Stability of Cis, Trans, and Nonplanar Peptide Groups[1]

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ABSTRACT: Conformational energy calculations using ECEPP (Empirical Conformational Energy Program for Peptides) were performed on the molecular fragment $C^{\alpha}_1C'ONHC^{\alpha}_2$, on *N*-methylacetamide, and on several peptide molecules including *N*-acetyl-*N'*-methylglycineamide (Gly single residue), *N*-acetyl-*N'*,*N'*-dimethylglycineamide, and *N*-acetyl-*N'*-methylamide dipeptides of Gly-Gly and Gly-Pro. Energy minimization was carried out with peptide groups taken in both the cis and trans conformations, and the librational entropy and conformational free energy were determined at each minimum. It was found that the instability of cis in Gly-Gly comes primarily from interactions of the C^{α}_1 and $H_{C^{\alpha}_1}$ atoms with the C^{α}_2 and $H_{C^{\alpha}_2}$ atoms, and also from avorable interactions present in the trans form which are disallowed in the cis form, and from conformational entropy. The instability of cis in Gly-Pro is much less than in Gly-Gly because unfavorable interactions of the type $C^{\alpha}H\cdots C^{\alpha}H$ present in the cis conformation of Gly-Gly are present in *both* the cis and trans forms of Gly-Pro. The instability of cis in Gly-Pro arises mainly from the change in electrostatic energy caused by the restricted rotation about the $N-C^{\alpha}$ bond of Pro. Entropy accounts for about 0.5 kcal/mol of the instability of cis in Gly-Pro compared with about 1.5 kcal/mol in Gly-Gly. The calculated fraction (4%) of cis in Gly-Pro is in good agreement with the experimental value (5%) for related peptides in nonpolar solvents. When the dihedral angle ω of the central peptide bond in these dipeptides is allowed to vary during energy minimization, the deviations from planarity are only 1–3° in low-energy minima of Gly-Gly but as much as 10° in Gly-Pro. A comparison of these results with calculations in which the peptide bond was held fixed in the planar trans conformation shows that conformation-dependent properties of blocked dipeptides can be represented adequately without allowing ω to vary.

Theoretical[3-6] and experimental[3b,7-11] studies on peptides have shown that, in general, the peptide group is more stable in the trans than in the cis conformation, but that the cis conformation can be observed in *N*-substituted peptide groups, as in compounds with X-Pro or X-Sar peptide bonds. Recently, much discussion[12-20] has centered on the question of whether or not the peptide group is planar, as was originally assumed in the Pauling–Corey–Branson model[21,22] and still assumed in many theoretical studies.[23,24]

The purposes of this work are (a) to calculate the relative stabilities of cis and trans conformations in model peptide systems, using empirical conformational energy parameters,[25] and to compare these results with those from other theoretical and experimental studies, (b) to ascertain the origin of the relative cis–trans stability, (c) to determine the degree of nonplanarity in the peptide unit in blocked dipeptides, and (d) to assess the validity of the assumption of planar trans peptide groups in conformational analysis.

Methods and Definitions

The nomenclature and conventions used here are those adopted by an IUPAC-IUB Commission.[26]

Conformational Energy Calculations. Conformational energy calculations were carried out using ECEPP (Empirical Conformational Energy Program for Peptides) available from the Quantum Chemistry Program Exchange.[27] The empirical potential energy functions and energy parameters are those described earlier.[25] The total conformational energy E_{tot} is the sum of the electrostatic energy E_{el}, nonbonded energy E_{nb}, and torsional energy E_{tor}.[25] The partial atomic charges of NMA were taken from the standard data[25] for the $NHCOCH_3$ *N*-terminal end group and the $CONHCH_3$ *C*-terminal end group. The hydrogen-bond energy E_{HB} is included in the nonbonded energy component.[25] The torsional energy $U(\omega)$ for rotation about peptide bonds is given by

$$U(\omega) = (U_{\omega}/2)(1 - \cos 2\omega) \quad (1)$$

where U_{ω} is set at 20 kcal/mol,[8,25,28] and ω is the dihedral angle. The conformational energy is expressed as $\Delta E = E_{tot} - (E_0)_g$, where $(E_0)_g$ is the value of E_{tot} at the global minimum for the particular compound.

Geometry. The bond lengths and bond angles of the model peptide unit (Figure 1) are the same as those of *N*-methylacetamide (NMA) shown in Figure 2. The geometry of NMA was taken from standard amino acid residue and end group data given in Tables I, X, and XI of ref 25; in particular, the $C^{\alpha}-C'$ bond length was taken as 1.53 Å (as in Table I of ref 25; also, see Table VIII of this paper) instead of 1.49 (of Table X of ref 25), the latter value applying to the acetyl end group of *N*-acetyl-*N'*-methylprolineamide.

In the blocked dipeptide *N*-acetylglycylproline-*N'*-methylamide (Gly-Pro), the Pro residue was taken in the L configuration and in the puckered "down" conformation.[25,29] The standard residue data for ECEPP,[25] derived from x-ray and neutron diffraction structures of peptides and model compounds, were used throughout this study without modification. The length of the peptide bond is 1.325 Å (see Figure 2) except in an X–Y unit which has a substituent on the N atom of Y (e.g., in Gly-Pro and in the *N'*,*N'*-dimethylamide end group), in which case the value is 1.36 Å.[25] When the X-Pro peptide bond is cis, the bond angle $\tau(C'NC^{\alpha})$ is 124.0° rather than 121.0° as in the trans form (see Figure 2), and the value of $\tau(C'NC^{\delta})$ becomes 121.0° rather than 124.0°.[25]

Grid of ϕ–ψ Space of Gly. *N*-Acetyl-*N'*-methylglycineamide contains two peptide bonds which, in principle, can exist as both trans (tt), one trans and one cis (tc and ct), or both cis (cc). All four possibilities were considered, and the conformational energy of Gly (for each of these sets of conformations of the peptide groups) was calculated at 10° intervals over the whole ϕ–ψ space, from which energy contour plots were drawn. The energy contour map of *N*-acetyl-*N'*,*N'*-dimethylglycineamide was determined in the same manner, where the first peptide bond was taken as planar trans; the second peptide unit, being symmetrically substituted at the nitrogen, has no cis–trans isomerism.

Energy Minimization. The conformational energy of NMA was minimized from various starting points, $\psi(C^{\alpha}_1-C')$, $\omega(C'-N)$, and $\phi(N-C^{\alpha}_2)$ (see Figure 1 for nomenclature). Blocked dipeptides (see Figure 3) of Gly-Gly and Gly-Pro were analyzed by energy minimization, with ω (corresponding to the peptide bond connecting the two residues) being taken initially in both the trans (180°) and cis (0°) forms. Starting points consisted of all combinations of single residue minima, trans minima being taken from Lewis et al.[30] and cis minima

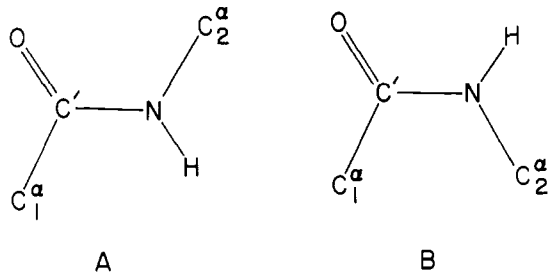

Figure 1. Peptide fragment in (A) trans and (B) cis conformations.

