3_{10} Helix *Versus* α-Helix: A Molecular Dynamics Study of Conformational Preferences of Aib and Alanine

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Abstract: The α-helix and the 3_{10} helix are two common constituents of protein architecture. Spectroscopy and crystallography indicate that short peptides containing alanine tend to assume α-helical conformations while short peptides containing Aib (α-aminoisobutyric acid) tend to assume 3_{10}-helical conformations. Here we have used molecular dynamics simulations to study the equilibrium between helix types both *in vacuo* and in water and found that decaalanine prefers the α-helix over the 3_{10} helix by 16 kcal/mol in water, or 8.0 kcal/mol *in vacuo*, while Aib_{10} shows no clear preference in water, and prefers the 3_{10} helix by 4.3 kcal/mol *in vacuo*. In either medium, introduction of a single Aib residue in the middle of a 14-residue 3_{10} helix containing only Ala (alanine) residues very much stabilizes the 3_{10}-helical conformation ($\Delta\Delta G° = -2.6$ kcal/mol , in water), while in the α-helical conformation the effect of the introduction is quite small ($\Delta\Delta G° = -0.7$ kcal/mol, in water). We argue that the conformation of the Aib residue predisposes this residue to helix formation, in terms of both entropy and energy, and that the low propensity for α-helix formation comes from unfavorable interactions between the methyl groups of an Aib residue in position i and the Ala residue in position $i + 3$, interactions which are absent in the 3_{10} helix. This is supported by results of simulations with oligomers with Aib in position i and glycine (Gly) in position $i + 3$, which show that the Aib/Gly pair makes as stable an α-helix as does the Ala/Ala pair, entirely suppressing the inherent helix destabilizing effect of Gly.

Introduction

The 3_{10} helix differs in its hydrogen-bonding pattern from the much more common α-helix: the carbonyl oxygen-to-amide hydrogen bonds are made between residues i and $i + 3$ in the former and between residues i and $i + 4$ in the latter. Changes in the backbone dihedrals ϕ and ψ of less than 15° distinguish these two helical conformations.

The 3_{10} helix is a minor conformation in the structure of globular proteins, occurring often at the ends of α-helices.[1] Dynamics simulations of helices have shown a tendency to some formation of 3_{10} helix in predominantly α-helical molecules, and the 3_{10}-helical conformation has been implicated as an intermediate in unfolding of α-helices to form extended conformations.[2,3]

Oligomers containing Aib (α-aminoisobutyric acid) have been observed to form 3_{10}-helical structures in crystals and in nonaqueous solution.[4] The assumption of 3_{10}- rather than α-helical conformation is apparently favored by high content of Aib residues and short chain length.[5-7] The preference of *short* molecules for the 3_{10} helix is understood to be the result of the additional hydrogen bond in the 3_{10}-helical conformation, while an intrinsic higher propensity of Aib for the 3_{10}-helical conformation is not entirely understood.

Alanine (Ala) has been historically recognized as a good α-helix former.[8] Recently, it has been suggested that certain amino acid oligomers rich in alanine, and not containing Aib, form the 3_{10} helix in aqueous solution.[9-11] The evidence for this is based on spin label ESR spectroscopy; the conclusion that these molecules indeed form 3_{10} helices has not yet been generally accepted.

Studies of the 3_{10} helix are much less complete than studies of the α-helix, for a number of reasons. The crystal structures of 3_{10}-helical peptides provide invaluable structural information, but contribute only limited understanding of molecular helical preferences, as these crystals contain few or no water molecules, and frequently contain end-to-end hydrogen-bonded interhelical contacts. Helices containing much Aib are more readily soluble in apolar solvents than in water; also nuclear magnetic resonance or optical properties of aqueous solutions do not allow an easy distinction between the two helical types. Early mechanical analysis of the systems suggested that Aib has lower energy in the α-helix than that in the 3_{10} helix,[12,13] or essentially the same energy in the two helical conformations.[14] The 3_{10} helices containing Aib were thought to be stabilized by the effects of low dielectric constant[13] and helix packing in crystals.[15] It was also suggested that the assumption of asymmetric geometry by the Aib residue could favor the 3_{10} helix over the α-helix.[16] A

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more recent study has shown that the entropy difference significantly favors the 3_{10} helix.[17]

In this paper we present some results of simulations of amino acid oligomers containing Ala, Aib, and glycine (Gly) in order to characterize the equilibria between the two helical conformation types. The advantage of simulations is that these allow the study of both stable and meta-stable conformations, i.e., of different helical conformations and the unfolded state. Also, it is always advantageous to study the simplest molecules that show the behavior of interest, which in this case would mean oligomers of apolar amino acids (Ala, Aib, and Gly) in water. With simulations one can study single molecules of these simple oligomers, which in actual fact are insoluble in water. The disadvantage of simulations is that the results allow a view of the underlying reality through the filter of an imperfect model used to represent it.

The model used in the present simulations has been tested by simulations of differences in α-helical propensity of a series of amino acids, which include Ala, Gly, Aib, and proline.[18,19] Comparison of the results of the simulations with experimentally observed differences[20-22] in helix propensity justifies some degree of confidence in the model's applicability to problems of conformational equilibria.

