treatment. More detailed conformational analyses will be required on this account. The very small characteristic ratios arising from the same physical origin have been predicted for a few biopolymers.[9,26,27]

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A Case Study of the Conformation of Poly(α-aminoisobutyric acid): α- or 3_{10}-Helix

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ABSTRACT: The relative stabilities of α- and 3_{10}-helical structures for polymers of α-aminoisobutyric acid (Aib) have been worked out, using the classical potential energy functions. To make a comparative study, we have used Buckingham "6-exp" and Kitaigorodsky's potential functions. Conformational analysis of the dipeptide segment with Aib residue indicates the necessity for nonplanar distortion of the peptide unit, which is a common feature in the observed crystal structures with Aib residues. In the range of $\Delta\omega$ -10 to $+10°$ studied, α-helical conformations are preferred in the region $-3° < \Delta\omega < +10°$, and 3_{10}-helical conformations are preferred in the region $-3° > \Delta\omega > -10°$. Minimum energy conformations for right-handed structures are found in the $+ve$ region of $\Delta\omega$ and correspondingly for left-handed structures in the $-ve$ region of $\Delta\omega$. For $\Delta\omega \sim 6°$, α-helical structures have four- or near fourfold symmetry with $h \sim 1.5$ Å. Such a helix with $n = 4$ and $h = 1.5$ Å is termed an α'-helix. This structure is found to be consistent with the electron diffraction data of Malcolm[3] and energetically more favorable than the standard 3_{10}-helix.

α-Aminoisobutyric acid (Aib) differs from the usual amino acids in that it has two methyl groups attached (corresponding to LD positions) to the C$^\alpha$ atom. The simultaneous presence of two methyl groups reduces the allowed region in the conformational space quite considerably as has been reported earlier.[1,2] Burgess and Leach[2] have suggested an obligatory α-helix for the polymer of Aib. Malcolm,[3] on the basis of electron diffraction and infrared studies, has indicated a 3_{10}-helical structure for poly(Aib). This prompted us to investigate in detail the relative stabilities of α- and 3_{10}-helices for poly(Aib), taking into account the available structural informations about the Aib residue. Crystal-structure analyses of oligopeptides containing Aib residues indicate nonplanar distortion of the peptide unit.[4-10] Nonplanar distortion of the peptide unit has been taken into account in our conformational energy calculations on the dipeptide segment of Aib and poly(Aib), and the results of such calculations are presented in this paper. The most important result is that a modified α-helix of pitch nearly 6 Å is found to be energetically more stable than a 3_{10}-helical structure.

Results and Discussion

The dipeptide fragment of the Aib residue is shown in Figure 1. The conformational angles φ, ψ, and ω are defined according to the IUPAC-IUB Commission.[11] Standard Pauling-Corey geometry for the trans-peptide unit was assumed throughout the calculations. While in-

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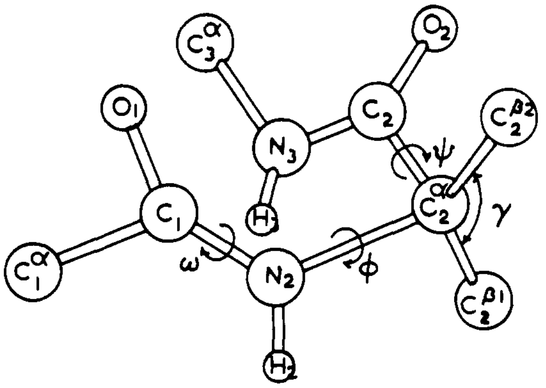

Figure 1. A schematic diagram of the dipeptide segment with Aib residue.

cluding the nonplanar distortion of the peptide unit, two variables $\Delta\omega$ and θ_N, as defined earlier by Ramachandran et al.,[12] were considered. The hydrogen atoms on the β carbons were fixed in the staggered conformations.