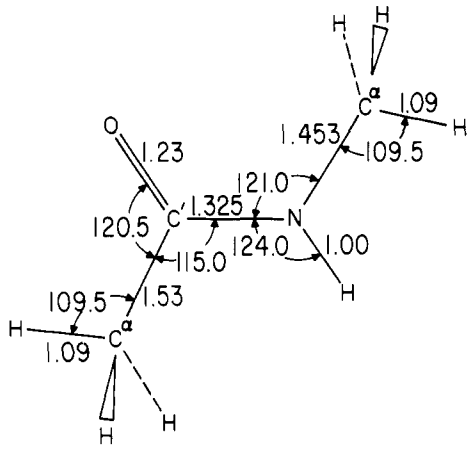

Figure 2. Geometry of *N*-methylacetamide. Bond lengths are in Å and bond angles in degrees.

being taken from the results of the ϕ–ψ maps of blocked Gly in the tc and ct state (see Figures 4B and 4C). The two end peptide bonds were fixed at 180°.

During minimization, which was carried out using the algorithm of Powell,[31] ϕ, ψ, and ω of NMA, ϕ_{i+1}, ψ_{i+1}, ω_{i+1}, ϕ_{i+2}, and ψ_{i+2} of Gly-Gly, and ϕ_{i+1}, ψ_{i+1}, ω_{i+1} and ψ_{i+2} of Gly-Pro were allowed to vary. The peptide bonds connecting each end group to the dipeptide residues and the dihedral angles for rotation about the end methyl groups were held fixed at 180°.

Definitions of Conformation-Dependent Quantities. The probability of occurrence (or mole fraction) of a particular ith dipeptide conformational energy minimum is approximated by (a) the quantity v_i, defined by

$$v_i = [\exp(-\Delta E_i/RT)]/Q \tag{2}$$

where the function Q, which can be treated as a partition function, is given by

$$Q = \sum_{i=1}^{n} \exp(-\Delta E_i/RT) \tag{3}$$

where ΔE_i is the difference between the conformational energy at the ith minimum and that at the global minimum, n is the number of minima, and RT is the ideal gas constant times temperature (in this study, T = 300 K); and (b) the quantity w_i, defined by[32,33]

$$w_i = [(2\pi RT)^{m/2}(\det \mathbf{F}_i)^{-1/2} \exp(-\Delta E_i/RT)]/Z \tag{4}$$

where m is the number of variables (degrees of freedom), $\mathbf{F}_i$ is the matrix of second derivatives[32] at the ith minimum, and the partition function Z is given by

$$Z = (2\pi RT)^{m/2} \sum_{i=1}^{n} (\det \mathbf{F}_i)^{-1/2} \exp(-\Delta E_i/RT) \tag{5}$$

The **F** matrix was computed numerically using a double precision version of ECEPP. Since the intervening barriers are not excessively high, all minima in the sums in eq 3 and 5 are accessible to each other.[34] The librational entropy s_i of the ith minimum is given by[32]

$$s_i = -(R/2) \ln (\det \mathbf{F}_i) + (Rm/2)[1 + \ln (2\pi RT)] \tag{6}$$

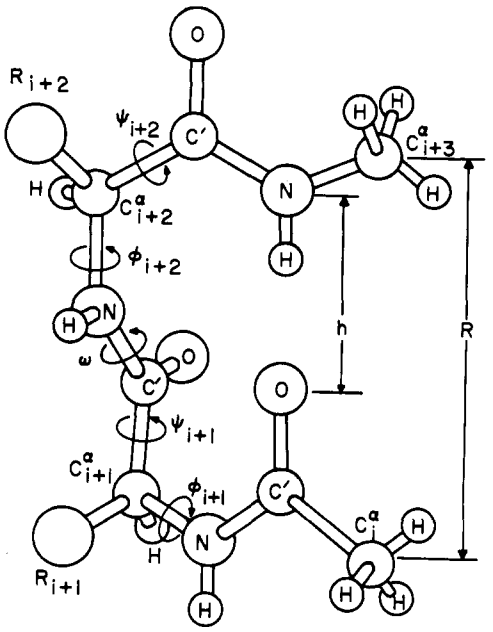

Figure 3. Diagram of *N*-acetyl-*N*′-methylamide dipeptides, showing the definition of the $C^{\alpha}_i \cdots C^{\alpha}_{i+3}$ distance R and of the $O_i \cdots N_{i+3}$ distance h.

The quantity h is defined as the distance between O_i and N_{i+3} (see Figure 3) in blocked dipeptides; R is the distance between C^{α}_i and C^{α}_{i+3}. The partition functions Q and Z are used to determine average quantities such as $\langle h \rangle_Q$, $\langle h \rangle_Z$, $\langle R \rangle_Q$, and $\langle R \rangle_Z$ defined by the general equation

$$\langle A \rangle_X = \sum_{i=1}^{n} A x_i \tag{7}$$

where A is h or R, x_i is v_i or w_i, and X is Z or Q. Similarly, the probabilities of occurrence of a bend, $P_{Q,b}$ and $P_{Z,b}$, are defined by

$$P_{X,b} = \sum_{i=1}^{l} x_{i,b} \tag{8}$$

where $x_{i,b}$ is the quantity v_i or w_i for the ith bend structure, a bend being defined as a conformation in which $R \leq 7$ Å (see Figure 3), and l is the number of energy minima which are bends. The quantities in eq 7 and 8 have found application in the general analysis of dipeptides.[35]

Quantities of more direct application to the study of cis–trans isomerism are the probabilities $P_{Q,cis}$ and $P_{Z,cis}$ of occurrence of the cis conformation, defined as follows:

$$P_{X,cis} = \sum_{i=1}^{k} x_{i,cis} \tag{9}$$

where $x_{i,cis}$ is the quantity v_i or w_i for the ith cis minimum-energy structure, k is the number of cis minima and, as before, X is Q or Z. $P_{Z,cis}$ is used to calculate the free energy change $\Delta G_{trans \rightarrow cis}$ for conversion of trans to cis,

$$\Delta G_{trans \rightarrow cis} = G_{cis} - G_{trans} = -RT \ln [P_{Z,cis}/(1 - P_{Z,cis})] \tag{10}$$

The entropy change $\Delta S_{trans \rightarrow cis}$ is given by

Table I
Differences in Interaction Energies in Trans and Cis Conformations of the Peptide Unit[a]

Interaction	ΔE_{el}[b]	ΔE_{nb}[c]	$\Delta E_{trans \rightarrow cis}$
$C^{\alpha}_1 \cdots H$	0.31	−0.18	0.13
$C^{\alpha}_1 \cdots C^{\alpha}_2$	−0.11[d]	1.74	1.63
O···H	−0.74	−0.14[e]	−0.88
$O \cdots C^{\alpha}_2$	0.25	−0.30	−0.05
Total	−0.29	1.12	0.83

[a] $C^{\alpha}_1C'ONHC^{\alpha}_2$; see Figure 1. [b] $\Delta E_{el} = E_{el,cis} - E_{el,trans}$ in kcal/mol. [c] $\Delta E_{nb} = E_{nb,cis} - E_{nb,trans}$ in kcal/mol. [d] See Figure 3B of ref 42 for the (opposite) charges on the end carbon atoms of NMA. [e] The nonbonded energy for this interaction was calculated using the hydrogen-bond function described in ref 25.

$$\Delta S_{trans \rightarrow cis} = S_{cis} - S_{trans} = \sum_{i=1}^{k_{cis}} [w_i' s_i - R w_i' \ln w_i']_{cis} - \sum_{i=1}^{k_{trans}} [w_i'' s_i - R w_i'' \ln w_i'']_{trans} \quad (11)$$

where k_{cis} and k_{trans} are the number of cis and trans conformations, respectively, and s_i is given above by eq 6. The statistical weight w_i' (or w_i'') is defined similar to w_i in eq 4, except that it is normalized over only those conformational states in which the peptide bond is cis (or trans).

