1.83 (112.6)	0.0035	0.1
μ helix¶	81	98	169	−45	−68	0.8	–	1.84 (112.6)	0.0035	0.1
Poly-L-phenylalanine										
α helix	−53	−52	178	−52	147	–	–	1.68 (111.4)	0.0050	0.0
μ helix	81	98	169	−45	112	–	–	1.84 (112.6)	0.0035	0.0
Poly-L-tryptophan										
α helix	−53	−55	180	−62	−20	–	–	0.98 (110.9)	0.0028	0.0
γ helix	80	97	178	−54	−63	–	–	2.11 (109.8)	0.0033	−0.4
μ helix	81	98	170	−55	−64	–	–	2.17 (113.0)	0.0041	−0.2
Poly-L-asparagine										
α helix	−54	−53	179	−88	−94	−0.7	–	1.27 (111.5)	0.0032	0.0
μ helix	81	98	170	−47	−36	0.3	–	2.25 (113.0)	0.0043	−1.9
Poly-L-glutamine										
α helix	−54	−53	178	−76	173	100	0.9	1.04 (111.6)	0.0038	0.0
γ helix	79	97	180	−76	65	−106	−2.9	3.04 (110.2)	0.0072	−2.4
μ helix	80	99	171	−82	67	−108	−3.0	2.98 (113.0)	0.0066	−2.4
α helix‖	−54	−53	178	−76	173	100	0.8	1.00 (111.7)	0.0034	0.0
γ helix‖	79	97	180	−74	65	−107	−2.8	2.47 (110.3)	0.0060	−2.7
μ helix‖	80	99	171	−80	68	−109	−2.8	2.42 (113.2)	0.0053	−2.7

* The rms deviations of bond angles and lengths from their equilibrium values.
‡ Values in parentheses represent the intrachain bond angle at the α-carbon ($\angle NC^{\alpha} C'$).
§ Conformation energy, expressed relative to that of the α helix of the same polypeptide.
¶ The second-lowest-energy conformation.
‖ All the bond angles and bond lengths are relaxed.

Moreover, even when ω is freely relaxed in energy minimization, *μ helices are never attained if the starting value of ω is in the vicinity of 180°.*

Fig. 1 is the cylindrical plot of the backbone atoms of the minimum-energy μ helix. The helix was composed of 6.2 residue per turn with an axial translation of 0.81 Å per residue (Table 1). Its pitch was 5.0 Å per turn, which was 0.5 Å shorter than that (=5.5 Å) of the α helix calculated by the same force field. The difference of such magnitude in helical pitch will have critical effects on the modes of inter-side-chain interactions in these two helices (see below).

The helix parameters of the μ helix given above are comparable with those for the $\beta^{6.3}$ helix (Urry et al., 1971; Venkatachalam and Urry, 1983; Koeppe II and Kimura, 1984; Roux and Karplus, 1991; Monoi, 1993b). The radial distances of the carbonyl carbons and of the amino nitrogens were approximately equal to each other (~3.3 Å). The carbonyl oxygens and the amino hydrogens took slightly more inner positions (~3.2 Å). The α-carbons were at ~0.6 Å outside from those atoms. The μ helix has a cylindrical pore along its longitudinal axis (see Fig. 4 below). The diameter of the pore is 3.7 Å when the van der Waals closest-approach radii of C and N atoms are assumed to be 1.45 Å on average (also see Turano et al., 1992), or it is 6.6 Å on the basis of the atom centers of carbonyl carbons and amino nitrogens. The dimensions of this pore are comparable with those of the pore of the $\beta^{6.3}$ helix. It may be expected that the pore of the μ helix can accommodate small molecules and ions as in the gramicidin channel (Myers and Haydon, 1972; Eisenman et al., 1978; Levitt et al., 1978; Rosenberg and Finkelstein, 1978).

In the γ-series helices the orientation of the hydrogen bond deviates considerably from the plane containing the helix axis and the donor N atom, which gives a peculiar appearance to the backbones of these helices. In the μ helix the deviation was ~22° (on the basis of the donor atom N and the acceptor-antecedent atom C′).

