of incorporation of metal ions into the "SAT" complexes of TPyP is through the free base form, which is always in equilibrium with the di- and monocations. Several groups have done temperature-jump kinetic studies on TPyP with various cations and found no relaxations in the range that could be ascribed to SAT equilibria of the sort postulated.[6] This is because the proton equilibria actually present are too fast to measure by such methods.[20]

(20) R. R. Das, R. F. Pasternack, and R. A. Plane, *J. Amer. Chem. Soc.*, **92**, 3312 (1970).

SAT complexes, adducts between metal ions and porphyrins which form prior to metal ion incorporation, have not been demonstrated either in aqueous or nonaqueous[9] solution. The sitting-atop notion must now be regarded as indicating a probable but yet unobserved configuration of the system along the reaction coordinate toward metalloporphyrin formation.

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An Estimate of the Barriers Hindering Rotation about the C^{α}–C′ Bond between the Cis′ and Trans′ Conformations in an Isolated L-Proline Residue

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Abstract: The barriers to rotation about the C^{α}–C′ bond between the cis′ ($\psi \approx 125°$) and trans′ ($\psi \approx 325°$) minimum energy conformations in an isolated **trans peptide bond L-proline** residue are estimated using semiempirical potential functions. Nonbonded repulsive and London dispersion interactions are evaluated using a 6–12 potential, while electrostatic interactions are treated in the monopole–monopole approximation. Contributions made by hydrogen bonding between the carbonyl oxygen preceding and the amide proton succeeding the pyrrolidine ring are accounted for by the method of Brant. In addition, valence angle distortion and peptide bond rotation resulting in nonplanar conformations are considered. All bond lengths are fixed, while the valence angles and the angles of rotation φ, ψ, and ω are varied. The calculated barriers at $\psi = 60$ and 210° between the cis′ and trans′ conformations are found to be significantly lower than the cis–trans barrier (*ca.* 20 kcal/mol) about the peptide bond. In fact, both barriers are less than 10 kcal/mol and would not lead to separate nuclear magnetic resonances (one for cis′ and another for trans′) at room temperature. Distortion of the valence angles from their crystalline values, which greatly relieve the steric interactions of the proline carbonyl group and the N–H group succeeding the pyrrolidine ring with the β-CH_2 group of the pyrrolidine ring and with the carbonyl group preceding the pyrrolidine ring, and when $\psi \approx 210°$, the intramolecular hydrogen bond between the carbonyl group preceding and the N–H group succeeding the pyrrolidine ring, as suggested previously, account for the small barriers.

During the course[1-12] of determining the solution conformations of several cyclic peptides (synthetic[1-6] and biologically active[7-11]) containing isolated L-proline residues (an L-proline residue not succeeded by another proline residue[13]) using nuclear magnetic resonance spectroscopy (nmr) and conformational energy estimates, the question of the magnitude of the barrier to rotation about the C^{α}–C′ bond (see Figure 1) between the cis′ ($\psi = 125°$) and trans′ ($\psi = 325°$) minimum energy conformations[14] became important (see ref 12 for a review of cyclic peptide conformation). If the barriers are of a magnitude similar to the cis–trans peptide bond barrier,[17] *ca.* 20 kcal/mol, then separate nuclear magnetic resonances could be expected for the cis′ and trans′ conformations of the L-prolyl residues, because both conformations have nearly the same energy.[13,18] On the other hand, if both barriers (at $\psi \approx 60$ and 210°) are much larger

