On the Stability of Cis and Trans Amide Bond Conformations in Polypeptides

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Abstract: The intramolecular energies and entropies of the randomly coiling conformations of poly-L-alanine are calculated as functions of the cis or trans character of the amide bond and the geometry of the peptide group. Intrinsic torsional and nonbonded van der Waals potentials and dipolar electrostatic interactions which depend on the cis or trans character of the amide bond, the conformations about the N–C^{α} (φ) and C^{α}–C (ψ) backbone bonds, and the geometry of the peptide group are considered. When only those interactions dependent upon a single pair of residue rotation angles φ and ψ are considered, a single isolated cis amide bond in randomly coiling poly-L-alanine is found to be as stable or as favorable as trans amide bond conformations. However, when interactions dependent upon the residue rotations φ and ψ in more than a single residue are accounted for, the all-trans amide bond conformations are favored by *ca.* 2.0 kcal/mol of residue over those conformations with a single cis amide bond. Thus, the absence of observed cis amide bonds in polypeptides and proteins appears to have its origin in nonbonded intramolecular interactions spanning more than a single pair of residue rotations φ and ψ. The same potential functions were employed in the calculation of the energy difference between the cis and trans conformations of *N*-methylformamide and *N*-methylacetamide. The trans conformer of *N*-methylacetamide is found to have an energy lower than the cis conformer by 1.6 kcal/mol in agreement with experiment. However, the cis conformer of *N*-methylformamide is predicted to have an energy 1.5 kcal/mol lower than the trans conformer in opposition to experimental observation. Similar disparities have been noted between the observed and calculated energy differences when approximate quantum mechanical methods were employed.

The partial double bond character[1] of the CO–NH bond results in a planar conformation of the amide groups in polypeptides and proteins. In addition to planarity, the amide bonds in linear or open-chain polypeptides and in proteins adopt the trans conformation exclusively[2-4] (see Figure 1). Only in the N-substituted peptide residues proline, sarcosine, *N*-methylalanine, etc., where the peptide bond is an imide bond, is the presence of the cis conformation observed.[5-8]

The simplest analog of the amide bond in polypeptides is *N*-methylacetamide whose preference for the trans conformation by at least 2 kcal/mol over the cis conformation has been established by La Planche and Rogers[4] using nuclear magnetic resonance evidence. In the same study[4] the trans conformer of *N*-methylformamide was found to be preferred by *ca.* 1.5 kcal/mol. Evidence of a similar nature for N,N-disubstituted acetamide (the simplest analog of the imide bonds in N-substituted peptide residues) is lacking, thereby precluding any comparison that might be made between the intrinsic stability of the cis and trans conformers in the mono- and di-N-substituted amides or by extension to the comparison of the stability of cis and trans amide and imide bonds in polypeptides.

The present study seeks to determine whether or not the preponderant preference for trans amide bonds in polypeptides can be rationalized solely in terms of nonbonded intramolecular interactions apart from the intrinsic contributions as measured for the simple amides and mentioned above. To realize this objective, approximate potential energy calculations are performed on L-alanine polypeptides as a function of the cis or trans character of the amide bonds. In addition, the same potential functions are used in the calculation of the energy difference between the cis and trans conformers of *N*-methylformamide and *N*-methylacetamide.

Description of the Calculations. The calculation of the conformational energies of random coil poly-L-alanine is accomplished by employing semiempirical potential functions[9,10] which partition the intramolecular conformational energy into separate contributions resulting from the intrinsic torsional potentials, the nonbonded van der Waals interactions, and the electrostatic interactions between dipolar amide groups. The potential functions used by Brant, Flory, *et al.*,[9-11] are adopted. In the first approximation, only those interactions between atoms and groups dependent on a single pair of residue rotation angles φ_2 and ψ_2 (see Figure 2) and the cis or trans character of the two intervening amide bonds are considered. The usual Pauling–Corey (P–C) geometry[12] of the trans peptide group is adopted and assumed to be rigid. The geometry proposed by Ramachandran and Venkatachalam[13] (R–V) for the cis peptide group is adopted and compared to the results calculated using P–C geometry for the cis-peptide group. [The R–V and P–C geometry differ in the values of the valence angles at N and C′ (carbonyl); $\angle C^{\alpha}C'N$ = 114 (P–C) and 118° (R–V), $\angle C^{\alpha}C'O$ = 121 (P–C) and 119° (R–V), $\angle C'NC^{\alpha}$ = 123