Table 2 also shows that the dihedral angles of the γ helix are approximately equal to those of the μ helix, except for ω, which is close to 180° in the former helix. It is therefore possible to transform a γ helix into a μ helix by decreasing ω from ~180° to ~170° (without altering the other dihedral angles significantly) and thereby moving the hydrogen

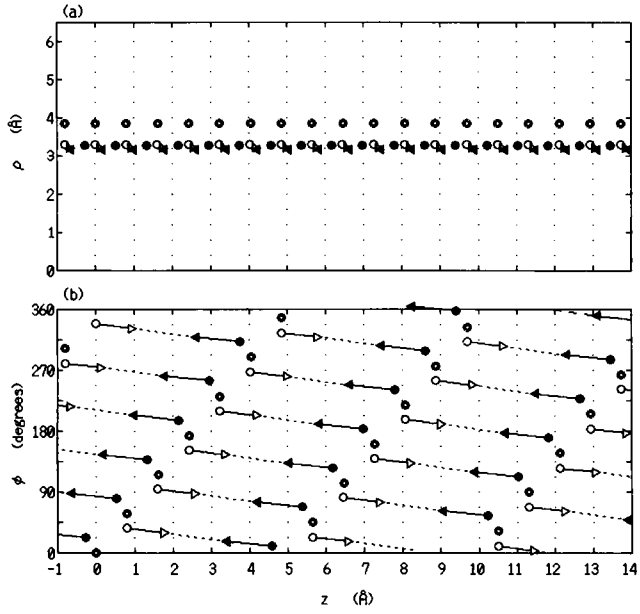

FIGURE 1 Cylindrical plot of the backbone atoms of the minimum-energy μ helix of poly-L-alanine. Ordinates ρ and ϕ are the radial distance and the azimuthal angle, respectively, in the cylindrical coordinate system. The longitudinal axis of the helix is taken as the z axis. The N-terminal end is to the left. Broken lines denote hydrogen bonds. ◎, C^{α}; ○, amino N; ▷, amino H; ●, carbonyl C; ◀, carbonyl O.

bonds along the chain. Transient intermediates of this transformation may involve furcate hydrogen bonds. The height of the energy barrier to be overcome in this process will be estimated elsewhere.

Conformation energy of the μ helix

The conformation energies of polypeptides can not be compared directly when the peptides have different amino acid compositions. In what follows, the term "relative conformation energy" refers, for the sake of simplicity, to the conformation energy expressed relative to that of the right-handed α helix of the same polypeptide.

The relative conformation energy of the μ helix was heavily dependent on amino acid species. As shown in Table 2 (rightmost column), the μ helix of poly-L-alanine was considerably unstable; its conformation energy was higher than that of the α helix of this polypeptide by 2.5 kcal/mol per residue (on an infinite-chain-length basis). With homopolymers of L-amino acids that possess aromatic side chains, the μ helices had conformation energies comparable with those of the respective α helices. Among the homopolypeptides in this table, poly-L-glutamine gave the most stable μ helix. Its relative conformation energy was −2.4 kcal/mol per residue. When all the bond angles and bond lengths of glutamine side chains were relaxed, the relative conformation energy of the μ helix decreased slightly to be -2.7 kcal/mol per residue (the last row in Table 2).

The γ and the μ helices have similar patterns of hydrogen-bond arrangement. Their conformation energies did not differ greatly (Table 2). With poly-L-alanine and poly-L-tryptophan the conformation energy of the γ helix was lower than that of the μ helix by 0.2 kcal/mol per residue. With poly-L-glutamine the two helices had approximately equal conformation energies.

Effects of bond-angle constraints; importance of the relaxation of bond angles in the μ helix

The force field employed in the present study is basically that of ECEPP83, but it has been extended to involve two additional energy terms, E_{BEND} and E_{STR} (see the Methods section). Thus it seems necessary to report here what structure the minimum-energy μ helix assumes when bond angles and/or bond lengths are constrained at their equilibrium values. The results for poly-L-alanine are summarized in Table 3. Essentially the same trends were observed for other polypeptides.

As Table 3 shows, the application of bond-length constraints alone did not appreciably affect the conformation and the conformation energy of the minimum-energy μ helix. The case was quite different when bond angles were fixed. Although ϕ and ψ did not vary significantly, even in the presence of bond-angle constraints (with and without bond-length constraints), ω definitely changed under these constraints: its deviation from the planar angle increased from 10° to 15°. The dihedral angle χ_1 for the side chain of the μ helix also deviated considerably from the lowest-energy position. (The conformation of the α helix did not vary significantly even in the presence of these constraints.)