The conformational energy composed of contributions from nonbonded, electrostatic, hydrogen bond, and torsional energies was evaluated as a function of φ, ψ, and ω for a dipeptide or a helix as the case may be, using the method described by Ramachandran and Sasisekharan.[13] The general method for helical energy calculations is described in detail in ref 14. We have made use of two potential functions [Buckingham "6-exp" potential with the set of constants in Table XI of ref 13 and Kitaigordsky potential with the set of constants (K1) in Table 1 of ref 15] for computing the nonbonded energy. The electrostatic interactions were estimated taking the monopole charges on the four atoms C, O, N, and H of the peptide unit to be +0.4, −0.4, −0.3, and +0.3 (electron unit), respectively, and an effective dielectric constant of 4.0. The hydrogen bond energy function was taken from ref 16. The variations in energy with changes in conformational angles φ, ψ, and ω have been evaluated using the following formulas: $V_\varphi = (K_1/2)(1 - \cos 3\varphi)$, $V_\psi = (K_2/2)(1 + \cos 3\psi)$, and $V_\omega = (K_3/2)(1 - \cos 2\omega)$, where K_1, K_2, and K_3 represent the barrier to rotations φ, ψ, and ω, and their values are 0.6, 0.2, and 20.0 kcal/mol, respectively.

Marshall and Bosshard[1] and Burgess and Leach[2] have independently obtained the dipeptide energy plots for Aib, the former using Kitaigordsky's potential function and the latter using the Lennard-Jones potential function for the evaluation of the nonbonded interactions. We also computed the energy map with the planar peptide unit using the Buckingham "6-exp" potential function, and the results are similar to those obtained earlier by them[1,2] (see Figure 2).

The (φ, ψ) dipeptide energy map indicates that the regular structures possible for poly(Aib) are α- and 3_{10}-helices of both left- and right-handed types. Burgess and Leach[2] have remarked from their semiempirical helical energy calculations that the most stable conformations are the two right- and left-handed α-helices. A consistent feature observed in most of the (φ, ψ) space corresponding to both φ and ψ varying from −100 to 0° is that steric contacts between C_1 and $C_2^{\beta 2}$ (superscript 2 of a C^β atom refers to the methyl group in the D position and superscript 1 refers to the methyl group in the L position), O_1 and one of the hydrogen atoms on $C_2^{\beta 2}$, N_2 and N_3, and N_2 and H_3 (see Figure 1) fall slightly short of the respective extreme limits.[13] Calculations performed with nonplanar peptide units indicate that these contacts are relieved for the most part of the conformational space specified only for positive values of $\Delta\omega$. Note that for left-handed helical conformations the same is true for negative values of $\Delta\omega$. In what follows we have considered the right-handed helical structures.

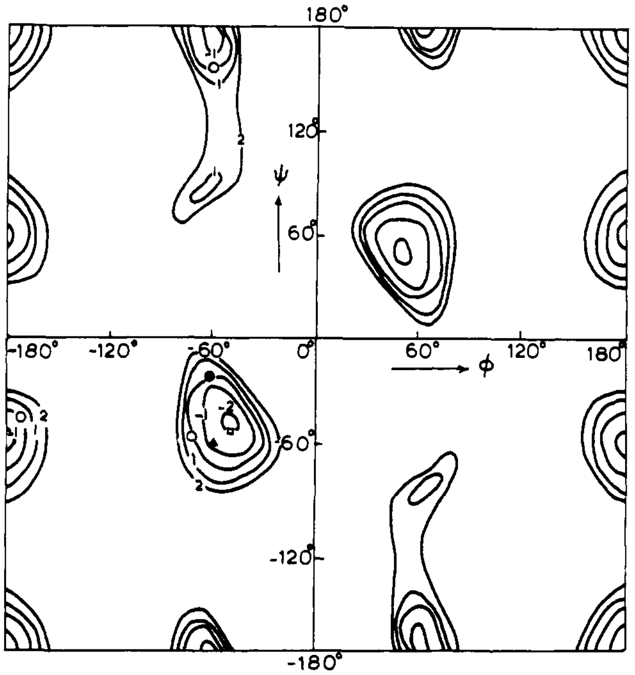

Figure 2. The (φ, ψ) energy map for the Aib residue, using the Buckingham "6-exp" potential function. The contours are drawn at 1 kcal intervals with respect to the innermost contour enclosing the minimum: (□) α-helix, (●) 3_{10}-helix, (▲) α'-helix (see text).