In this study these calculations are extended to assess the tendency to form the 3_{10} helix rather than α-helix. During the preparation of this manuscript, two other groups published studies of equilibria and kinetics of the α-helix/3_{10} helix transitions of undecaalanine[23] and Aib_{10}.[17] The general sense of the results on transition free energies presented in these papers has been confirmed in our study, which has the advantage of use of an all-atom potential and of identical methods and force field for calculations with both types of residues. Also reported here are the results of calculations of relative 3_{10}-helical propensities, something that has not been done in earlier work. The results show a large relative propensity of Aib for the 3_{10} helix, much larger than for the α-helix. In a sequence with a nearby glycine residue the relative α-helical propensity of Aib is found to be larger (by 2.2 kcal/mol), an effect that should be testable by experiment. We then show how these effects can be understood in terms of (atomic) interactions in the helix and coil states.

Materials and Methods

Molecular Models. The peptides studied are as follows:

Ala_{10}: Ac-AAAAAAAAAA-NMe

Ala_{10}-G: Ac-AAAAAAAGAA-NMe

Ala_{14}: Ac-AAAAAAAAAAAAAA-NMe

Ala_{14}-G: Ac-AAAAAAAAAGAAAA-NMe

Aib_{10}: Ac-BBBBBBBBBB-NMe

where A = Ala, B = Aib, Ac = acetyl, G = Gly, NMe = *N*-methyl. **A** represents the site of Ala-to-Aib replacement.

Free energy changes associated with the α-helix/3_{10} helix transition were calculated for Ala_{10} and Aib_{10}. Oligomers of alanine in which a central residue could be replaced by Aib or glycine were used in free energy simulations to study the relative stabilities. Also studied was replacement of Ala by Aib in the latter oligomers modified by substituting one of the alanines with a glycine residue.

Molecular Dynamics Simulations. Molecular dynamics simulations were carried out with the program Cedar using the all-atom representation. (In one calculation the corresponding united-atom force field was used.) The simulations were performed with constant mean pressure (1 atm) and mean temperature (300 K), SPC[24] water solvent, a 8 Å cut-off distance for nonbonded interactions, a 2 fs time step, and bond lengths held fixed with the Shake[25] algorithm, as described in recent publications.[18,19,26] For simulations in water, periodic boundary conditions were used; each oligomer was placed in a box of approximately 25 × 25 × 35 Å^3, together with approximately 300 water molecules. The direction of the helix was maintained parallel to the long dimension of the box (z) with restraints[27] on the x-, y-, and z-coordinates of the first and on the x- and y-coordinates of the last carbon atom of the oligomer.

Conformational restraints were used in order to prevent conformation changes from one low-energy state to another. The restraints were designed so that they restricted the sampling to a single conformation without perturbing the distribution within it.

Two types of restraints have been used in our simulation. (1) In order to study helical conformations without incurring unfolding to extended conformations, torsional bounds have been used. These are flat-bottomed potentials that are zero, and hence nonperturbing, in the neighborhood of free energy minimum and rapidly rise to a high value (here chosen to be 10 kcal/mol) over a small range of torsion angle (20°). These were applied to restrict the conformations to the ranges of $-180° < \phi < 0°$ and $-180° < \psi < 0°$. It is essential to use this restraint in simulations of helices in water. (2) In order to select a *particular* helical conformation to the exclusion of another, upper bound restraints on the appropriate hydrogen bond distances have been used. With these, the energy rises to 60 kcal/mol over an interval from 2.3 to 3.9 Å in the H-to-O distance. Without this restraint it would be impossible to study each oligomer in two different helical conformations.

Free Energy of 3_{10}-Helix/α-Helix Transition. In the free energy simulations we have applied a potential function that will force the exchange of the set of hydrogen bonds appropriate for α-helix for the set appropriate for the 3_{10} helix. This function can drive the conformation (back and forth) between the two types of helix by a slow change of the coupling parameter λ between 0 and 1. The driving force potential is

$$U_r = (K_f/2)\sum\{|[\lambda \underline{\underline{r}}_{H_{i+3}} + (1-\lambda)\underline{\underline{r}}_{H_{i+4}} - \underline{\underline{r}}_{O_i}]| - d_0\}^2 \quad (1)$$

where $\underline{\underline{r}}_{O_i}$, $\underline{\underline{r}}_{H_{i+3}}$, and $\underline{\underline{r}}_{H_{i+4}}$ are the vectors of atomic positions of the carbonyl oxygen of residue i and the amide hydrogen of residue $i+3$ and $i+4$, the vertical bars indicate the vector length, K_f is a force constant (40 kcal/ mol), and $d_0 = 1.75$ Å. [Because Ala_{10} has a very strong preference for the α-helical conformation in vacuo, the term $(1-\lambda)\underline{\underline{r}}_{H_{i+4}}$ is not needed and, therefore, has been omitted in calculating the free energy of 3_{10} helix/α-helix transition of Ala_{10} in vacuo.] Free energies are calculated as the work done on the system along a path determined by the changing value of the coupling parameter, λ

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$$\Delta G^\circ = \int \langle \partial U/\partial \lambda \rangle \, d\lambda \tag{2}$$

and approximated with the slow-growth method.