TABLE 3 Effects of bond angle and/or bond-length constraints on the minimum-energy μ helix of poly-L-alanine

Constraints	Dihedral Angle (deg)				$E_{ES} + E_{NB}$ (kcal/mol/res)			E_{TOR}	E_{BEND}	E_{STR}	Total Conformation Energy‡
	ϕ	ψ	ω	χ_1	bb–bb	bb–sc*	inter-sc				
None	81	98	170	−173	−3.4	−1.8 (−1.5)	0.1	0.7	1.2	0.06	−3.1
Bond lengths	81	98	170	−173	−3.3	−1.7 (−1.5)	0.1	0.7	1.3	0.0	−3.0
Bond angles	84	96	165	−158	−3.3	1.3 (1.6)	0.1	2.1	0.0	0.36	0.6
Bond angles and lengths	84	96	165	−157	−3.1	1.8 (2.2)	0.1	2.2	0.0	0.0	1.0

Abbreviations bb and sc denote backbone and side chain, respectively (for details, see text). The intra-side-chain nonbonded interactions in alanine are null in conventional molecular mechanics force fields.

* Values in parentheses represent the contribution of E_{NB}.

‡ Sum of all the energy terms.

The conformation energy of the μ helix was severely altered by bond-angle constraints (with and without bond-length constraints). For the μ helix of poly-L-alanine the conformation energy increased by 4.1 kcal/mol per residue when both bond angles and bond lengths were constrained (whereas the conformation energy of the α helix increased by only 0.6 kcal/mol per residue).

This instability caused by the bond-angle constraints is due to the increase (1.5 kcal/residue) in the torsional energy and the unfavorable nonbonded (Lennard–Jones) interactions (3.7-kcal/residue increase) between the side chains and the backbone (see columns 7 and 9 in Table 3). Further detailed analysis indicates that the origin of this instability is mainly i) the increased deviation of ω from the planar configuration and ii) the steric hindrance between one of the β-hydrogens of a residue and the backbone carbonyl oxygen of the residue preceding it along the chain (the unfavorable change in χ_1 reflects this steric hindrance).

Therefore the free relaxation of bond angles is essential for achieving the true minimum-energy state of the μ helix. The above results afford an example showing that minimum-energy structures that, under bond-angle constraints, are possible only at a high cost of van der Waals and tortional energies, may be possible with modest degrees of bond-angle bending. It has also been suggested that there is a strong coupling between (ω, χ_1) and bond angles in the μ helix.

Normal-mode analysis; contribution of the conformational entropy

In evaluating the relative stabilities of different conformations, one should compare their free energies. The conformational entropy was calculated in terms of normal-mode analysis, in which only n consecutive residues in the middle of a sufficiently long chain were assumed to have variable internal coordinates. The conformational entropy (per residue) for an infinite chain can be obtained as the value at the limit of infinite n. The range of n examined was 2 to 48, which corresponds to approximately 0.3 to 8 helical turns of the μ helix.

The results are summarized in Fig. 2, where the conformational entropic energy (relative value) per residue for poly-L-alanine is plotted against $1/n$. This energy was calculated from the F matrix (Eq. 6). All the elements of this matrix and hence all the normal-mode frequencies had positive definite values. The entropy term for an infinite chain can be obtained as the y intersect of this plot.

Fig. 2 shows that the conformational entropic energy of the μ helix was not significantly different from that of the γ helix and that there was a small but significant difference (~0.3 kcal/mol per residue) in this energy between the μ and the α helices. In this calculation, however, the bond angles were fixed. As was shown above, the relaxation of the bond angles markedly stabilizes the conformation of the μ helix, and a strong coupling will exist between dihedral