Table II
Location and Energy of Lowest Minima of Blocked Gly in tt, tc, ct, and cc States[a]

State	Location ϕ, deg	Location ψ, deg	ΔE,[b] kcal/mol
tt	−83	76	0.0
tc	180	180	6.54
ct	180	60	7.32
cc	180	180	13.32

[a] Conformation of the two peptide bonds. t = trans, c = cis. [b] ΔE is the energy with respect to the tt global minimum, for which $(E_0)_g = -4.71$ kcal/mol.

Results

The Peptide Group. The changes in the individual interaction energies between atoms within the peptide group upon going from trans to cis (see Figure 1) are given in Table I. In this molecular fragment, the trans conformation is more stable by a total energy $\Delta E_{trans \rightarrow cis}$ of 0.83 kcal/mol. The largest term which contributes to the destabilization of cis is ΔE_{nb} of the

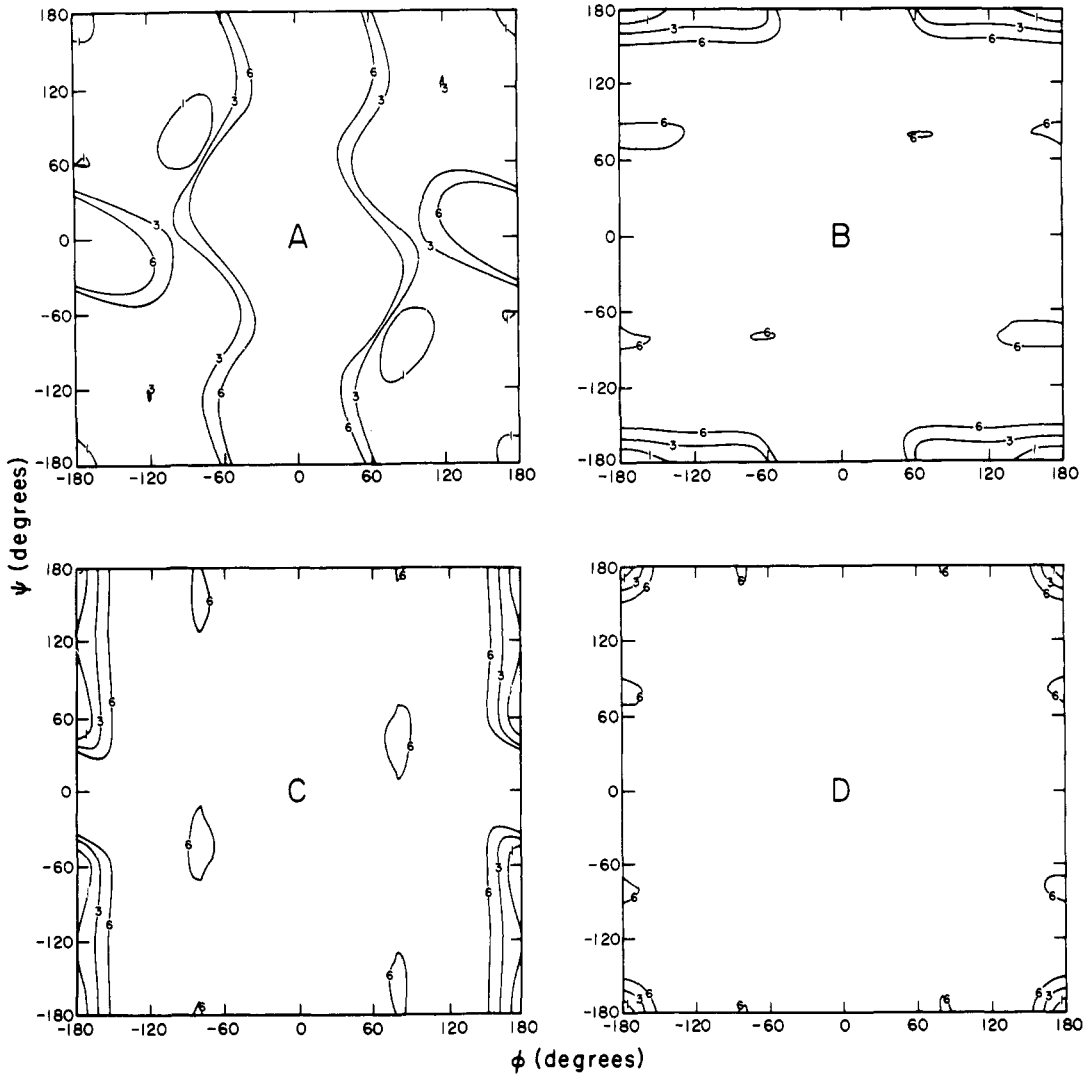

Figure 4. ϕ–ψ contour maps of the blocked Gly residue. The contours are in kcal/mol above the minimum-energy point on each individual map. The two peptide bonds are fixed in the states (A) trans–trans, (B) trans–cis, (C) cis–trans, and (D) cis–cis.

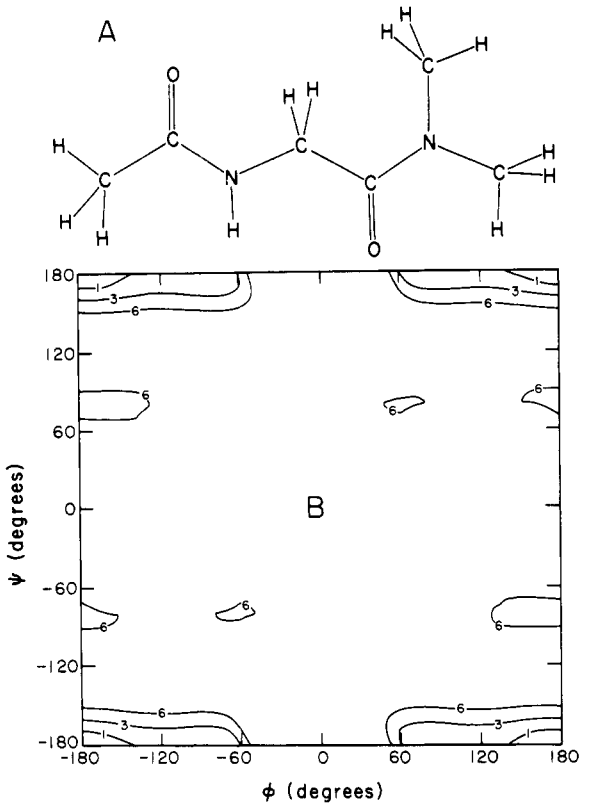

Figure 5. (A) Structure of *N*-acetyl-*N'*,*N'*-dimethylglycineamide and (B) its ϕ–ψ energy contour map. The first peptide bond is planar trans; the second peptide bond is planar but, since the *N* is symmetrically substituted, cis and trans are equivalent.