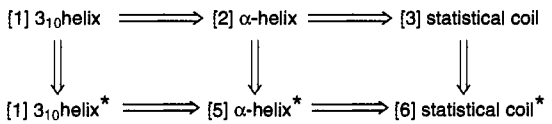
The driving force potential also perturbs the state of the system with a corresponding free energy contribution. This was calculated in separate simulations in which the perturbation potential was slowly grown in (or faded out), i.e., for the α-helix

$$U'_\alpha = \lambda'(K_f/2)\sum[|\underline{r}_{H_{i+4}} - \underline{r}_{O_i}| - d_0]^2 \tag{3}$$

and for the 3_{10} helix

$$U'_{3_{10}} = \lambda'(K_f/2)\sum[|\underline{r}_{H_{i+3}} - \underline{r}_{O_i}| - d_0]^2 \tag{4}$$

Differences in Helix Propensity. Differences in helix propensity have been calculated as described in earlier papers from this laboratory.[19] The nature of the calculation can be understood by consideration of the following thermodynamic cycle: where the asterisks indicate the



(5)

mutated peptide, which in *one* position has Aib instead of Ala (which is the reference). The sum of the free energy changes for any cycle must be zero, so that

$$\Delta\Delta G^\circ_{3_{10}\text{-}\alpha} = \Delta G^\circ_{12} - \Delta G^\circ_{45} = \Delta G^\circ_{14} - \Delta G^\circ_{25} \tag{6a}$$

$$\Delta\Delta G^\circ_{3_{10}\text{-coil}} = \Delta G^\circ_{13} - \Delta G^\circ_{46} = \Delta G^\circ_{14} - \Delta G^\circ_{36} \tag{6b}$$

$$\Delta\Delta G^\circ_{\alpha\text{-coil}} = \Delta G^\circ_{23} - \Delta G^\circ_{56} = \Delta G^\circ_{25} - \Delta G^\circ_{36} \tag{6c}$$

In earlier work, $\Delta\Delta G^\circ_{\alpha\text{-coil}}$ has been referred to as the differential α-helix propensity of Aib; a negative value would indicate that a helix with Aib is more stable relative to the statistical coil conformation than a reference helix with Ala.[19,24] Similarly, $\Delta\Delta G^\circ_{3_{10}\text{-coil}}$ indicates the differential propensity of Aib for folding of the 3_{10} helix, and $\Delta\Delta G^\circ_{3_{10}\text{-}\alpha}$ indicates the difference in propensity of Aib (relative to Ala) for refolding from α-helix to 3_{10} helix. In each of these equations, the first difference is related to experimentally measurable data, and the second one can be assessed by computation.

Results

Free Dynamics Simulations of 3_{10} Helix/α-Helix Transition. In the *in vacuo* simulations, Ala_{10} can maintain quite well the α-helical conformation and Aib_{10} the 3_{10}-helical conformation, while starting from the 3_{10}-helical conformation Ala_{10} changes into the α-helix in about 15 ps (Figure 1, left side) and Aib_{10} changes into the 3_{10} helix from the α-helix in about 20 ps (Figure 1, right side). The reverse transitions have never been seen. The dynamic behavior of these molecules is fairly reproducible as shown by repeated observations in independent simulations. The geometry of the Aib_{10} 3_{10} helix is characterized by almost equal values of the dihedral angles ϕ and ψ of close to −40° (averages over the molecule). This may be compared with the geometry observed in crystals of an Aib decamer, where the average value of ϕ is 54° and that of ψ is 31°.[4] (The crystal contains both right- and left-handed helices.) The mean geometry around the C^α atom is not significantly different from the symmetric equilibrium geometry used in the model. This is observed in simulations in water and *in vacuo*.

In solvated systems, the α/3_{10} transitions took much more time. In the selected example it took nearly 160 ps for Ala_{10} to change from the 3_{10} helix to the α-helix (Figure 2, lower left). In two other simulations, the transition to α-helix took respectively 80 and 100 ps. For Aib_{10}, the transition was never completed (Figure 2, right side).

Free Energy of 3_{10} Helix/α Helix Transition. Rather long simulations were needed to calculate free energy differences for 3_{10}/α transitions with the needed precision. Each *in vacuo* calculation, consisting of simulations of 200 ps in each direction (i.e., with increasing and with decreasing λ), separated by 10 ps equilibrations, was done twice; the estimated error in the mean free energy difference is circa 1.0 kcal/mol. The results are reported in Table 1. A value similarly obtained for Aib_{10} with a united-atom potential is also given there.