Table I

Distribution of α- and 3_{10}-Helices in the First Ten Conformations in the Ascending Order of Energy from the Minimum Energy Conformation as Given by the Two Potential Functions

polymer	potential function	α	3_{10}	γ, deg	ΔE
Aib	Buckingham	9	1	109.5	3.59
		9	1	112.0	3.35
		9	1	114.0	3.21
	Kitaigordsky	5	5	109.5	1.36
		4	6	112.0	0.71
		5	5	114.0	0.28
Ala	Buckingham	9	1	109.5	3.58
	Kitaigordsky	9	1	109.5	3.30

We have performed helical energy calculations in the conformational space spanned by φ and ψ varying from −100 to 0° for poly(Aib) with planar and nonplanar peptide units. In our calculations, all of the helices with the 4 → 1 type of intrachain hydrogen bonding, i.e., N–H of the fourth residue hydrogen bonding to C=O of the first, are grouped as 3_{10}-helices, and helices with the 5 → 1 type where the donor is N–H of the fifth residue are grouped as α-helices. α- and 3_{10}-helices are generated in the conformational space specified above. As a result of the hydrogen bonding pattern in the two types of helices, α-helices are formed for lower ψ values and 3_{10}-helices for higher ψ values. Because of the presence of the methyl group corresponding to the D position, for lower ψ values, the contacts between this methyl group and the carbonyl group of the succeeding peptide unit fall in the borderline region of the extreme limits. Also, such is the case for the contacts between $C_i^{\beta 2}$ and $C_{i+4}^{\beta 1}$ and O_i and one of the hydrogen atoms on $C_{i+4}^{\beta 1}$ for most of the α-helical conformations. However, these contacts are greater than the normal limits for 3_{10}-helical conformations. Because of such borderline contacts, the choice of the potential

Table II
Conformational Angles and Characteristics of Helical Structures with Pitch Around 6.0 Å

H bond pattern	conformational angles, deg				helical parameters			H bond parameters		total energy[a]	
	φ	ψ	$\Delta\omega$	θ_N	n	h, Å	p, Å	N···O, Å	∠H-N···O, deg	B	K
4 → 1	−60	−20	+4	−8	3.05	1.98	6.04	3.02	5.90	−13.20	−1.21
	−55	−25	+4	−8	3.07	1.97	6.05	2.92	6.64	−13.92	−2.17
	−50	−30	+4	−8	3.08	1.96	6.04	2.84	9.31	−13.97	−2.48
	−45	−30	+4	−8	2.99	2.01	6.00	2.83	3.15	−12.98	−1.82
5 → 1	−55	−60	+7	13	4.00	1.48	5.92	3.07	9.29	−16.31	−4.39
	−50	−65	+7	13	4.03	1.49	6.00	3.10	6.67	−15.65	−4.00

[a] kcal/monomer unit.

function becomes important. We have considered two potential functions: Buckingham "6-exp" (B) and Kitaigordsky's potential function (K).

Table I summarizes the results obtained by using the two potential functions for poly(Aib), assuming the planarity of the peptide unit. Both potential functions lead to the α-helix as the most stable conformation for poly(Aib). It may be noted from the last column of Table I that ΔE, the energy difference between the most stable conformations in the α- and 3_{10}-helical sets, depends on the potential function used. Thus with the B function the energy difference is around 3.0 kcal/monomer unit, whereas with the K function it is around 1.0 kcal/monomer unit or less. However, it is well known that both of these functions give similar results for poly(L-Ala).[15] The ΔE for poly(L-Ala), using the B function, is almost the same as that obtained using the K function (see Table I). The dissimilar behavior of these two functions toward poly(Aib) is also brought out by considering the distribution of α- and 3_{10}-helices in the first ten conformations in the ascending order of energy from the most stable conformation. The calculations have been performed for different values of $\gamma(\angle C^{\beta 2}C^{\alpha}C^{\beta 1})$. We have chosen to vary γ in order to find out which of the two potential functions is more sensitive to borderline contacts due to the additional methyl group. With the B function, ΔE remains almost the same when γ is varied from 110 to 114°. However, with the K function, ΔE is significantly reduced with the variation of γ from 110 to 114°. The B function is a softened potential function, whereas the K function is not. As a result, the K function attributes higher energy for contacts slightly less than the extreme limits. Thus the K function destabilizes α-helical structures because of such contacts (see also Figure 3).