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(5) A. I. Brewster and F.A. Bovey, *ibid.*, **68**, 1199 (1971).
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(8) D. J. Patel, *ibid.*, **12**, 667, 677 (1973).
(9) A. E. Tonelli, *ibid.*, **12**, 689 (1973).
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(11) A. E. Tonelli, D. J. Patel, H. Faulstich, and Th. Wieland, *ibid.*, in press.
(12) F. A. Bovey, A. I. Brewster, D. J. Patel, A. E. Tonelli, and D. A. Torchia, *Accounts Chem. Res.*, **5**, 193 (1972).
(13) P. R. Schimmel and P. J. Flory, *J. Mol. Biol.*, **34**, 105 (1968).
(14) The angles of rotation, φ, ψ, and ω (see Figure 1), are taken[15] as zero in the planar zigzag conformation and are measured in a right-handed sense. To avoid confusion, a more recently proposed convention,[16] which assigns $\varphi = \psi = \omega = 180°$ to the planar zigzag conformation, is not adopted here.
(15) J. T. Edsall, P. J. Flory, J. C. Kendrew, A. M. Liquori, G. Nemethy, G. Ramachandran, and H. A. Scheraga, *Biopolymers*, **4**, 121 (1966); *J. Biol. Chem.*, **241**, 1004 (1966); *J. Mol. Biol.*, **15**, 399 (1966).
(16) J. C. Kendrew, W. Klyne, S. Lifson, T. Miyazawa, G. Nemethy, D. C. Phillips, G. N. Ramachandran, and H. A. Scheraga, *Biochemistry*, **9**, 3471 (1970); *J. Biol. Chem.*, **245**, 6489 (1970); *J. Mol. Biol.*, **52**, 1 (1970).
(17) (a) W. D. Phillips, *J. Chem. Phys.*, **23**, 1363 (1955); (b) F. A. Bovey, "Nuclear Magnetic Resonance Spectroscopy," Academic Press, New York, N. Y., 1969, pp 195–197.
(18) B. Maigret, D. Perahia, and B. Pullman, *J. Theor. Biol.*, **29**, 275 (1970).

and only one of the conformers (cis′ or trans′) is generated during synthesis,[19] or if the barriers are much smaller than the 20 kcal/mol peptide bond rotation barrier, then only a single resonance corresponding to the cis′ or trans′ conformation or to an average over both conformations, respectively, would be observed.

The nmr evidence[1,2] and the conformational energy estimates[3] clearly pointed to the presence of trans′ L-prolyl residues in the synthetic cyclic hexapeptides (-pro-ser-gly-)$_2$ and (-ser-pro-gly-)$_2$. Chemical shift differences between the α protons in the adjacent L-proline residues in the cyclic nonapeptide cyclolinopeptide A[5,6] implied a cis′ conformation for the second L-proline residue in the pair. In conjunction with the apparent severity of the steric interactions of the prolyl carbonyl group and the N–H group succeeding the pyrrolidine ring with the β-CH_2 group of the pyrrolidine ring and the carbonyl group preceding the pyrrolidine ring (see Figure 1), as observed in molecular models when interconverting between the cis′ and trans′ conformations, it was tentatively concluded[3,6] that the barrier to cis′–trans′ interconversion is comparable to or greater than the cis–trans peptide bond barrier. Hence, the suggestion that the probable high magnitude of this barrier prevents cis′ and trans′ conformations from being in rapid equilibrium was advanced.

A quantum mechanical calculation[18] of the energy of rotation about the C^α–C′ bond in an isolated trans peptide bond L-proline residue yielded a barrier of 9.0 kcal/mol at $\psi = 230°$ when the imide group is rotated $\omega_1 = -40°$ out of the planar trans conformation. The reduction of the barrier at $\psi = 230°$ in comparison to the barrier at $\psi = 60°$, when $\omega_1 = -40°$, was attributed[18] to the seven-membered hydrogen bond formed between the carbonyl group preceding and the N–H group succeeding the pyrrolidine ring in the former conformation ($\psi = 230°$). The resulting disparity in the magnitude of the cis′–trans′ barrier as indicated by the conformational studies of cyclic peptides[1,2] and by the quantum mechanical energy calculations[18] prompted the present study.