$C^{\alpha}_1 \cdots C^{\alpha}_2$ interaction, which is 1.74 kcal/mol. All other nonbonded interaction energy differences are slightly negative, leaving the total ΔE_{nb} = +1.12 kcal/mol. It should be noted that the difference in the total electrostatic energy is negative (−0.29 kcal/mol).

From an analysis of this molecular fragment, made up of the six atoms which constitute a peptide unit, the conclusion can be drawn that in any peptide molecule, in which there is no substituent on the N atom, the trans conformation is favored over the cis by about a 1 kcal/mol contribution from this fragment.

However, this molecular fragment is an incomplete model for cis–trans isomerism because, first, it has a net charge (in the ECEPP treatment) and, second, it lacks atoms that are normally attached to the α carbons and which also could contribute to $\Delta E_{trans \rightarrow cis}$. Hence, the more realistic model, NMA, in which hydrogens are attached to the α carbons, and whose net charge is zero, was studied.

NMA. Energy minimization of NMA gives two minima: the global minimum at ψ, ω, ϕ = 60°, 180°, 60° (trans) and a second minimum at ψ, ω, ϕ = 60°, 0°, 60° (cis). The energy difference between the two, $\Delta E_{trans \rightarrow cis}$, is 5.77 kcal/mol. Equations 4, 9, and 10 were used to calculate the statistical weights of each conformation and to determine the free energy of cis–trans isomerism, yielding $\Delta G_{trans \rightarrow cis}$ = 7.22 kcal/mol.

In the Discussion section, the minimum-energy values of ϕ and ψ of NMA (and the barriers to variation of ϕ and ψ) calculated in this study will be compared with (a) values determined with ECEPP functions and parameters but with geometry used in other theoretical studies, (b) values determined in theoretical studies using other empirical potentials and using quantum mechanical methods, and (c), where possible, values obtained from experiment.

Contour Maps of *N*-Acetyl-*N'*-methylglycineamide. The stability of trans peptide units in polypeptides may be influenced not only by the conformational energy but also by the conformational entropy. The effect of a cis bond on the ϕ–ψ space of Gly is shown in Figure 4. Blocked Gly has two peptide bonds; when both are in the trans conformation, the familiar contour map in Figure 4A results, but when one or both are cis, the contour maps in Figures 4B, 4C, and 4D are obtained. The maps show energy contour lines at 1, 3, and 6 kcal/mol above the minimum energy of the particular state (tt, tc, ct, or cc), and not above the global minimum at ϕ, ψ = −83°, 76° of the tt state (except, of course, in the tt state); hence, the maps reflect the conformational freedom in a particular state. It is clear that there is less conformational entropy in the tc, ct, and cc states, compared to the tt state. Table II gives the location and energy (with respect to the tt global minimum) of the point of lowest energy in each contour map.

Contour Map of *N*-Acetyl-*N'*,*N'*-dimethylglycineamide. The conformational freedom of a residue which precedes an *N*-substituted group, as in molecules with X-Sar or X-Pro peptide bonds, is illustrated by the contour map of *N*-acetyl-*N'*,*N'*-dimethylglycineamide. Figure 5A depicts the covalent structure of the molecule, and Figure 5B shows its conformational energy contour map. Both peptide bonds are fixed at 180°. For the *N*-terminal peptide bond, however, 0 and 180° are equivalent due to the symmetry of the dimethylamide group, and hence there is no cis–trans isomerism at that bond.

Gly-Gly and Gly-Pro. In polypeptides and proteins, the probability of formation of a cis bond is determined by the nature of the entire conformational space in both the trans and cis conformations. As an approximate method of analyzing the complete conformational space of blocked Gly-Gly and Gly-Pro, the partition functions *Q* and *Z* were determined by considering only conformations at energy minima. The total number *n* of minima obtained by the procedure described in the Methods section is 46 (or 90 when nonsuperimposable mirror images are included) for Gly-Gly (28 trans and 18 cis) and 32 for Gly-Pro (22 trans and 10 cis). All Gly-Gly minima of the trans isomer have relative energies from 0.0 to 2.64 kcal/mol; the minima of the cis isomer range in relative energy from 7.67 to 13.73 kcal/mol. The Gly-Pro trans minima range from 0.0 to 3.57 kcal/mol and the cis minima range from 1.73

Table III
Calculated Probability of Cis Peptide Bond in Model Compounds

Model	$P_{Q,cis}$	$P_{Z,cis}$	$\Delta G_{trans \rightarrow cis}$[a]	$-T\Delta S_{trans \rightarrow cis}$[b]
Peptide fragment	0.13	[c]	0.83[d]	[c]
NMA	6.2×10^{-5}	5.5×10^{-6}	7.22	1.45
Gly-Gly	1.1×10^{-6}	0.85×10^{-6}	8.33	1.56
Gly-Pro	4.2×10^{-2}	4.1×10^{-2}	1.87	0.46

[a] Definition given by eq 10, expressed in kcal/mol. [b] Definition given by eq 11, expressed in kcal/mol, with T = 300 K. [c] Librational entropy was not calculated for the peptide fragment. [d] This value is $\Delta E_{trans \rightarrow cis} = E_{cis} - E_{trans}$ in kcal/mol.

Table IV
Representative Minima of Blocked Gly-Gly and Gly-Pro

Dihedral angles, deg										
ϕ_{i+1}	ψ_{i+1}	ω_{i+1}	ϕ_{i+2}	ψ_{i+2}	h, Å	R, Å	Bend type[a]	ΔE	v	w
						Gly-Gly				
−82	77	179	84	−75	5.19	6.20	V	0.0[b]	0.194	0.044
83	−76	180	83	−76	5.93	8.87		0.22	0.133	0.031
−78	83	−178	77	45	3.73	4.91	II	0.22	0.133	0.133
−66	−47	177	−69	−45	3.54	4.81	III	1.59	0.013	0.010
179	180	1	179	−62	7.28	10.06		7.67[c]	0.0	0.0
82	−176	−5	−176	59	7.06	9.95		8.72	0.0	0.0
						Gly-Pro[d]				
178	−175	177	−75	79	7.08	7.81		0.0[e]	0.286	0.291
76	−170	176	−75	77	4.34	6.42	II′ [f]	0.32	0.166	0.105
−62	−70	170	−75	75	3.66	5.13	I[g]	0.92	0.061	0.018
165	−72	174	−75	79	6.91	6.92	IV	1.16	0.041	0.035
180	178	−2	−75	163	5.34	7.74		1.73[c]	0.016	0.019
71	81	−18	−75	−50	4.80	4.39	VI	4.50	0.0	0.0

[a] Defined in ref 36. [b] $(E_0)_g = -2.62$ kcal/mol. [c] Cis structure of lowest energy. [d] ϕ_{i+2} is fixed by the Pro ring at −75°. [e] $(E_0)_g = -15.60$ kcal/mol. [f] A distorted type II′ bend.[36] [g] A distorted type I bend.[36]

Table V
Comparison of Average Quantities and Bend Probabilities Calculated With and Without Variation of ω