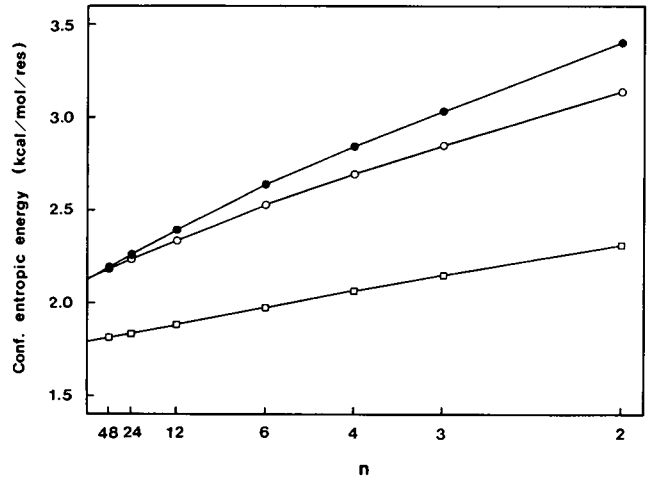

FIGURE 2 Conformational entropic energy as a function of the number n of residues having variable internal coordinates in an infinite chain of poly-L-alanine. The other residues are fixed at the optimized conformation. The ordinate is the total conformational entropic energy (relative value) at 298°, devided by n. The abscissa is the reciprocal of n. ●, μ helix; ○, γ helix; □, α helix. For details, see text.

angles (ω and χ_1) and bond angles in this helix. Accordingly, the entropic term of the μ helix will decrease significantly if bond angles are treated as additional variables in the normal-mode calculation. In fact, calculations along this line indicated that the entropy term of the μ helix is comparable with, or slightly less than, that of the α helix. It can, therefore, be concluded that the conformational entropy makes a minor contribution, if any, to the relative stability of the μ helix.

Conformational periodicity of minimum-energy structures of homopolymers

It was pointed out in a preceding paper (Monoi,1993b) that, if an infinitely long polypeptide has a primary structure characterized by an N-residue periodicity, an energy-minimized conformation of the polypeptide obtained within the framework of the conformational N-residue periodicity corresponds to an equilibrium structure (and is not necessarily at an energy minimum) when no conformational periodicity is postulated. There is, therefore, a possibility that the energy-minimized structures of homopolypeptides obtained above (under the assumption of the conformational one-residue periodicity) are not necessarily at true energy minima.

As mentioned above, however, no negative modes of normal vibrations appeared for all the values (2–48) of n examined. In addition, the optimized structures were independent of N that characterizes the conformational periodicity (data not shown). It can thus be concluded that the conformations of the minimum-energy helices of homopolymers given above correspond to true minimum-energy species, even though they were obtained under the assumption of the conformational one-residue periodicity.

Origin of the diversity in the conformation energy of the μ helix

It was shown above that there is a marked diversity in the relative conformation energy of the μ helix. What is the origin of this diversity then? In Table 4 are tabulated the components of the conformation energies of the minimum-energy μ helices of several polypeptides. Those for the corresponding α helices are also shown for comparison. In this table the sum of E_{ES} and E_{NB} represents the total interaction energy (per residue) between nonbonded atoms. This energy is composed of four contributions, i.e., interactions between backbone atoms (backbone–backbone), between backbone atoms and side-chain atoms (backbone–side chain), between atoms belonging to different side chains (inter-side-chain), and between atoms belonging to the same side chain (intra-side-chain). The four contributions are given in the second to the fifth columns of Table 4.

For the sake of clarity, the energy components in Table 4 are diagrammed in Fig. 3, in which each horizontal bar represents the magnitude of an energy component of the μ helix expressed relative to the corresponding component of the α helix. The stretching energy and the energy of intra-side-chain interactions are not plotted in this figure.

Fig. 3, together with Table 4, indicates that the diversity in the relative conformation energy of the μ helix is attributable mainly to the diversity of the inter-side-chain interactions in this helix. As can be seen from Table 4 (column 4), the inter-side-chain interactions in the μ helix of poly-L-alanine were weak and not significantly different from those in the α helix of this polypeptide. With poly-L-asparagine and poly-L-glutamine, the attractive inter-side-chain interactions were markedly strong in the μ helix; this is due to the formation of hydrogen bonds between NH and CO groups of neighboring side chains. In contrast, no inter-side-chain hydrogen bonds were formed in the α helices of these polypeptides. With homopolypeptides of aromatic amino acids, their μ helices exhibited inter-side-chain interactions of intermediate magnitudes, which were modestly greater than those in the respective α helices.

In the μ helix the greater part of the inter-side-chain interactions can be ascribed to the interactions between the nearest-neighbor side chains belonging to adjacent helical turns, i.e., the side chain of a residue and the side chain of the sixth residue ahead or behind along the backbone (Table 4, values in parentheses in the fourth column). The remaining part of the inter-side-chain interactions can be assigned chiefly to the interactions between the side chains adjacent along the peptide chain (data not shown).