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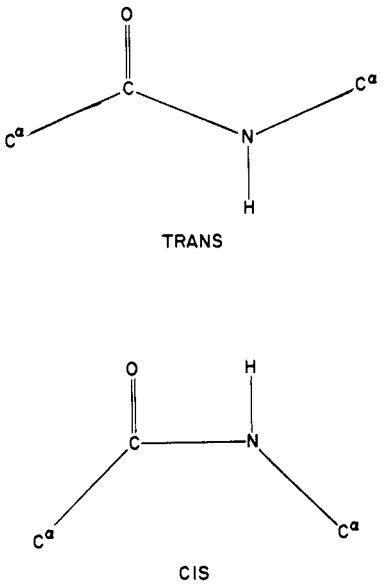

Figure 1. A schematic representation of the cis and trans conformers of the amide bond in polypeptides.

(P–C) and 126° (R–V), and ∠C′NH = 123 (P–C) and 121° (R–V).]

All of the parameters (intrinsic torsional barriers, partial charges, dielectric constant, etc.) required in the potential energy calculation are taken from Brant, Flory, *et al.*[9–11] The intrinsic torsional potential about the amide bond is neglected (equal inherent torsional energies are assigned to the cis and trans conformers of this bond) and the same partial charges on the atoms of the amide group are retained for both cis and trans conformers based on the calculations of Yan, *et al.*[14]

The rotation angles φ_2 and ψ_2 are varied first in 30° and then in 10° increments. For both calculations the peptide unit partition function, average conformational energy, entropy, and free energy are evaluated at 25° following Brant, Miller, and Flory.[10] Free energy calculated in this manner is used as the criterion of stability.

In the second approximation, the calculations described above are repeated with the inclusion of all the interactions between atoms and groups which depend on two φ and two ψ rotations ($\psi_1, \varphi_2, \psi_2, \varphi_3$; see Figure 3) as well as on the cis or trans character of the intervening amide bonds. Only those values of ψ_1 and φ_3 which correspond to energetically favorable conformations according to the calculated results obtained assuming independent residue rotations and energies (the first approximation mentioned above) are used. Comparison of these two sets of conformational calculations provides a measure of the relative importance of longer range intramolecular interactions to the stability of cis and trans amide bonds in polypeptides. (Similar semiempirical calculations[15–17] have been conducted on proline and proline derivative oligomers.)

In addition, the energies of the cis and trans conformers of *N*-methylformamide and *N*-methylacetamide are also evaluated for purposes of comparison.

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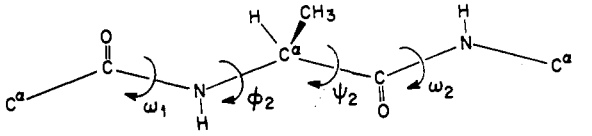

Figure 2. A portion of a planar zigzag poly-L-alanine chain including all atoms and groups whose distance of separation depends on a single residue rotation(s), *i.e.*, φ_2 and/or ψ_2.

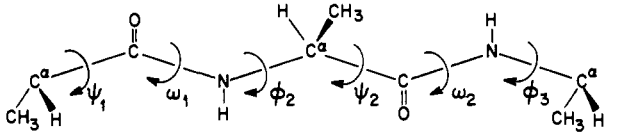

Figure 3. A portion of a planar zigzag poly-L-alanine chain including all atoms and groups whose distance of separation depends on more than a single residue rotation(s); *i.e.*, they depend on $\psi_1, \varphi_2, \psi_2, \varphi_3$.

Results and Conclusions

The average conformational energy, $\langle V \rangle$, the entropy, S, and the free energy, $A = \langle V \rangle - TS$, each evaluated at 25° assuming independent residue rotations (see Figure 2), are presented in Table I. Table II contains the same quantities calculated with inclusion of all interactions between atoms and groups whose distance of separation depends on one or more neighbor dependent residue rotations (see Figure 3). The most striking observation resulting from a comparison of the results in Tables I and II is the reversal in stability of the all-trans and -cis peptide bond containing conformers,

Table I. Calculated Conformational Characteristics of Cis and Trans Peptide Bond, Random Coil Poly-L-alanine Assuming Independent Residue Rotations