The same protocol was inadequate for the simulations in water, where 200 ps simulations still gave an extremely large hysteresis (Figure 3a,b). However, the value of $\langle \partial U/\partial \lambda \rangle$ in simulations going in opposite directions was seen to differ only in the interval $0.1 < \lambda < 0.4$. This is the transition region; as we have seen the transition between α- and 3_{10}-helical conformations is slow, and therefore, the change of the configuration of the peptide lags significantly behind the change of the coupling parameter λ. Thus, the convergence of $\langle \partial U/\partial \lambda \rangle$ was found to be slow, while the convergence outside the transition region was found to be much more rapid. The solution to this problem has been to run two time simulations (200 ps each) at constant λ, for several values of λ in this interval, one starting from the predominantly α-helical conformation and the other from the predominantly 3_{10}-helical conformation, reached at this value of λ in the prior slow-growth simulations. In this way, it was possible to obtain converged $\langle \partial U/\partial \lambda \rangle$ values in the transition region. The resulting averages have been indicated by squares and crosses in Figure 3, parts a and b. Integration of these results then produced the curves shown in Figure 4.

The free energy differences between helical conformations were corrected for the presence of the restraints imposed on the hydrogen bond distances. Restraint free energies, which were computed according to eqs 3 and 4, varied between 2 and 5 kcal/mol for the simulations in water and were less than 0.5 kcal for the simulations *in vacuo*. The corrections are equal to the differences between restraint free energies for the two helical conformations and were at most 0.5 kcal/mol.

After correction for the artificially imposed restraints, the following results were obtained: For Ala_{10} the free energy for the transition from α-helix to 3_{10} helix is 16 kcal/mol, and for Aib_{10} it is 0.10 kcal/mol (both values are for the system in water). The free energies *in vacuo* were found to be 8.0 kcal/mol for Ala_{10} and −4.3 kcal/mol for Aib_{10}. (The results have been summarized in Table 1.) Thus, for Ala_{10} the α-helix is considerably more stable, both in solution and *in vacuo*, while for Aib_{10}, the 3_{10} helix is more stable *in vacuo*, and in water there is no obvious preference. (We hesitate to give any estimate of the uncertainty in these numbers.)

Differences in Helix Propensity ($\Delta\Delta G^\circ$). We have calculated the free energies for replacing one residue of Ala with Aib in several sequences with and without the solvent in α- and 3_{10} helices.

The free energy differences for replacement of Ala with Aib are given in Table 2. In agreement with what was observed in simulations of Aib_{10} and Ala_{10}, a single Aib residue also was found to stabilize the 3_{10} helix (in water $\Delta\Delta G^\circ_{3_{10}\text{-coil}} = -2.6$ kcal/mol; Table 3). The replacement of Aib with Ala was also done with a Gly residue adjacent to the replacement position (position $i + 3$). Details of the results of the simulations are given in Tables 2 and 3. The free energy difference for replacement of Ala with Aib in the 3_{10} helix was found to depend little on the chain length.

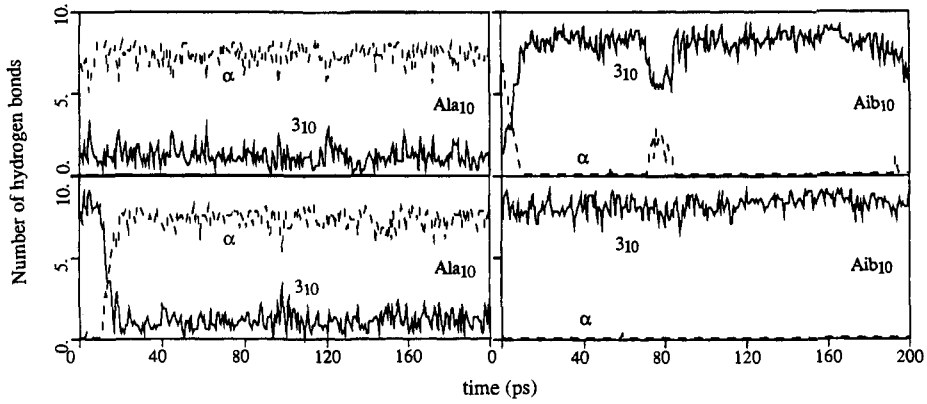


Figure 1. Number of hydrogen bonds (O···H distance below 2.3 Å) during *in vacuo* simulations of the oligomers. The left two simulations were done with Ala_{10} and the right two with Aib_{10}. The upper two simulations started from the all-α-helix and the lower two started from the all-3_{10} helix. The solid line indicates 3_{10}-helical and the dashed line α-helical hydrogen bonds.

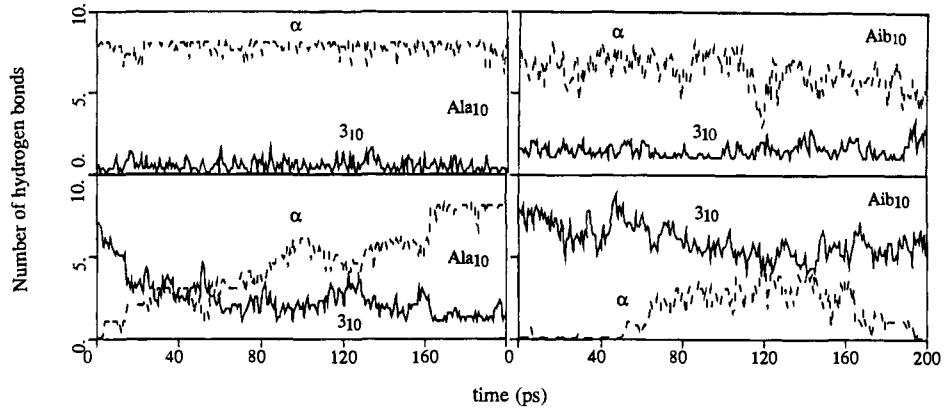


Figure 2. Same as Figure 1, but for simulations in water.