The backbone–side-chain interactions also affected the relative conformation energy of the μ helix. In general, this component of interactions was more favorable to α helices. It was prominent in the α helices of poly-L-asparagine and poly-L-glutamine because of the formation of hydrogen bonds between side-chain NH groups and backbone CO groups. Each of the backbone CO groups is also hydrogen-

TABLE 4 Components of the conformation energies of the minimum-energy μ helices of homopolymer poly-L-amino acids

Polypeptide	$E_{ES} + E_{NB}$ (kcal/mol/res)				E_{TOR}[§]	E_{BEND}[§]	E_{STR}	Total Conformation Energy[¶]
	bb–bb*	bb–sc	inter-sc[‡]	intra-sc				
Poly-L-alanine								
α helix	−3.5 (−7.9)	−2.4	0.1	0.0	0.01 (0.0)	0.2 (0.2)	0.04	−5.6
μ helix	−3.4 (−8.3)	−1.8	0.1	0.0	0.7 (0.6)	1.2 (1.0)	0.06	−3.1
Poly-L-tyrosine								
α helix	−3.4 (−7.7)	−6.3	−2.1	−1.2	0.4 (0.0)	0.8 (0.4)	0.08	−11.6
μ helix	−3.3 (−8.2)	−5.7	−3.5 (−2.2)	−1.2	1.2 (0.7)	0.9 (0.7)	0.04	−11.5
Poly-L-phenylalanine								
α helix	−3.4 (−7.7)	−6.0	−1.9	−0.3	0.4 (0.0)	0.8 (0.4)	0.08	−10.3
μ helix	−3.3 (−8.2)	−5.4	−3.5 (−2.1)	−0.3	1.2 (0.7)	0.9 (0.7)	0.04	−10.3
Poly-L-tryptophan								
α helix	−3.4 (−7.9)	−6.6	−3.2	−0.2	0.3 (0.0)	0.3 (0.2)	0.03	−12.8
μ helix	−3.4 (−8.3)	−6.3	−5.3 (−3.1)	−0.2	0.9 (0.6)	1.3 (1.0)	0.06	−13.0
Poly-L-asparagine								
α helix	−3.5 (−7.8)	−9.2	−0.3	−15.8	0.7 (0.0)	0.4 (0.3)	0.04	−27.5
μ helix	−3.3 (−8.1)	−5.7	−7.6 (−7.1)	−15.3	1.0 (0.6)	1.3 (1.1)	0.08	−29.4
Poly-L-glutamine								
α helix	−3.5 (−7.8)	−6.8	−0.5	−12.0	0.5 (0.0)	0.3 (0.2)	0.05	−21.9
μ helix	−3.1 (−8.0)	−4.7	−8.4 (−7.5)	−11.8	1.2 (0.5)	2.3 (1.8)	0.17	-24.3
α helix[‖]	−3.5 (−7.8)	−6.8	−0.5	−12.4	0.5 (0.0)	0.4 (0.2)	0.08	−22.1
μ helix[‖]	−3.1 (−8.0)	−4.8	−8.5 (−7.4)	−12.3	1.2 (0.5)	2.5 (1.7)	0.19	−24.8

Abbreviations bb and sc denote backbone and side chain, respectively (for details, see text).

* Values in parentheses represent E_{NB} for the backbone–backbone interactions.

‡ Values in parentheses represent the interaction energy between the nearest-neighbor side chains belonging to adjacent helical turns in the μ helix.

§ Values in parentheses represent the contribution of the backbone alone. The value 0.0 means <0.05. See text for the definition of backbone bond angle.

¶ Sum of all the energy terms.

‖ All the bond angles and bond lengths are relaxed.