The magnitudes of the barriers to ψ rotation at $\psi \approx 60$ and 210° in an isolated L-proline residue are estimated by calculating the energy of interaction of the carbonyl group preceding the pyrrolidine ring and the β-CH_2 group of the pyrrolidine ring with the carbonyl and N–H groups succeeding the pyrrolidine ring. Nonbonded van der Waals, electrostatic, and hydrogen-bonding interactions are accounted for, in addition to the intrinsic torsional potentials about the C^α–C′ and peptide bonds and the potentials encountered in valence angle bending.

Details of the Barrier Calculations

The crystalline structure[20] of L-leucyl-L-prolylglycine is taken as the reference geometry. Both peptide bonds adjoining the L-prolyl residue are allowed to adopt the conformations corresponding to $\omega = 0, -20$, and $-40°$, where $\omega = 0°$ for trans peptide bonds. Rotation about the N–C^α bond is restricted by the pyrrolidine ring; hence $\varphi = 102$ and 122°, which are the values observed in crystalline poly-L-proline form II[21] and L-leucyl-L-prolylglycine,[20] respectively, are selected. Rotation about the C^α–C′ bond is restricted in 10° increments to the ranges of $\psi = 40$–80° and $\psi = 190$–250°. The valence angles N–C^α–C′, C^α–C′–N, and C′–N–C^α are varied ±0, ±3, and ±6° from their values in crystalline L-leucyl-L-prolylglycine,[20] and it is assumed, as an example, that if $\Delta(\angle C^\alpha\text{–}C'\text{–}N) = +6°$, then the two remaining valence angles at C′ ($\angle C^\alpha\text{–}C'{=}O$ and $\angle O{=}C'\text{–}N$) are each reduced by 3°.

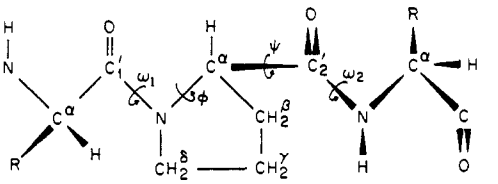

Figure 1. A schematic representation of a portion of a poly-L-peptide chain containing an isolated L-proline residue. All φ's, ψ's, and ω's are at 0°, except $\varphi_{pro} \approx 120°$ which is fixed by the pyrrolidine ring.

The nonbonded van der Waals (6–12), electrostatic (monopole–monopole), and hydrogen-bonding interactions are evaluated according to the potentials of Brant, *et al.*[22–24] Intrinsic torsional potentials about the peptide[25] and C^α–C′ bonds are taken from Maigret, *et al.*,[18] and Brant, *et al.*,[22–24a] respectively. The valence angle bending potential [$V_{\Delta\theta} = 40.0 \times (\Delta\theta)^2$ ($V_{\Delta\theta}$ is in kcal/mol when $\Delta\theta$ is in radians)] used by Ramachandran and Venkatachalam[26] is adopted for each of the valence angles.

Calculated Results and Discussion

The barriers to rotation about the C^α–C′ bond between the cis′ and trans′ conformations in an isolated L-prolyl residue are calculated to be less than 10 kcal/mol and are located at $\psi \approx 60$ and 210°. The geometry[27] and rotation angles corresponding to the minimum barrier heights found at each position ($\psi \approx 60$ and 210°) are listed in Table I. It is clear that both

(19) If the barriers are large (≫20 kcal/mol), but both cis′ and trans′ isomers are formed during synthesis, then the populations of the separately observed resonances would be expected to be nearly independent of temperature.

(20) Y. C. Leung and R. E. Marsh, *Acta Crystallogr.*, **11**, 17 (1958).

(21) V. Sasisekharan, *ibid.*, **12**, 897 (1959).

(22) D. A. Brant and P. J. Flory, *J. Amer. Chem. Soc.*, **87**, 2791 (1965).

(23) D. A. Brant, W. G. Miller, and P. J. Flory, *J. Mol. Biol.*, **23**, 47 (1967).

(24) (a) D. A. Brant, *Macromolecules*, **1**, 291 (1968); (b) D. A. Brant, A. E. Tonelli, and P. J. Flory, *ibid.*, **2**, 228 (1969).