Table 1. (Free) Energies (kcal/mol) for the α-Helix to 3_{10} Transition of Ala_{10} and Aib_{10}[a]

molecule	$\Delta G°$ (in water)	$\Delta G°$ (*in vacuo*)	$\Delta E°$ [b] (*in vacuo*)	$-T\Delta S°$ (*in vacuo*)
Ala_{10}	16.0	8.0	12.0	−4.0
Aib_{10}	0.1	−4.3 [0[c]]	0	−4.3

[a] $\Delta G°$: Gibbs free energy. $\Delta E°$: energy (kinetic + potential). $\Delta S°$: entropy change from α-helix to 3_{10} helix, calculated as $-T\Delta S° = \Delta G° - \Delta E°$. T: temperature 300 K. [b] Difference in mean total energy in dynamics simulations of the two helix types. [c] For the united-atom potential.

Free energy differences for the α-helix in water have been determined in a previous study.[19] For replacement of Ala with Aib, the values are $\Delta G°_{14} = 5.3$ kcal/mol, $\Delta G°_{36} = 5.9$ kcal/mol, and $\Delta\Delta G°_{\alpha\text{-coil}} = -0.6$ kcal/mol. The minus sign indicates that, compared with the reference, all-Ala α-helix, the α-helix in which one Ala in the middle of the helix is replaced by Aib, is more stable. The effect of increasing chain length is larger in this case.

Discussion

3_{10}/α-Helix Transition. The 3_{10} helix/α-helix transition serves as a good model for studying conformational changes in proteins. There is no experimental measure of the free energy change associated with the transition except some observations in the crystal structures that alanine-based peptides form α-helices and Aib-based peptides can form either 3_{10} helix or α-helix under various conditions.[5] Our free energy numbers as well as free dynamics simulations provide a theoretical explanation of these observations in crystal structures.

Our results of free dynamics simulations and free energy calculations are self-consistent. *In vacuo* free dynamics simulations show that the α-helix is favored by Ala_{10} and the 3_{10} helix is favored by Aib_{10}. Free energy calculations *in vacuo* yield the same conclusion. The 3_{10} helix is favored entropically, as was also observed by Huston and Marshall.[28] This is, of course, not unexpected for a longer and thinner structure.

In water, free dynamics simulations show a clear preference for the α-helix for Ala_{10}, while Aib_{10}'s conformational preference is ambiguous, as for several independent 200 ps simulations of Aib_{10} starting from α-helical conformation some show incomplete transition to a 3_{10} helical conformation and some no transition at all. As expected, a free energy change for the 3_{10} helix/α-helix transition of Aib_{10} in water of about zero is found in free energy simulations. In water, the free energy difference of Ala_{10} also has shifted (further) in favor of the α-helix. The difference between *in vacuo* and in water results is as expected from considerations of hydrogen bond stability: the 3_{10} helix has one additional backbone hydrogen bond, and this naturally produces a larger stabilizing effect *in vacuo* than in water.

Tirado-Rives et al.[23] have calculated the free energy change of the 3_{10} helix/α-helix transition of Ac-Ala_{11}-NMe. In this study the helical conformation was defined in terms of the values of the backbone dihedral angles ψ as reaction coordinate, which were gradually changed in Monte Carlo simulations with the OPLS united-atom potential of a partially rigid helix model. (To be precise, the 3_{10} helix was defined as $\{\phi,\psi\} = \{-60°,-35°\}$ and the α-helix as $\{\phi,\psi\} = \{-60°,-45°\}$.) These calculations resulted in a preference for α-helical conformation by 10.5 kcal/mol in water and 13.4 kcal/mol *in vacuo*; i.e., in this model hydration was found to make the 3_{10} helix more,

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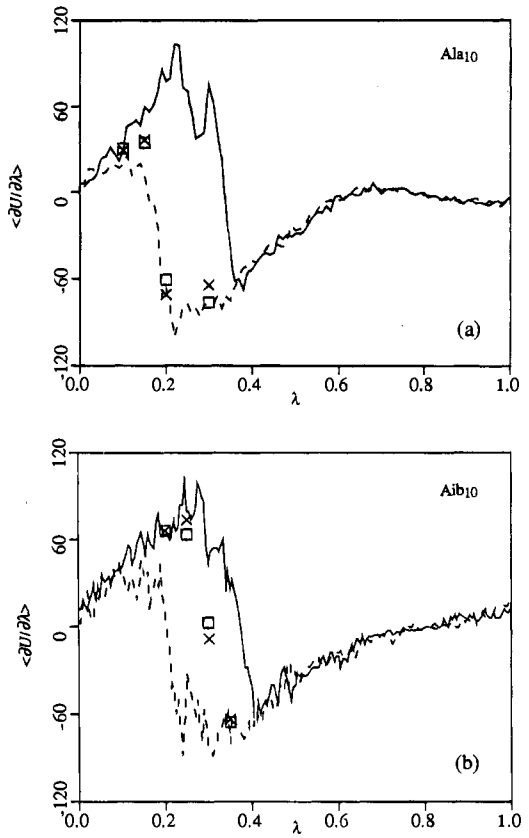