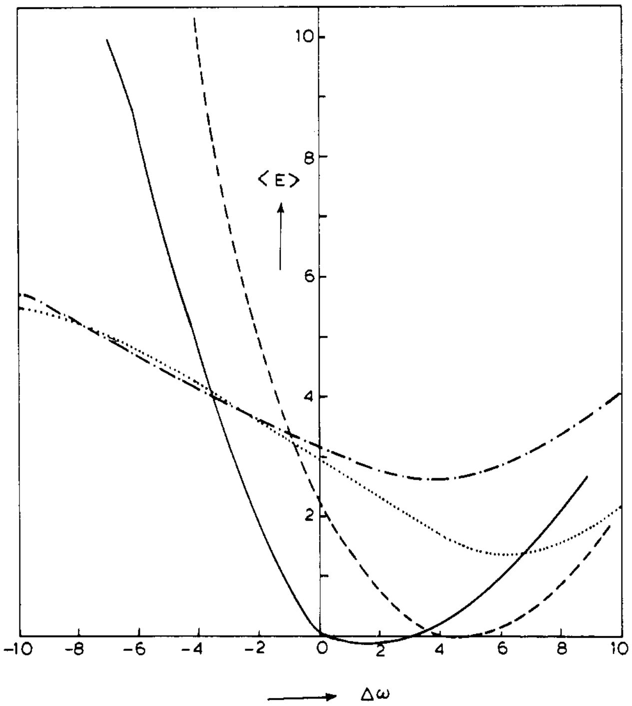

Figure 3. The variation of average energy $\langle E\rangle$ with $\Delta\omega$ for α- and 3_{10}-helices, using B and K functions. The variations of energy for α-helix, using B and K functions, are denoted by — and - - -, respectively, and for 3_{10}-helices by ··· and ····.

We have carried out calculations for six values of $\Delta\omega$, namely ±4, ±7, and ±10, and θ_N in each case varied from $-2\Delta\omega$ to $+2\Delta\omega$. To simplify the presentation of the results, as suggested by one of the referees, we have calculated the Boltzman average of conformational energies for each value of $\Delta\omega$. The averaging has been performed over φ, ψ, and θ_N, following the procedure given in ref 17 (see also ref 18 and 19). The averages ($\langle E\rangle$) were obtained for both 3_{10}- and α-helical structures, whose conformational energies have been computed by using B and K functions. For each case, $\langle E\rangle$ is plotted against $\Delta\omega$ in Figure 3. It is evident from these curves that poly(Aib) prefers conformations with positive values of $\Delta\omega$. Using the K function, we found the minimum for the α-helix around $\Delta\omega = +5°$ and that for the 3_{10}-helix around +7°, whereas with the B function the minimum for the α-helix occurs near $\Delta\omega = 0$ and that for the 3_{10}-helix at $\Delta\omega = +4°$. The K function shifts the minimum for both α- and 3_{10}-helical conformations toward higher values of $\Delta\omega$ and attributes lower energy values to 3_{10}-helical conformations than the B function, thereby bringing down the difference in the average energy between the two types of helices. It is to be noted at this point that in the crystal structures of oligopeptides containing the Aib-Aib sequence the average value of $\Delta\omega$ is around +6°.[5,8]

In the case of the α-helical structures, the contacts between $C_i^{\beta 2}$ and $C_{i+4}^{\beta 1}$ and O_i and $C_{i+4}^{\beta 1}$ are very sensitive to the variation in $\Delta\omega$. For $\Delta\omega < -3°$, these contacts become very severe, and because of this the α-helical structures are destabilized with respect to 3_{10}-helical structures (see Figure 3). However, in the region of $\Delta\omega$ around 0°, these contacts lie on the borderline of the extreme limits; for this reason only, as already discussed, the two potential functions behave in a dissimilar fashion, and the α-helical structure is more stable than the 3_{10}-helical structure by about 3.0 kcal/monomer unit using the B function and about 1.0 kcal/monomer unit using the K function (see Figure 3). When these contacts are relieved in the region of $\Delta\omega$ around 5°, the α-helical structure is unambiguously more stable than the 3_{10}-helical structure. For higher values of $\Delta\omega$, say $\Delta\omega = +10°$, α-helices are not possible due to nonformations of the 5 → 1 type of intrachain hydrogen bonds, whereas satisfactory 3_{10}-helical structures with good hydrogen bonds are possible.