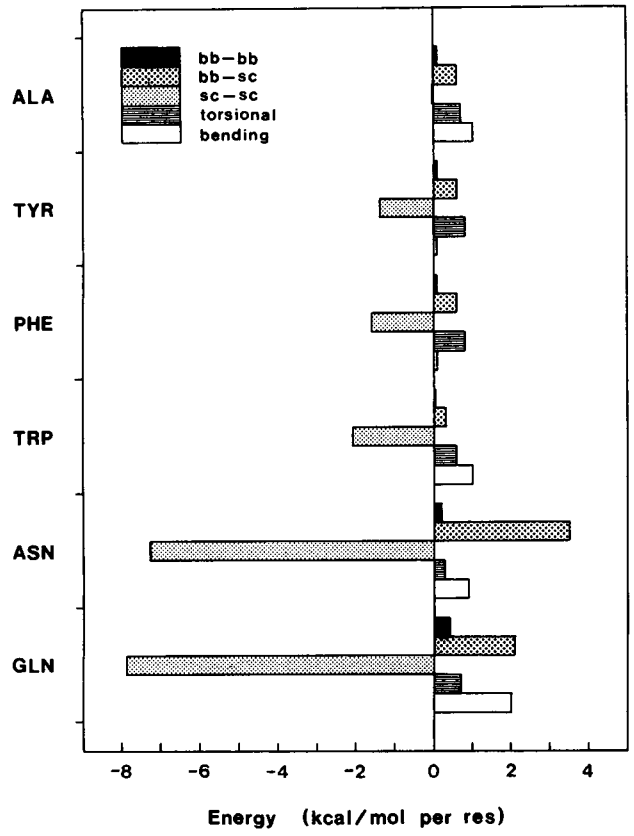

FIGURE 3 Components of the conformation energy of the μ helix of homopolymer poly-L-amino acids. Each energy component is expressed relative to the corresponding energy component of the α helix of the same polypeptide. *bb–bb*, Backbone–backbone interactions; *bb–sc*, backbone–side-chain interactions; *sc–sc*, interactions between different side chains; *torsional*, torsional energy of dihedral angles; *bending*, bending energy of bond angles. The other energy components are not shown.

bonded with a backbone NH group. Thus there is a furcate hydrogen bond for each backbone CO group in the α helices of these polypeptides.

Table 4 also shows that the backbone of the μ helix is per se less stable than that of the α helix. There are two principal factors responsible for this instability. The first is that, as mentioned above, the peptide linkages in the μ helix deviate from the planar *trans* configuration by approximately 10° ($\omega \sim 170°$), which increases the conformation energy by approximately 0.6 kcal/mol per residue within the framework of the ECEPP force field (see the values in parentheses in the sixth column). On the other hand, the ω of the α helix is approximately 180°, its torsional energy being negligibly small. (No other rotatable bonds in the backbone contribute to the torsional energy.) The second factor is that the backbone bond angles of the μ helix are, in general, more distorted than those of the α helix (the term "backbone bond angle" used here means a bond angle that involves at least two of the three backbone atoms N, C^{α}, and C′). This is inferred by the rms deviations of bond angles given in Table 2 (although the rms values in this table also involve the contribution of the side chains, they are approximately equal to the rms values for the backbone alone). The instability of the backbone of the μ helix (relative to that in the α helix) caused by the second factor is 0.3–1.2 kcal/mol per residue, depending on amino acid species (see the values in parentheses in the seventh column). The combined effect of those two factors on the relative conformation energy is 0.9–1.6 kcal/mol per residue, depending on amino acid species.

In many problems concerning the conformation of polypeptides the intrachain bond angle $\angle NC^{\alpha}C'$ at the α-carbon atom plays an important role. This angle was approximately 113° in the μ helix (Table 2), greater than that of the α helix only by 1–2°; it is still in an energetically allowable range of the bond angle of the "tetrahedral" carbon.

It seems generally accepted that the presence of empty space within a helix decreases the van der Waals interactions within the backbone (e.g., Schultz and Schirmer, 1979). Table 3 (values in parentheses in the second colomn), however, suggests that the nonbonded interactions in the backbone of the μ helix are comparable with, or, rather, slightly greater than, those in the backbone of the α helix, in spite of the presence of a hollow pore within the μ helix.

The total interaction energy, $E_{ES} + E_{NB}$, between nonbonded atoms of the backbone was not significantly different between the μ and the α helices. There were no appreciable effects of the stretching energy of bonds on the relative stabilities of these helices.

Relative position of α-carbons; its implication to the inter-side-chain interactions

As pointed out above, the inter-side-chain interactions in the μ helix exhibited a marked diversity, depending on the species of side chains. They were also often greatly different in magnitude from the inter-side-chain interactions in the α helix. This can be explained, as follows, by the difference in the relative position of α-carbons between the two helices.