Figure 3. Mean "force", $\langle \partial U/\partial \lambda \rangle$, in free energy calculations of 3_{10}-to-α-helix transitions (averaged over 1 ps intervals in slow-growth calculations, the unit is in kcal/mol): (a) Ala_{10}, (b) Aib_{10}. $\lambda = 0$ corresponds to the 3_{10} helix and $\lambda = 1$ to the α-helix. Solid line: coupling parameter λ increased with time. Dashed line: λ decreased. Mean values of the force at constant values of λ, each over 200 ps, are indicated as squares (starting point was on the dashed curve) and crosses (starting point was on the solid curve).

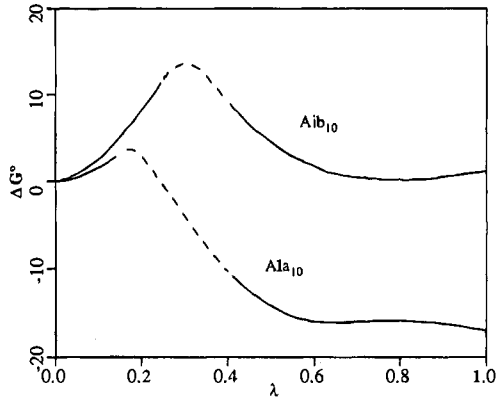

Figure 4. Free energy (in kcal/mol) of 3_{10}-to-α-helix transitions obtained by integration of the data of Figure 3. $\lambda = 0$ corresponds to the 3_{10} helix and $\lambda = 1$ to the α-helix. The solid portions of the curves have been obtained from the intervals in which the curves for increasing and decreasing λ in Figure 3 coincide. The dashed portions have been obtained from transition curves drawn to fit the points indicated by squares and crosses in Figure 3.

rather than less, stable relative to the α-helix. In free dynamics simulations with a flexible model, these authors also observed a spontaneous transition of Ala_{10} from the 3_{10} helix to the α-helix, but within a shorter time period of circa 15 ps.

Smythe et al.[17] calculated the free energy change of the 3_{10} helix/α-helix transition of Aib_{10}. They used dynamics simulations with the AMBER/OPLS potential (also with united atoms)

Table 2. Free Energies for Replacing Ala and Aib from Molecular Dynamics Simulations ($\Delta G°$ and Hysteresis Are Shown in kcal/mol)[a]

conformation	molecule	medium	$\Delta G°$	hysteresis	rmsd[b]
3_{10} helix	Ala_{10}	vacuum	2.0	0.2	0.2
3_{10}	Ala_{10}-G	vacuum	2.1	0.1	0.2
α-helix	Ala_{10}	vacuum	3.5	−0.3	0.2
α-helix	Ala_{10}-G	vacuum	2.5	0.4	0.3
3_{10} helix	Ala_{10}	water	2.5	0.6	0.3
3_{10} helix	Ala_{10}-G	water	3.1	−0.2	0.3
α-helix	Ala_{10}	water	3.7	−0.1	0.1
α-helix	Ala_{10}-G	water	3.7	0.1	0.1
3_{10} helix	Ala_{14}	water	3.2	−0.4	0.2
3_{10} helix	Ala_{14}-G	water	3.1	−0.2	0.3
α-helix	Ala_{14}	water	4.8 5.3[c]	−0.2	0.1
α-helix	Ala_{14}-G	water	3.9	−0.2	0.1
coil state		water	5.9[c]		

[a] These results are from 3 or 4 successive pairs of slow-growth simulations. The free energy values were calculated according to eq 5. [b] Root-mean-square deviation. [c] From Hermans et al.[19]

Table 3. Differences in Helix Propensity ($\Delta\Delta G°_{\alpha\text{-coil}}$ and $\Delta\Delta G°_{3_{10}\text{-coil}}$) for Aib Relative to Ala, Calculated with Eqs 6c and 6b (in kcal/mol)[a]

conformation	molecule	medium	$\Delta\Delta G°$
3_{10} helix	Ala_{10}	water	−3.4
3_{10} helix	Ala_{10}-G	water	−2.8
α-helix	Ala_{10}	water	−2.1
α-helix	Ala_{10}-G	water	−2.2
3_{10} helix	Ala_{14}	water	−2.6
3_{10} helix	Ala_{14}-G	water	−2.8
α-helix	Ala_{14}	water	−0.8
α-helix	Ala_{14}-G	water	−1.9

[a] The minus signs indicate that in all cases introduction of Aib increases helix stability.

to calculate the potential of mean force in various media, defining the reaction coordinate in terms of the helix end-to-end distance, which is shorter in the α-helix and, if controlled, can be used to force a transition from one helix type to the other (an end-to-end distance of 19 Å corresponded to the 3_{10} helix and of 13 Å to the α-helix). They concluded that the α-helical conformation is always favored (12.9 kcal/mol in water, 7.7 kcal/mol in acetonitrile, 3.4 kcal/mol in vacuum).