Conclusion

Our conformational energy calculations on poly(Aib), using the Buckingham "6-exp" function, show that the conventional α-helix with planar or nearly planar peptide units has the lowest energy. On the other hand, calculations using the Kitaigordsky function lead to the minimum for the α-helical structure, with $\Delta\omega \sim 5°$. In this minimum energy region, the helical structures have fourfold or near fourfold symmetry and pitch around 6 Å. The helix with $n = 4$ and a $5 \rightarrow 1$ hydrogen bonding scheme has been referred to as the ω-helix in the literature, but the height per residue is around 1.35 Å.[20] Though the choice is purely arbitrary, we have preferred to call the helix with $n \sim 4$ and $h \sim 1.5$ Å an α'-helix, since the h is the same as that of the conventional α-helix. In the region corresponding to average nonplanar distortion of the peptide unit observed in the crystal structures of oligopeptides containing the Aib-Aib sequence ($\Delta\omega \sim 6°$), both B and K functions lead to the α'-helix. In that region of $\Delta\omega$, the minimum for 3_{10}-helical structures attributed by the K function is within 1.5 kcal/monomer unit from that of α-helical structures. The 3_{10}-helical conformations in this minimum energy region have pitch around 6.25 Å. So, for poly(Aib) the conventional α-helix is not obligatory and an α'-helix and a 3_{10}-helix are possible, but with nonplanar distortion of the peptide unit. The electron diffraction data of poly(Aib) suggest helical structures with pitch of 6 Å, and Malcolm has interpreted the data in terms of the 3_{10}-helix, although near meridional reflection of 1.49 Å could be indicative of an α-helix.[3] We have listed all of the minimum energy helical conformations having pitch around 6 Å in Table II. All of the meridional or near-meridional reflections of the electron diffraction data, we find, can be interpreted in terms of the α'-helix. The 1.49 reflection thus fits well with the α'-helix. It is of particular interest to note that the crystal structure of Tosyl(Aib)$_5$-OMe which has been recently reported by Shamala et al.[5] showed that the pentapeptide adopts a 3_{10}-helical conformation and the average nonplanar distortion corresponds to $\Delta\omega$ around +6°. However, it should be noted that the energy difference between α'- and 3_{10}-helices (Table II) is more than compensated for by the additional hydrogen bond present in the 3_{10}-helical conformation of the pentapeptide.

We believe that further diffraction data are needed to unequivocally establish the structure of poly(Aib) as the α'-helix or the 3_{10}-helix.

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Electron Spin Resonance Study of Poly(α-L-glutamic acid)- and Poly(acrylic acid)-Copper(II) Complexes in the Frozen State with Emphasis on the Complex Species [1]

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ABSTRACT: ESR spectra of Cu(II) in the presence of poly(sodium α-L-glutamate) (poly(Glu)) and poly(sodium acrylate) (poly(Acr)) were measured in frozen aqueous solutions at various degrees of neutralization (α') (0.2–1.0), pH 3–11 at room temperature, and at various molar mixing ratios of polymer residue to cupric ion (8–64). The parallel hyperfine constants, $g_{\parallel}$ values, line widths, and line heights of the polyanion–Cu(II) complexes were determined by means of computer simulation of the observed ESR spectra. The Cu(II)-residue complexes of two different classes (classes 1 and 2) were concluded to exist in the poly(Acr)–Cu(II) system. In the poly(Glu)–Cu(II) system, the Cu(II)-residue complex containing one ligand nitrogen atom (class N) was found to exist in addition to the same complexes of classes 1 and 2 as found in the poly(Acr)–Cu(II) system. The magnetic parameters of the Cu(II)-residue complexes were compared with those of Cu(II)–glutarate and –malonate complexes. The Cu(II)-complex species of $Cu(COO)_4^{2-}$ and $Cu(COO)_3^{-}$ were assigned to class 1 and the species of $Cu(COO)_2$ to class 2. The Cu(II)–residue complex of class N was found to be the $Cu(N\text{-}H)(COO)_2$.

Interactions between polyelectrolytes and metal ions have been investigated from various points of view, e.g., catalytic activity[2-5] and conformational change of polymer–metal ion complexes.[6-28] In particular, polyelectrolyte–copper complexes have been utilized as model compounds to elucidate the mechanism of the biological ac-

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