	ω fixed[a]						ω varied[b]					
Dipeptide	$\langle h \rangle_Q$	$\langle h \rangle_Z$	$\langle R \rangle_Q$	$\langle R \rangle_Z$	$P_{Q,b}$	$P_{Z,b}$	$\langle h \rangle_Q$	$\langle h \rangle_Z$	$\langle R \rangle_Q$	$\langle R \rangle_Z$	$P_{Q,b}$	$P_{Z,b}$
Gly-Gly	5.20	5.99	6.97	7.47	0.53	0.37	5.18	5.95	6.96	7.44	0.53	0.37
Gly-Pro	6.55	6.91	7.87	8.15	0.22	0.13	6.30	6.74	7.64	8.00	0.30	0.19

[a] ω fixed at 180° during minimization. [b] ω allowed to vary during minimization with starting values at 180 and 0°.

to 4.52 kcal/mol. The probability $P_{Z,cis}$ of formation of a cis conformation at the peptide bond connecting the two residues is 8.5×10^{-7} for Gly-Gly and 4.1×10^{-2} for Gly-Pro. In Table III, these values, along with other calculated thermodynamic quantities for cis–trans isomerism, are compared with values for the peptide fragment and for NMA.

Table IV gives data on selected energy minima of the two dipeptides. Included are the energy global minimum, the structure of highest statistical weight (which is the energy global minimum in Gly-Pro but, due to librational entropy, is not the global minimum in Gly-Gly), examples of bend structures, the cis conformation of lowest energy, and examples of a conformation in which the peptide bond deviates substantially from planarity.

To show the effect of variation in ω on the conformational properties, the values of $\langle h \rangle_X$, $\langle R \rangle_X$, and P_X ($X = Q$ or Z) obtained in a previous study[35] by fixing ω at 180° are compared with those obtained by allowing ω to assume both cis and trans values and to vary during minimization. The results are given in Table V.

The average quantities for Gly-Gly are almost unaffected by deviations from planarity of the peptide bond. This is true not only for average properties determined from Q and Z but also for conformational properties at the individual minima. For example, of the 28 low-energy minima in the trans conformation, only one has an energy ΔE which changes by more than 0.01 kcal/mol when ω is allowed to vary during the minimization. In this particular case, a type III bend, it was found that ΔE is 1.62 kcal/mol with $\omega = 180°$, and that ΔE changed to 1.59 kcal/mol when $\omega = 177°$ after minimization; h changed from 3.59 to 3.54 Å and R from 4.95 to 4.81 Å. Most other conformations of Gly-Gly, however, show much less change when ω is allowed to vary. Thus, the deviations from the planar trans conformation are too small (always ≤3°), and the energies of cis conformations are too high, to have any effect on the values of $\langle h \rangle_X$, $\langle R \rangle_X$, and $P_{X,b}$ in Gly-Gly (Table V), where $X = Q$ or Z.

On the other hand, Gly-Pro is noticeably affected by deviations from the planar trans conformation of the peptide bond. For example, taking an extreme case, ω deviates from planarity by 10° in the low-energy type I bend given in Table IV. The related structure[35] with $\omega = 180°$ and $(\phi_{i+1}, \psi_{i+1}, \phi_{i+2}, \psi_{i+2}) = (-63°, -72°, -75°, 77°)$ has the values $h = 4.09$ Å, $\Delta E = 1.29$ kcal/mol, and $w = 0.010$. Hence, when ω is allowed to vary during minimization (Table IV), the bend becomes tighter (i.e., R decreases by 0.63 Å) and the relative energy is lowered (by 0.37 kcal/mol). Moreover, in Gly-Pro, cis is much more stable than in Gly-Gly, making the cis probability $P_{Z,cis}$ almost 50 000 times greater than in Gly-Gly (see Table III). The contributions to Q and Z of the deviations in the peptide bond from planar trans are thus reflected in the average conformational properties, as given in Table V. It should be pointed out that the increase in the bend probability $P_{Z,b}$, determined from eq 8, when ω is allowed to vary is due mainly to the type IV bend (see Table IV) which was found[35] not to be a bend but to have an R value of 7.23 Å, when ω was fixed at 180°.

Discussion

In the following discussion, we examine the results of the conformational energy calculations in an effort to understand the origin of the relative stabilities of the cis and trans conformations. Care must be taken, however, in specifying a particular interaction as the origin of differences in stability,

for two reasons: (a) individual interatomic interactions are identifiable only to the extent that the fundamental assumptions of the empirical method are applicable; and (b) the total conformational energy is the sum of many interactions, all of which, in principle, must be considered in determining the origin of differences in energy. Nevertheless, an examination of the calculated individual interaction energies is instructive if the basis for their determination is kept in mind and if the results of the calculations are compared, whenever possible, with appropriate experimental data.

The Origin of Trans Stability in Peptides with no Substituent on the N Atom. Conformational energy calculations show that the peptide molecular fragment $C^{\alpha}{}_1C'ONHC^{\alpha}{}_2$ is more stable in the trans than in the cis conformation, but only by about 1 kcal/mol. The instability of the cis form is due mainly to the unfavorable $C^{\alpha}{}_1\cdots C^{\alpha}{}_2$ nonbonded interaction. This suggests that, in general if other atoms were connected to the α carbons, which is the case in any real molecule, the cis form would be even less stable. Calculations on NMA, which is a simple case in which three hydrogen atoms are attached to the α carbons, confirm this point; the cis conformation of NMA has an energy 5.77 kcal/mol above the trans (Table III). Interactions of the types $H_{C^{\alpha}{}_1}\cdots C^{\alpha}{}_2$, $H_{C^{\alpha}{}_1}\cdots H_{C^{\alpha}{}_2}$, and $H_{C^{\alpha}{}_2}\cdots C^{\alpha}{}_1$ lead to an additional 5-kcal/mol destabilization of the cis form. These interactions also restrict rotations of the methyl groups, causing a decrease in the entropy and hence an increase in the free energy (ΔG = 7.22 kcal/mol).

The question arises as to what effect other groups attached to the α carbons have on the stability of the cis conformation. This is answered partially by considering the model compound *N*-acetyl-*N'*-methylglycineamide, referred to as blocked Gly. It has two peptide bonds, the most stable conformation being the one in which they both are trans. When either or both are fixed in the cis conformation, the energy is very high (>6 kcal/mol above the global minimum of the tt state; see Table II), and the conformation space is severely restricted (see Figure 4), suggesting that entropy as well as energy favors the trans peptide bond in this compound.

In the blocked dipeptide Gly-Gly, the central peptide bond has a full residue on each side; therefore, inter-residue interactions could affect the relative stabilities of cis and trans. As seen in Table IV, ΔE of the cis conformation of lowest energy is 7.67 kcal/mol, compared with 6.54 kcal/mol for the same quantity in the tc conformation of blocked Gly (see Table II), and 5.77 kcal/mol in NMA, indicating that the inter-residue interactions in Gly-Gly stabilize trans more than cis. The change in entropy $\Delta S_{\text{trans}\rightarrow\text{cis}}$ upon conversion from trans to cis (Table III) increases the free energy of cis over that of trans by 1.56 kcal/mol at 300 K, so that $\Delta G_{\text{trans}\rightarrow\text{cis}}$ = 8.33 kcal/mol.