In the μ helix the helical pitch was 5.0 Å/turn, and the difference in azimuthal angle between the nearest-neighbor α-carbons belonging to adjacent helical turns was only ~10° (Table 5; also see Fig. 1). On the other hand, they were 5.5 Å and ~−70°, respectively, in the α helix when calculated by the same force field. It follows that in the μ helix the distances between corresponding atoms of the nearest-neighbor side chains belonging to adjacent helical turns are approximately 5.0 Å (Table 5). On the other hand, they were 5.9 Å or greater in the α helix, depending on the radial distance (or the distance from the helical axis) of the atoms in question.

Such a difference in the relative position of side chains has differential effects on the modes of inter-side-chain interactions in the two helices. With small and nonpolar side chains such as those of alanyl residues, the interactions between adjacent side chains are at low levels in both helices (Table 4, fourth column). With poly-L-tryptophan,

TABLE 5 Interatomic distances between corresponding atoms of the amino acid residues with the nearest-neighbor α-carbons belonging to adjacent helical turns in minimum-energy α and μ helices

Polypeptide	Helix	Pitch (Å/turn)	Difference in Azimuthal Angle* (deg)	Interatomic Distance (Å)		
				α-Carbon	β-Carbon	γ-Carbon
Poly-L-alanine	α helix	5.5	−69 (29)	5.2 (6.0)	5.9 (6.2)	–
	μ helix	5.0	13	4.9	5.0	–
Poly-L-tryptophan	α helix	5.5	−70 (26)	5.2 (6.0)	5.9 (6.1)	6.6 (6.2)
	μ helix	5.0	13	4.9	5.0	5.1
Poly-L-glutamine	α helix	5.5	−66 (32)	5.2 (6.0)	5.9 (6.2)	7.0 (6.4)
	μ helix	5.0	9	4.9	4.9	5.0

Values in parentheses are for the residues with the second-nearest-neighbor α-carbons in the α helix.
* Difference in azimuthal angle around the helical axis between corresponding atoms of the residues with the nearest-neighbor α-carbons.

which possesses bulky side chains, the nearest-neighbor side chains in its periodic α helix are still too far apart to be in the most favorable van der Waals contact, and the inter-side-chain interactions are much weaker than the side-chain–backbone interactions, whereas the nearest-neighbor indole rings in the μ-helical form can take a stacked position, and the stabilization that is due to the inter-side-chain interactions is greater than that in the α helix by ~2 kcal/mol per residue. In the case of the α helix of poly-L-glutamine, no hydrogen bonds can be formed between the side chains because of its long inter-side-chain distance, which stands in marked contrast to the μ helix (see below).

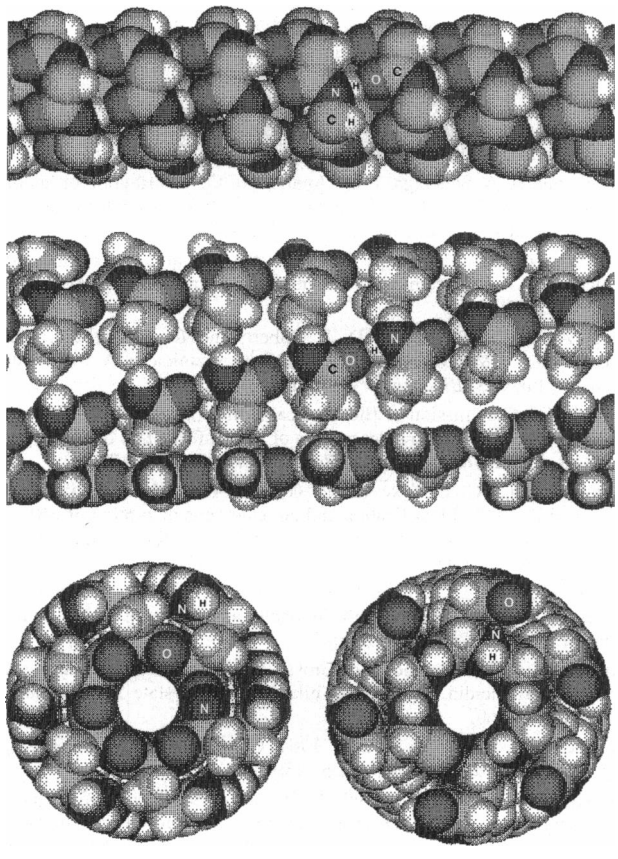

FIGURE 4 Dual hydrogen-bonded network of the minimum-energy μ helix of poly-L-glutamine. The N-terminal end is to the left. (*Top*) Side view of the backbone. (*Middle*) Side view of the hydrogen-bonded lacing of side chains; only the frontal half is shown. (*Left bottom*) View from the N-terminal end. (*Right bottom*) View from the C-terminal end. End groups are removed. The radii of atoms employed are H, 1.00 Å; C, 1.50 Å; N, 1.45 Å; O, 1.35 Å.