The results for Ala_{10} reported in this paper are in agreement with those obtained for Ac-Ala_{11}-NMe by Tirado-Rives and Jorgensen,[23] in that both studies find the α-helix to be strongly favored over the 3_{10} helix. Differences in method, rather than differences in the used potentials (OPLS and Cedar), can easily account for the differences in the results. Thus, the use of rigid instead of flexible geometry for the backbone dihedral angles ϕ and ψ will underestimate the large entropic contribution in favor of the 3_{10} helix ($-T\Delta S° = -4.3$ kcal/mol for Ala_{10}, cf. Table 1). Also, as has already been noted by Tirado-Rives and Jorgensen, selection of a value of the backbone dihedral (ϕ) of −60° produces a geometry for the 3_{10} helix in which the backbone can interact well with the solvent; this interaction is less when the model finds the most stable conformation (which is, for our model, at ϕ near −40°), and this is probably the reason why the effect of hydration is opposite in the two studies. (Hydration favors the 3_{10} helix in their study and the α-helix in ours.)

The results obtained here for Aib_{10} differ more dramatically from those obtained by Smythe et al.:[17] that study found the α-helix to be very much more stable in water, whereas our results indicate that the two helix types are equally stable; *in vacuo* the studies conclude in favor of different conformations.

In large part, this difference is probably due to differences in behavior with all-atom and united-atom potentials. This conclusion is based on observations of conformational energies, in which solvent effects were dealt with by various treatments of the dielectric constants; with AMBER united-atom potentials, Aib_{10} was found to favor the α-helical conformation,[13] but with AMBER all-atom potentials, it was found to favor the 3_{10}-helical conformation.[15] Comparison of free energies calculated with CEDAR all-atom and united-atom potentials (Table 1) confirms the direction and magnitude of this effect. The difference between these two kinds of potentials will show up strongly only when methyl or methylene groups are in close contact; it is probably significant here because of the presence of unfavorable methyl−methyl contacts in the 3_{10} helices of Aib, *cf.* discussion below. We note that the results reported in *this* paper have been obtained with the inherently more accurate all-atom potential, with flexible geometry (except for bond lengths), and with use of exactly the same methods for oligomers of alanine as for oligomers of Aib.

Until very recently it had been generally assumed that amino acid oligomers rich in Ala content, when helical, would, except maybe at the helix ends, assume the α-helical conformation, regardless of the kind of solvent, as well as in crystal.[8] This view has been challenged in a series of papers[9-11] in which it has been proposed, on the basis of ESR measurements, that certain peptides with high alanine content, first made and studied by Marqusee and Baldwin,[29] actually assume a 3_{10}-helical conformation. As has already been pointed out by Tirado-Rives et al.,[23] the free energy difference between the α-helix and the 3_{10} helix of alanine oligomers in simulations enormously favors the former and much exceeds the limits of uncertainty inherent in the computer model. In free dynamics simulations of the Ala_{10} helix in water, little formation of 3_{10}-helical hydrogen bonds is observed (Figure 2), again at odds with the proposal that these oligomers assume partial 3_{10}-helical structures.[11]

Differences in Helix Propensity ($\Delta\Delta G°$). The high relative propensity of Aib for formation of the 3_{10} helix has been known from experimental studies. Nevertheless, the difference with alanine found here by simulation is exceptionally large ($\Delta\Delta G°_{3_{10}\text{-coil}} = -2.6$ kcal/mol, while $\Delta\Delta G°_{\alpha\text{-coil}} = -0.57$ kcal/mol; the discussion focuses on the results obtained for hydrated molecules). It is almost as large as the difference in propensity between alanine and proline to form α-helix, where proline lacks the peptide hydrogen atom needed to form the helical hydrogen bond, and instead of it has a CH_2 group that can be accommodated if a kink occurs in the helix.[18] The difference in refolding propensity, $\Delta\Delta G°_{3_{10}\text{-}\alpha}$, equal to $\Delta\Delta G°_{3_{10}\text{-coil}} - \Delta\Delta G°_{\alpha\text{-coil}}$, is -2 kcal/mol. This is consistent with our finding that for Aib_{10} the free energy for the $3_{10}-\alpha$ equilibrium differs by −16 kcal/mol from that for Ala_{10} (cf. Table 1).

One wishes to understand why Aib's relative propensity for formation of one helix type is so high, and also why that of a very closely related helix type is so much lower. The high 3_{10} helix propensity of Aib has been attributed to the limited conformational freedom of this residue.[30] This plays a role, because the propensity is measured from the basis of the random-coil state, and because a residue with high conformational freedom will have lower free energy (higher entropy) in the coil state, and therefore a lower propensity to form a folded structure, *a priori*, to form *any* folded structure. The conformational freedom of a residue can be assessed from a study of the terminally blocked residue (dipeptide); the requisite conformational probabilities of dipeptides of glycine, alanine, and Aib in aqueous systems have been calculated in earlier papers from this laboratory and reported in terms of free energy maps.[19,31] A comparison shows that the probability distributions of these three residues are successively more restricted. The difference between alanine and Aib is significant; however, the symmetry of Aib, so to speak, allows each distinct free energy minimum to occur twice, and this lessens the difference. From the computed probability distributions one estimates that the reduced conformational freedom of Aib can account for only a part of the high propensity.