The conclusion drawn from the comparison of relative stabilities of cis and trans in these peptide molecules is that the *major* interactions responsible for the higher energy in cis can be deduced from the NMA model. The value of ΔE of the cis conformation of lowest energy in both blocked Gly and blocked Gly-Gly is greater than the value of ΔE of the cis conformation in NMA (i.e., greater than 5.77 kcal/mol) by about 1–2 kcal/mol. This additional energy can be accounted for by the favorable interactions (e.g., i to $i+2$ and i to $i+3$ hydrogen bonds) which are present in the trans conformations but which are disallowed in the cis conformations.

Thus, the instability of the cis isomer in peptides with no substituent on the N atom is due to four factors: (a) interactions between the $C^{\alpha}{}_1$ and $C^{\alpha}{}_2$ atoms of the peptide unit, accounting for about 1 kcal/mol; (b) interactions which result from the close approach of hydrogens attached to the C^{α} atoms, accounting for about 4–5 kcal/mol of the instability of cis; (c) favorable interactions (e.g., hydrogen bonds) which exist in trans but not in cis, accounting for another 1–2 kcal/mol; and (d) conformational entropy, accounting for the final 1–2 kcal/mol of the total $\Delta G_{\text{trans}\rightarrow\text{cis}}$ of about 8 kcal/mol (Table III).

In a study to determine the origin of the instability of the cis form in polymers of alanine, Tonelli[6a] carried out empirical conformational energy calculations on alanine peptides and on NMA, concluding that long-range (i.e., inter-residue) interactions rather than short-range (i.e., intra-residue) interactions favor trans over cis. The present work substantiates the conclusion[6a] that long-range interactions favor the trans isomer, but is not in agreement with the conclusion[6a] that short-range interactions lead to only a slight preference for trans. The data of Tonelli[6a] suggest that the cis conformation should be detected in small peptide molecules, but this is in disagreement with experimental results, as will be discussed later (see Table IX) in more detail.

The Origin of Trans Stability in *N*-Substituted Peptide Groups. Although NMA serves as a model for the peptide unit in regular amino acid residues, it is not a good model for imino acid (*N*-substituted) residues such as proline, hydroxyproline, and sarcosine. The analogous model for these residues is *N,N*-dimethylacetamide (NDMA). In NDMA, however, there is no cis–trans isomerism; cis and trans become meaningful only upon substitution at one of the *N*-methyl carbons and, hence, the stability of the trans form in imino acid peptide bonds must be accounted for on the basis of interactions involving atoms attached to but not part of the peptide unit.

The effect of an imino group on the conformational space of a residue which precedes it is shown by comparing the ϕ–ψ map of *N*-acetyl-*N'*,*N'*-dimethylglycineamide with that of *N*-acetyl-*N*-methylglycineamide (Figures 4 and 5). Interestingly, the ϕ–ψ space of the imino-blocked Gly is almost identical with that of the regular blocked Gly in the tc state. Obviously, the interactions which destabilize the cis isomer in peptides that have no substituent on the N atom are present in both the cis and trans isomers. Hence, we expect that the value of $\Delta G_{\text{trans}\rightarrow\text{cis}}$ of *N*-substituted peptides will be much less than the value for regular residues. This is indeed the case; as seen in Table III, $\Delta G_{\text{trans}\rightarrow\text{cis}}$ for Gly-Gly is 8.33 kcal/mol but only 1.87 kcal/mol for Gly-Pro.

The question then remains as to what causes $\Delta G_{\text{trans}\rightarrow\text{cis}}$ to be 1.87 kcal/mol in Gly-Pro. The answer lies primarily in the restricted rotation about the N–C^{α} bond of Pro. Because ϕ_{Pro} is fixed at −75° by the pyrrolidine ring, a different set of interactions dominate than in Gly-Gly. Specifically, the restricted rotation around the N–C^{α} bond of Pro leads to a favorable $O_{i+1}\cdots C'_{i+2}$ electrostatic interaction in the trans form, which is much weaker (by about 2 kcal/mol) in the cis conformation. It is important to note that, although this $O_{i+1}\cdots C'_{i+2}$ electrostatic interaction energy is the major contribution to $\Delta E_{\text{trans}\rightarrow\text{cis}}$ in *most* cis conformations of Gly-Pro, in some cis conformations the peptide unit deviates substantially from planarity (e.g., $\omega = -18°$ in the type VI bend of Gly-Pro shown in Table IV), resulting in a more favorable $O_{i+1}\cdots C'_{i+2}$ interaction energy. In these cases, the torsional energy $U(\omega)$ then becomes a major source of instability of cis with respect to trans.

In summary, the instability of the cis X-Pro peptide bond (a) is less than that in residues having no substituent on the N atom because unfavorable interactions between atoms attached directly to the peptide unit are present in both cis and trans, (b) is caused mainly by the change in electrostatic energy of the $O_{i+1}\cdots C'_{i+2}$ interaction, which results from the restriction on rotations about the N–C^{α} bond of Pro due to the pyrrolidine ring, and (c) also results from loss of entropy in going from trans to cis (see Table III).

Nonplanarity of the Peptide Bond. Energy minimization of NMA, carried out with the peptide bond dihedral angle ω being allowed to vary, results in a global minimum in the

planar trans conformation ($\omega = 180°$), and a second minimum in the planar cis conformation ($\omega = 0°$). These results are in disagreement with CNDO/2 studies of Ramachandran and co-workers[16,17] who found the trans minimum to deviate considerably from planarity ($\omega = 167.5°$). Whereas the bond lengths and bond angles were held fixed in our study, they were allowed to vary in the CNDO/2 calculations;[16] this flexibility resulted in a deviation from planarity of the three atoms connected to the N atom. Ramachandran and co-workers[16] compared their results with the nonplanarity of formamide as deduced from the microwave spectrum by Costain and Dowling;[37] however, an analysis by Hagler et al.[38] of crystallographic data of 12 *N*-methylamides, including NMA and *N*-methylformamide (NMF), showed that the values of ω were all within 6° of planar trans. NMA and NMF deviated from planarity by 0 and 4°, respectively.

In the larger unstrained peptides, blocked Gly-Gly and Gly-Pro, the results presented here suggest that deviations from planarity are not significant in peptide bonds between regular amino acid residues but are more pronounced when the second residue is an imino acid (Pro, Hypro, Sar). In *N*-substituted peptide bonds, not only are deviations from planarity in the trans conformation greater than in normal peptide bonds but also cis conformations are relatively more stable. However, a comparison of average conformational properties calculated with ω fixed and with ω varied during minimization indicates (Table V) that, even for X-Pro peptides, the assumption of planar trans peptide bonds is adequate in determining *general* conformational character.