(25) The intrinsic rotation potential about the peptide bond is calculated by Maigret, *et al.*,[18] to be 0.0, 3.0, and 8.0 kcal/mol for $\omega = 0, -20$, and $-40°$ when $\psi = 330°$. Ramachandran and Venkatachalam[26] suggest the empirical intrinsic peptide bond potential $V_\omega = 10.0 \cdot (1 - \cos 2\omega)$, which yields $V_\omega = 0.0$, 2.34, and 8.26 kcal/mol for $\omega = 0, -20$, and $-40°$.

(26) G. N. Ramachandran and C. M. Venkatachalam, *Biopolymers*, **6**, 1255 (1968).

(27) The magnitudes of the barrier heights are not very sensitive to the choice of reference geometry. As an example, the sums of valence angle distortion energies $\Sigma(V_{\Delta\theta})$ for the barrier conformations with $\psi = 60$ and 210° in Table I are 0.77 and 0.88 kcal/mol, respectively. In fact, if the reference geometry is adjusted so that each of the valence angles deviates by 6° from the Leung–Marsh[20] geometry in a direction which would maximize $\Sigma V_{\Delta\theta}$ for the two-barrier conformations, the $\Sigma V_{\Delta\theta}$ for the two-barrier conformations in Table I is 4.3 ($\psi = 60°$) and 4.8 ($\psi = 210°$) kcal/mol. Even this extreme choice of reference geometry, which does not lead to an appreciable increase in nonbonded interactions when $(\varphi,\psi) = 122°, 325°$, results in barriers (9–12 kcal/mol) lower in magnitude than the peptide bond rotation barrier,[17] and would not be expected to produce separate nuclear magnetic resonances at room temperature.

Table I. Calculated Conformations of the Minimum Barrier Heights about the C^{α}–C′ Bond in an Isolated L-Prolyl Residue

φ,[a] deg	ψ, deg	ω_1, deg	ω_2, deg	$\Delta(\angle C^{\alpha}\text{–}C_1'\text{–}N)$,[b] deg	$\Delta(\angle C_1'\text{–}N\text{–}C^{\alpha})$, deg	$\Delta(\angle N\text{–}C^{\alpha}\text{–}C_2')$, deg	$\Delta(\angle C^{\alpha}\text{–}C_2'\text{–}N)$, deg	$\Delta(\angle C_2'\text{–}N\text{–}C^{\alpha})$, deg	$V_{barrier}$[c]
102	60	0	0	0	−3	+3	−6	+3	7.9 (8.0)[d] (7.9)[e]
102	210	0	0	0	0	−6	−6	0	4.0 (3.9)[d] (149)[e] (307)[f]

[a] $\varphi = 122°$ is the value found by Leung and Marsh[20] in crystalline L-leucyl-L-prolylglycine, while $\varphi = 102°$ was observed by Sasisekharan[21] in poly-L-proline form II. Both values of φ(102 and 122°) were used in the calculations, but $\varphi = 102°$ led to smaller barriers. [b] The Leung–Marsh[22] geometry of L-leucyl-L-prolylglycine is $\angle C^{\alpha}\text{–}C_1'\text{–}N = 119°$, $\angle C_1'\text{–}N\text{–}C^{\alpha} = 121°$, $\angle N\text{–}C^{\alpha}\text{–}C_2' = 111°$, $\angle C^{\alpha}\text{–}C_2'\text{–}N = 115°$, and $\angle C_2'\text{–}N\text{–}C^{\alpha} = 122°$ (see Figure 1). [c] The conformational energy at $(\varphi,\psi) = 122°, 325°$ and for Leung–Marsh[20] geometry is 0.28 kcal/mol. [d] Without the inclusion of the interactions between the carbonyl group preceding and the N–H group succeeding the L-prolyl residue (see Figure 1). [e] Without inclusion of the hydrogen-bonding interaction (inclusion of the usual 6–12 van der Waals and electrostatic interactions only) between the carbonyl group preceding and the N–H group succeeding the L-prolyl residue. [f] Leung–Marsh[20] geometry adopted.