Conformer[a]	(φ_2, ψ_2) rotational increments, deg	$\langle V \rangle$, kcal/mol	S, cal/(°K mol)	A, kcal/mol
Trans–trans	30	1.40	5.94	−0.37
Trans–cis	30	0.95	5.82	−0.79
Cis–trans	30	1.03	5.85	−0.72
Cis–cis	30	0.26	5.29	−0.95
Cis–cis[b]	30	4.08	5.02	2.58
Trans–trans	10	1.35	10.3	−1.72
Trans–trans[c]	10	0.66	10.4	−2.45
Cis–cis	10	0.32	9.51	−2.51

[a] All-trans peptide bonds have Pauling–Corey (P–C) geometry[12] and all-cis peptide bonds have Ramachandran–Venkatachalam (R–V) geometry[13] unless noted otherwise. [b] P–C geometry. [c] R–V geometry.

Table II. Conformational Characteristics of Cis and Trans Peptide Bond, Random Coil Poly-L-alanine Calculated for Neighbor Dependent Residue Rotations

Conformer[a]	$\langle V \rangle$, kcal/mol	S, cal/(°K mol)	A, kcal/mol
Trans–trans	5.40	9.06	2.70
Trans–cis	7.43	9.26	4.67
Cis–trans	5.93	5.38	4.33
Cis–cis	7.67	5.86	5.92

[a] All-trans peptide bonds have P–C geometry, and all-cis peptide bonds have R–V geometry. The residue rotations ψ_1, φ_2, ψ_2, φ_3 were varied in 30° increments.

as longer range interactions dependent upon more than one pair of residue rotations are included. More specifically, the cis peptide bond conformers become disfavored relative to the all-trans peptide bond conformers when the neighboring residue-dependent, longer range interactions are considered. This reduction in the stability of cis amide bond conformations relative to trans conformers is a result of the fact that some of the low-energy, independent residue conformations (φ_2, ψ_2) (see Figure 2) with cis amide bonds become prohibited when interactions depending on ψ_1 and/or φ_3 as well as on (φ_2, ψ_2) are included (see Figure 3). At the same time, none of the low-energy, independent residue conformations (φ_2, ψ_2) with trans amide bonds are found to be prohibited when interactions also dependent upon ψ_1 and/or φ_3 are considered. Although excessive computer time requirements prohibit a complete study, exploratory calculations of the conformational energy including interactions still longer in range than those considered in Figure 3 indicate a further stabilization of the all-trans conformers at the expense of the cis peptide bond conformers.

Thus, it appears that aside from any inherent torsional preference for trans amide bonds[4] the intramolecular nonbonded interactions in amide bond polypeptides appear to account for the absence of observed cis amide bond conformations. However, when these same potential functions are used to estimate the energy difference between the cis and trans conformers of *N*-methylformamide and *N*-methylacetamide, the results presented in Table III are obtained. The calculated preference for the trans conformer in *N*-methylactamide is in agreement with experiment,[4] but the calculated preference for the cis conformer in *N*-methylformamide is in opposition to the observed[4] preference for the trans conformer. Two approximate quantum mechanical methods, the EHT and SCF–MO (CNDO/2) theories, have recently[14] been used to calculate the conformational energies of some simple amides including *N*-methylformamide and *N*-methylacetamide. For *N*-methylformamide the trans conformer is favored by 0.09 kcal/mol according to the EHT theory and by 1.18 kcal/mol by the CNDO/2 theory. The trans conformer of *N*-methylacetamide is favored by 2.92 kcal/mol in the EHT theory and is 0.09 kcal/mol higher in energy than the cis conformer according to the CNDO/2 theory. Consequently, while the semiempirical (present work) and EHT calculations[14] correctly predict the stability of *trans*-*N*-methylacetamide and the CNDO/2 method[14] correctly predicts the stability of *trans*-*N*-methylformamide, none of the three different calculations correctly predicts the observed[4] inherent preference for the trans conformer in both of these simple amides.

Table III. Conformational Energies of the Cis and Trans Conformers of *N*-Methylformamide and *N*-Methylacetamide

Amide	Conformer[a]	*V*, kcal/mol
N-Methylformamide	Trans	−2.36
	Cis	−3.92
	Cis[b]	−3.33
N-Methylacetamide	Trans	−1.48
	Cis	0.12
	Cis[b]	5.48

[a] The P–C and R–V geometries are used for the trans and cis amides, respectively, unless noted otherwise. [b] P–C geometry.