Comparison of Calculated and Experimental Values of ϕ_{min} and ψ_{min} in NMA. Since NMA is often used as a model for the peptide group in proteins, it is of interest to compare the results obtained using ECEPP with calculations from other theoretical methods. Table VI gives results from this work, from other calculations[38] using empirical potentials,[39] and from quantum mechanical studies using ab initio methods,[5,38] INDO,[40] PCILO,[41] CNDO/2,[3a,40] and EHT.[3a,40] This table is similar to the comparison presented by Hagler et al.[38] except that, when those workers applied the ECEPP potential, they considered only the *inter*molecular part,[42] rather than the complete set of parameters[25] including those for *intra*molecular interactions. As we had concluded previously,[25] Hagler et al.[38] found that the 6–12 potentials derived from intermolecular considerations alone give barriers which are too large. However, as seen in Table VI, the results with the full potential[25] (including intramolecular interactions) indicate that the barriers are reasonable, and quite similar to those[38] obtained from the 6–9 potential.[39] The ϕ barrier (from the ECEPP calculation) is still somewhat higher than those given by any of the quantum mechanical methods; the value of the ψ barrier is about in the middle of all theoretical values. Of course the experimental values of these barriers are not known, and any argument at this point over the specific values obtained by a particular theoretical method would be meaningless.

The conclusion can be drawn, however, that ECEPP gives reasonable results when compared with those obtained by other theoretical methods and, where meaningful experimental data are available, gives as good or better results than most methods.

A comparison of these calculations with experiment would be beneficial in evaluating the validity of each theoretical method since there are disagreements, especially in the value of ϕ_{min}. However, the reported experimental results are rather sparse. Hagler et al.[38] have refined the x-ray data of Katz and Post[43] to locate the positions of the methyl hydrogens, but, a comparison of the crystal analysis[38] with the gas phase results shown in Table VI may or may not be valid. A valid comparison, however, can be made with the results of Kitano et al.[44] Their recent gas-phase electron diffraction results suggest that ϕ_{min} has a value of 60°; however, their analysis could not differentiate between $\psi_{min} = 60°$ and $\psi_{min} = 180°$. The empirical calculations (Table VI) are in agreement with the conclusions[44] that $\phi_{min} = 60°$, whereas some of the ab initio and semiempirical calculations are in discrepancy with this experimental result.

Table VI
Comparison of ϕ_{min} and ψ_{min} and Energy Barriers for NMA from Calculations by Various Methods[a]

Method	Ref	ϕ_{min},[b] deg	ϕ barrier[c]	ψ_{min},[b] deg	ψ barrier[c]
		Empirical Potentials			
ECEPP	This work	60	1.2	60	0.8
6–12 of Hagler et al.	38	60	2.3	60	0.9
6–9 of Hagler et al.	38	60	1.3	60	0.4
		Ab Initio			
Minimum basis set	38	60	0.03	60	0.91
Extended basis set	38	60	0.73	60	0.06
"Molecular fragment"	5	0	0.69	60	1.1
		Semiempirical			
PCILO	41	60	0.8	60	1.0
INDO	40	0	0.37	60	0.39
CNDO/2	3a	0	0.24	60	0.30
CNDO/2	40	0	0.37	60	0.41
EHT	3a	0	0.30	60	0.18
IEHT	40	0	0.74	60	0.94

[a] The geometry used for NMA was not the same for all calculations; consult individual references for the particular set of bond lengths and bond angles used. [b] Dihedral angles are defined such that at 0° (≡ ±120°, due to symmetry) the C^{α}–H bond is syn to the C′–N bond, and at 60° (≡ 180°) the C^{α}–H bond is anti to the C′–N bond. [c] Barriers are in kcal/mol.

Comparison of Calculated Values of $\Delta E_{trans \rightarrow cis}$ in NMA. A further comparison of various theoretical results is given in Table VII where the calculated values of $\Delta E_{trans \rightarrow cis}$ for NMA are given. All but one method gives a positive value of $\Delta E_{trans \rightarrow cis}$, although there is a large spread in the actual amount of energy by which trans is favored. Since most experimentalists report that NMA is detected only in the trans conformation (see Table IX), there is no way of judging which calculated value of $\Delta E_{trans \rightarrow cis}$ is the best.

The Effect of Variation in the Geometry of NMA on Calculated Results. Shipman and Christofferson[5] suggested that differences in geometry may be the source of discrepancies in the quantum mechanical calculated values of ϕ_{min}, ψ_{min}, and $\Delta E_{trans \rightarrow cis}$. This notion was tested by Kolaskar et al.[40] and found to be the case (see Table 2 of ref 40); when the geometry used by Maigret et al.[41] is used with CNDO/2, the CNDO/2 and PCILO methods give the same value of ϕ_{min} (=0°). Although this result is in disagreement with experiment,[44] it does point out the need for selecting proper values for bond lengths and bond angles. Similarly, Hagler et al.[38] investigated the effects of geometry extensively, and showed that even relatively minor variations in geometry can change the location of the barrier obtained from ab-initio calculations as well as almost double the height of the barrier as calculated with *their* empirical potentials.[39] We also analyzed the effect of variations in geometry on our results by carrying out calculations using the ECEPP[25] energy parameters with various geometries used by others, viz., the geometry of Katz and

Table VII
Comparison of Calculated Values of $\Delta E_{trans \rightarrow cis}$ in NMA[a]

Method	Ref	$\Delta E_{trans \rightarrow cis}$[b]
ECEPP	This work	5.8
Empirical	6a	1.56
Ab initio, "molecular fragments"	5	3.66
EHT	3a	2.92
EHT	45	14
EHT	46	1.9
CNDO/2	3a	−0.09
CNDO/2	46	7.0

[a] See footnote *a* in Table VI. [b] $\Delta E_{trans \rightarrow cis} = E_{cis} - E_{trans}$ in kcal/mol.

Table VIII
Comparison of Calculated Quantities Using Various Geometries[a] of NMA[b]

Geometry	ϕ barrier[c]	ψ barrier[c]	$\Delta E_{trans \rightarrow cis}$[d]
This work[25]	1.20	0.82	5.77
Katz and Post[43]	1.78	0.67	5.42
Kitano et al.[44]	1.41	0.76	5.84
Pauling–Corey[47]	0.70	1.18	4.75

[a] The geometric parameters given in ref 43, 44, and 47, respectively, follow. Bond lengths: C^{α}_1–C′, 1.536, 1.52, 1.53 Å; C′–O, 1.236, 1.225, 1.24 Å; C′–N, 1.290, 1.386, 1.32 Å; N–C^{α}_2, 1.465, 1.469, 1.47 Å. Bond angles: C^{α}_1–C′–N, 116.5°, 114.1°, 114°; C^{α}_1–C′–O, 120.5°, 124.1°, 121°; C′–N–C^{α}_2, 120.5°, 119.7°, 123°. Relative locations of hydrogen atoms used in all the calculations were those given in Figure 2. [b] In all cases $(\psi, \omega, \phi)_{min} = (60°, 180°, 60°)$. [c] See footnote *c* of Table VI. [d] See footnote *b* of Table VII.