A comparison of the dipeptide maps of alanine[31] (cf. Figure 2 in the paper) and Aib[19] (Figure 3 in the paper) shows a second major effect that may not earlier have been identified as contributing to Aib's high helix propensity. This effect is related to the relative depth of the free energy minima. For the alanine dipeptide, the lowest free energy minimum is for the β-conformation ($\phi \approx -110°$, $\psi \approx -120°$), and the minimum for what we have called the α_R-conformation ($\phi \approx -100°$, $\psi \approx -60°$) is between 1.2 and 2.4 kcal/mol higher in free energy. The backbone conformation of the α-helix lies in the region of this free energy minimum, but it is at least another 0.48 kcal/mol higher in free energy. The Aib dipeptide has four free energy minima of nearly equal free energy, one of which is for the α_R-conformation, at $\phi = -48°$, $\psi = -45°$, which is close to the α- and 3_{10}-helical backbone conformations. Thus, in order to become part of a helix, alanine residues must, nearly always, first assume a less stable conformation, something which is not the case for Aib. The free energy required to change alanine's conformation is in addition to the contribution that is caused by the difference in conformational freedom. By taking into account also the second effect, the −2.6 kcal/mol magnitude of $\Delta\Delta G°$ for the 3_{10} helix is reasonably well accounted for.

Nevertheless, the above argument produces a quandary, as for the α-helix the difference in propensity for Aib and alanine is much smaller (0.5−1.0 kcal/mol). Indeed, in a free-energy map of the Aib residue, the conformations preferred in the α-helix and the 3_{10} helix are equally favorable; on this basis, Aib is expected to show an equally strong preference for the 3_{10} helix and the α-helix. This problem has been solved by the identification of a contact which destabilizes the Aib residue in the α-helix and is not present in the 3_{10} helix.

By inspection of molecular models of the α-helix and the 3_{10} helix a possible close contact is found between the second β-methyl group of an Aib residue at position i and the β-methyl group of Ala at position $i + 3$, and this is unique to the α-helix. This bad contact has also been suggested by Marshall et al.[32] In order to investigate the contribution of this contact to the discrepancy between the differences in helix propensity of Ala and Aib for the two helix types, the replacements have been repeated with sequences containing a glycine residue at position $i + 3$ (Table 3). One sees that the differences in propensity for α-helix and 3_{10} helix are much more similar when a glycine residue is present at position $i + 3$.

Context Dependence. Context dependence can cause large differences in helix propensity for the same residue type. These differences can be exploited in order to construct more stable oligomers. For example, negatively charged residues near the amino end and positively charged residues near the carboxyl end can stabilize the helix by interactions with its dipole, and a judiciously placed pair of negatively and positively charged side chains can stabilize the helix by formation of a salt bridge.[29]

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Context dependence of helix propensity can also be a source of confusion. For example, the context of the early host–guest experiments,[33] in which the host residues were dihydroxypropyl and dihydroxybutyl esters of glutamic acid, is rather different from the context in more recent experiments[20,22] with oligomers containing only amino acids found in proteins with much shorter side chains. The differences in helix propensity observed in the former experimental system are systematically lower. Thus, different experimental systems can produce different estimates of relative helix propensities.

In this study, context dependence of the relative α-helix propensity of Aib and Ala has shown up in two instances. The first is the dependence of the relative propensity on the chain length of the oligomer. Apparently, the Ala_{10} molecule can adjust its conformation and relieve one or more bad contacts between Aib at position 5, and other residues, while this relief is much less or absent when the Aib residue is more distant from both ends of the helix (at position 8 in Ala_{14}). The second is the difference in relative propensity when a glycine is present in position $i + 3$ (position 8 in Ala_{10}-G; position 11 in Ala_{14}-G). This calculation has served to identify the nature of, at least, the most severe bad contact. Obviously, Aib residues at or near the C-terminus of an α-helical peptide also do not have this bad contact and, by implication, might be able to stabilize the helix (over alanine residues in the same positions). Such a stabilization would be an advantage in experimental studies of helix formation in synthetic peptides.

The effect of combining an Aib residue at position i and a glycine residue at position $i + 3$ is, in principle, accessible to experimental investigation. The results obtained here can be restated as follows: the replacement, in a peptide containing an Aib residue at position i, of an Ala residue at position $i + 3$ (or another type of L-amino acid residue with relatively high α-helix propensity) with a glycine residue should not cause the helical stability of that peptide to decrease, in contrast to what is normally expected. ($\Delta\Delta G°_{\alpha\text{-coil}}$ for glycine in an all-alanine model is +1.2 kcal/mol.[19]) Such a result would be supportive evidence that the peptide in question assumed an α-helical and not a 3_{10}-helical conformation.

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