minimum barrier heights occur for planar,[28] trans peptide bonds, and not for $\omega_1 = -40°$ as calculated by Maigret, *et al.*,[18] using the PCILO quantum mechanical method.[29] However, in agreement with the PCILO calculations, the barrier at $\psi \approx 210°$ does owe its low magnitude in part to the seven-membered hydrogen bond[30] between C'_1=O and N–H (see Figure 1), as well as to deviations in the valence angles from the Leung–Marsh[20] geometry.

Thus, it appears that the barriers to rotation about the C^{α}–C′ bond between the minimum energy cis′ and trans′ conformations in an isolated L-proline residue[31] are less than 10 kcal/mol. This means that cis′ and trans′ isolated L-prolyl residue conformations may be in rapid dynamic equilibrium (rapid on the nmr time scale) in cyclic and linear peptides, in contradiction to our earlier tentative proposal,[3,6] which denied such a rapid interconversion. Consequently, the possibility that the conformation of an isolated L-prolyl residue in a cyclic peptide is some average over both the cis′ and trans′ conformations must be considered when generating possible conformations[12] that are consistent with nmr data.

Finally, it should be mentioned that the possibility of cis peptide bonds connecting amino acid to imino acid residues, such as peptide bond 1 in Figure 1, has not been considered here. Although it is experimentally[1,2,8,12] well known that imino acid peptide bonds may adopt either the cis or the trans conformations in linear or cyclic polypeptides, the presence of a cis peptide bond renders[32] the rotations φ and ψ in the residues joined by the cis peptide bond interdependent. When $\omega_1 = 180°$ (cis), ψ rotations about the C^{α}–C′ bond in the L-prolyl residue in Figure 1 become dependent upon the conformation (φ,ψ) of the preceding residue and can no longer be treated as an isolated rotation.

Acknowledgment. Valuable discussions with Dr. D. A. Torchia are gratefully acknowledged.

(28) Both peptide bond potential functions preclude $\omega_1,\omega_2 = -40°$, because $V_{\omega=-40°} = 8.0$ and 8.26 kcal/mol. However, several low energy conformations for $\psi = 210°$ were found with either ω_1 or $\omega_2 = -20°$. In the most favorable case, the energy of the $\psi = 210°$ barrier height with ω_1 or $\omega_2 = -20°$ is *ca.* 3 kcal/mol higher in energy than the $\omega_1 = \omega_2 = 0°$ conformer presented in Table I. The higher energy originates primarily from V_{ω}. Thus, ω_1 or $\omega_2 = 0$ or $-20°$ is possible at the barrier height, but not ω_1 or $\omega_2 = -40°$ as suggested by Maigret, *et al.*[18]

(29) S. Diner, J. P. Malrieu, and P. Claveri, *Theor. Chim. Acta*, **13**, 1, 18 (1969); **15**, 100 (1969).

(30) The seven-membered hydrogen bond between C_1'=O and N—H in the barrier height conformation at $\psi = 210°$ is characterized by an O-to-H distance of 1.78 Å, an angle of 112° between C_1'=O and O···H, and an angle of 31° between N—H and N···O.

(31) When the isolated L-prolyl residue is situated in an unstrained cyclic peptide (six or more residues), the barrier to cis′–trans′ interconversion must at least equal, but more probably exceeds, the same barrier present in open chain peptides. This increase in the cis′–trans′ barrier height in cyclic peptides is a consequence of the cooperative nature of conformational transitions occurring in cyclic peptides. The conformational transitions are cooperative, because the integrity of the ring structure must be maintained during the transition.

(32) A. E. Tonelli, *J. Amer. Chem. Soc.*, **92**, 6187 (1970); **93**, 7153 (1971); *J. Mol. Biol.*, submitted for publication.