Post[43] used by Hagler et al.,[38] the geometry of Corey and Pauling[47] used by Maigret et al.,[41] and the geometry of Kitano et al.,[44] who gave the more accurate experimental values for NMA. Table VIII gives the results. In all cases $(\psi, \omega, \phi)_{min}$ was 60°, 180°, 60°, and the various energies are quite close in value. The only exception was found with the Pauling–Corey geometry, which of course was based upon very few experimental data compared with those available today. It should be noted that, even though the geometry of NMA in this work was chosen on the basis of the values of bond lengths and bond angles used in amino acid residues[25] (so that these results could be compared directly with those for single residues and blocked dipeptides), the results are very close to those obtained with the gas-phase geometry of Kitano et al.,[44] which are of course better data for purposes of comparing theoretical calculations than are the crystal data of Katz and Post.[43]

In these calculations, no explicit torsional potential $U(\phi)$ or $U(\psi)$ was used. Thus, the discussion by Kolaskar et al.[40] concerning $U(\phi)$ does not really apply to our study. Nevertheless, the conclusion of Kolaskar et al.[40] that $U(\phi)$ should be of the form $U(\phi) = (U_{\phi}/2)(1 - \cos 3\phi)$ to give a minimum at $\phi = 0°$ (or ±120°) rather than $U(\phi) = (U_{\phi}/2)(1 + \cos 3\phi)$ to give a minimum at $\phi = \pm 60°$ (or 180°) does not seem justified. The quantum mechanical calculations of Hagler et al.,[38] our analysis using ECEPP, and the experimental results[44] all give $\phi_{min} = 60°$. Therefore, NMA can still be considered a good model for the peptide unit in proteins, provided that correct geometry and methods of analysis are employed.

Comparison of Calculated and Experimental Values of the Cis Content in NMA and Blocked Dipeptides. Table IX gives the calculated values of $P_{Z,cis}$ and examples of experimentally determined values of the fraction of cis in NMA and in blocked Gly-Gly and Gly-Pro. The calculations indicate that the amount of cis in NMA or Gly-Gly should be below the level of detection by standard experimental techniques, and indeed this is the case. The only exception is that Barker and Boudreaux[48] report a cis content of about 3%; however, subsequent experiments[43,44] have failed to detect any conformation but trans.

The *N*-substituted peptide bond, however, has a significant probability for trans → cis isomerism. The calculated value is 4%. This compares very favorably with the experimental value[18] of 5% for the related Gly-Pro peptide bond in CF_3CO-Gly-Gly-Pro-Ala-OCH_3 in a nonpolar solvent. Since these calculations show that the stability of trans over cis is due mainly to favorable electrostatic interactions in the trans form, it is expected that the percent cis should increase in going from nonpolar to polar solvents. This is found to be the case, as shown in Table IX. The percent cis in the Gly-Pro peptide group in CF_3CO-Gly-Gly-Pro-Ala-OCH_3 is much greater in D_2O and in $(CD_3)_2SO$ than in $CDCl_3$.

Table IX
Comparison of Calculated and Observed Percent of Cis Isomer in Peptides

Peptide	Calcd ($P_{Z,cis}$, %)	Exptl			
		Obsd, %	Solvent	Method	Ref
NMA	5.5×10^{-4}				This work
		3	D_2O	^1H NMR	48
		0	D_2O	^1H NMR	11
		0	Pure liquid	^1H NMR	9
		0	Pure solid	X-ray diffraction	43
		0	Pure gas	Electron diffraction	44
Unsubstituted *N* peptides					
CH_3CO-Gly-Gly-$NHCH_3$	8.5×10^{-5} [a]				This work
CH_3CO-Gly-OH		0	D_2O	^1H NMR	11
H-Gly-Gly-OH		0	D_2O	^1H NMR	11
N-Substituted peptides[b]					
CH_3CO-Gly-Pro-$NHCH_3$	4.1				This work
CF_3CO-Gly-Gly-Pro-Ala-OCH_3		20	D_2O	^{13}C NMR	18
		40	$(CD_3)_2SO$	^{13}C NMR	18
		5	$CDCl_3$	^{13}C NMR	18

[a] The calculated percent cis refers to the peptide bond between the two Gly residues. [b] The calculated and observed cis content refers to the Gly-Pro peptide bond.

Conclusions

The results of this work yield the following conclusions.

1. Calculations using ECEPP[25] correctly predict that trans is more stable than cis in all peptide molecules studied, and gives reasonable values for $\Delta E_{trans \rightarrow cis}$.

2. The instability of cis in peptides with no substituent on the N atom (by ~8 kcal/mol) comes primarily from interactions of the $C^{\alpha}{}_1$ and $H_{C^{\alpha}{}_1}$ atoms with the $C^{\alpha}{}_2$ and $H_{C^{\alpha}{}_2}$ atoms. The instability is also due to favorable interactions present in the trans form which are disallowed in the cis form, and to conformational entropy.

3. The instability of cis in *N*-substituted peptides (e.g., Gly-Pro) is much less than in *N*-unsubstituted peptides. This is because unfavorable interactions of the type $C^{\alpha}H \cdots C^{\alpha}H$, present in the cis conformation of peptides with no substituent on the N atom (as in NMA), are present in both cis and trans in *N*-substituted peptides (as in NMDA). The instability of cis in X-Pro peptides arises mainly from the change in electrostatic energy of the $O_{i+1} \cdots C'_{i+2}$ interaction, which is, in turn, caused by the absence of rotation about the $N-C^{\alpha}$ bond of Pro. Entropy accounts for about 0.5 kcal/mol of the instability of cis in Gly-Pro compared with about 1.5 kcal/mol in Gly-Gly.

4. A slight amount ($\leq 3°$) of nonplanarity of the peptide bond can be observed in most minimum-energy structures of the blocked dipeptide Gly-Gly. However, this nonplanarity is not significant in the average conformational properties or in the relative conformational energies. In the *N*-substituted dipeptide Gly-Pro, however, deviations from planarity can be large (up to 10°) but, to a fairly good approximation, *general* aspects of average conformational properties can be determined adequately by assuming a planar trans conformation, at least in small molecules.

5. The calculated fraction (4%) of cis in blocked Gly-Pro is in good agreement with the experimental value[18] (5%) for related peptides in nonpolar solvents. The calculations also suggest that the fraction of cis should be higher in polar solvents, which is also in agreement with experiment.

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Addendum

After submitting this manuscript, Dr. F. A. Momany suggested that we determine the effect of a greater length of the Gly-Pro peptide bond (1.36 Å), compared to that of the Gly-Gly peptide bond (1.325 Å), on the stability of the cis form of Gly-Pro. When energy minimization was carried out on several trans and cis conformations of Gly-Pro with the peptide bond fixed at 1.325 Å, the relative energy ΔE (with respect to the trans global minimum) of the most stable cis conformation was 1.90 kcal/mol, compared with 1.73 kcal/mol obtained with a peptide bond length of 1.36 Å (see Table IV). Similarly, the other cis conformations had values of ΔE which were slightly higher than those obtained with the longer peptide bond. These differences in the values of ΔE are so small that it can be concluded that the greater length of the Gly-Pro peptide bond is not an important factor in making the cis form more stable in Gly-Pro than in Gly-Gly.

On the other hand, the relative stability of the cis and trans isomers in these peptides does depend on the difference in the bond angle $\tau(C'NC^{\alpha})$ between the cis and trans forms. In this study, $\tau(C'NC^{\alpha})$ was taken as 121° in both the trans and cis forms of Gly-Gly and in the trans form of Gly-Pro, but as 124° in the cis form of Gly-Pro (see Methods section). However, if $\tau(C'NC^{\alpha})$ is held fixed at 121° in both trans and cis forms of Gly-Pro, ΔE of the most stable cis conformation is 5.19 kcal/mol, rather than 1.73 kcal/mol (see Table IV). Thus, the different values of $\tau(C'NC^{\alpha})$ in the cis and trans forms, which were chosen[25] from x-ray crystal data, lead to a reasonable value for the cis–trans energy difference.

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