Structure of the μ helix of poly-L-glutamine; dual hydrogen-bonded network

Fig. 4 is a space-filling representation of the minimum-energy μ helix of poly-L-glutamine. In this helix the side-chain NH group of a residue is hydrogen-bonded to the side-chain CO group of the sixth residue before it along the chain (i.e., the side-chain CO group of a residue is hydrogen-bonded to the side-chain NH group of the sixth residue behind). Thus the hydrogen-bonded structure of the backbone is surrounded by another hydrogen-bonded network, or lacing, composed of the side chains. Accordingly, there is a dual hydrogen-bonded network in the μ helix of this polypeptide.

In this conformation the side-chain atoms are positioned close to the backbone; this leads to favorable van der Waals interactions between the side chains and the backbone. An inter-side-chain hydrogen bond also lies close to a backbone hydrogen bond, and their dipole moments are approximately parallel to each other but point in opposite directions (cf. the top and the middle figures of Fig. 4). This leads to favorable interactions between them.

Four types of hydrogen-bonded networks of the side chains were possible. In two types the NH groups of the side chains face toward the N-terminal end, and in the others they look toward the opposite direction. The side-chain conformation that gives the minimum-energy μ helix belongs to the first category. The other types of side-chain networks gave rise to conformations less stable by at least 0.8 kcal/mol per residue.

There was also a similar hydrogen-bonded lacing of side chains in the μ helix of poly-L-asparagine. Hydrogen-bonded networks of side chains also occured in the minimum-energy γ helices of those two polypeptides.

CONCLUSION

The present molecular mechanics calculations have formulated a new tubular single-stranded helix of poly-L-amino acids, which appears in the vicinity of the point (ϕ, ψ, ω) = (81°, 98°, 170°) in the conformational space. It corresponds to the 6.2_{20} helix. This helix, or the μ helix, has a cylindrical pore along the helical axis. The diameter of the pore is 6.6 Å on the basis of the atom centers of carbonyl carbons and amino nitrogens. Interestingly, this helix cannot be obtained when energy minimization is started from ω = 180°.

The backbone of the μ helix is per se less stable than that of the α helix. This is mainly due to the nonplanar peptide bonds and more distorted bond angles of the former helix. However, the conformation energy of the μ helix is usually decreased to various degrees by the inter-side-chain interactions, depending on the species of side chains concerned. It varies over a range of a few kilocalories per mol per residue above and below the conformation energy of the α helix of the same polypeptide. This diversity in the relative conformation energy of the μ helix (with reference to the conformation energy of the α helix) can be ascribed primarily to the difference in the relative position of α carbons between the two helices. The conformational entropy makes only a small contribution, if any, to the relative stability of the μ helix.

With homopolypeptides the stability of the μ helix is comparable with that of the γ helix. In the light of the importance of the inter-side-chain interactions, there is, however, a possibility that some specific amino acid sequences may cause a substantial difference between the stabilities of these two helices. In the μ helices of poly-L-glutamine and poly-L-asparagine, the conformations are stabilized by inter-side-chain hydrogen bonds. In this calculation, however, the presence of water molecules is not taken into consideration. It is interesting to see whether the hydrogen-bonded network of side chains is maintained even in the presence of water. And what is the free energy of the μ helix in the presence of solvent? To what degree is the μ helix stabilized by the presence of water molecules within the pore? Can hydrophobic side chains be introduced into the μ helix without destabilizing its whole structure? In a subsequent report these points will be examined, and the possibility of the occurrence of this helix will be discussed.

It is known that not a few proteins have homopolymer segments in the primary structure. The human TF2D protein, for example, has a stretch of 38 consecutive glutamine residues (Kao et al., 1990), and the yeast SNF5 protein contains a repeat of 37 glutamine residues (Laurent et al., 1990). There is a region consisting of 26 consecutive asparagine residues in a cyclic AMP receptor CAR3 of *Dictyostelium* (Johnson et al., 1993). The present results suggest that these polyglutamine and polyasparagine segments may assume a μ or a γ helical conformation.

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