Dynamics of Cis/Trans Isomerization of the Cysteine^6–Proline Peptide Bonds of Oxytocin and Arginine–Vasopressin in Aqueous and Methanol Solutions

Cynthia K. Larive† and Dallas L. Rabenstein*

Contribution from the Department of Chemistry, University of California, Riverside, California 92521

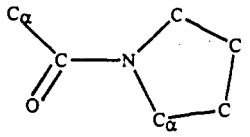
Received September 14, 1992

Abstract: The kinetics and thermodynamics of cis ⇌ trans isomerization by rotation around the Cys^6–Pro peptide bond of oxytocin (OT) and arginine–vasopressin (AVP) were characterized in aqueous and methanol solutions. Isomerization rate constants were determined over a range of temperatures by the inversion-magnetization transfer NMR method, and isomerization equilibrium constants were determined from resonance intensities. Activation parameters were obtained from Eyring plots of the rate constants. The trans conformation is the more abundant for both OT and AVP, and the abundance of the trans conformation is larger in methanol than in aqueous solution. Equilibrium constants for cis ⇌ trans isomerization are 12 and 20 for OT and 15 and 25 for AVP in aqueous and methanol solutions, respectively. Rate constants for cis-to-trans and trans-to-cis interconversions are 4.2×10^{-2} and 3.5×10^{-3} s^{-1} for OT and 6.7×10^{-2} and 4.6×10^{-3} s^{-1} for AVP in aqueous solution at 25 °C. Rate constants for both cis-to-trans and trans-to-cis interconversions are significantly larger for both OT and AVP in methanol solution, which is consistent with interconversion via a mechanism involving a less polar transition state characterized by partial rotation around the C–N bond. Comparison of trans-to-cis interconversion rate constants for OT and AVP with those for smaller proline-containing peptides suggests that the trans conformations of OT and AVP are not stabilized by intramolecular interactions between the macrocyclic hexapeptide rings and the acyclic tripeptide tails.

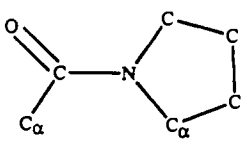
Introduction

The neurohypophyseal peptide hormones oxytocin (OT) and arginine–vasopressin (AVP) are nonapeptides having the amino acid sequences Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2 and Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2, respectively. Key structural elements of both OT and AVP are the macrocyclic ring formed by residues 1–6, the acyclic tripeptide tail, and the proline residue at position 7. The tripeptide tails are connected to the macrocyclic hexapeptide rings via cysteine^6–proline peptide bonds.

As compared to peptide bonds for other amino acids, the additional substitution of the amino group of proline destabilizes the trans (Z) conformation across the Xaa–Pro peptide bond relative to the cis (E) conformation.[1] The barrier to cis ⇌ trans



trans



cis

interconversion is sufficiently large that interconversion is slow on the NMR time scale, and resonances are observed for both the cis and trans isomers of proline-containing peptides.[2] However, on the basis of early ^1H and ^{13}C NMR results, the Cys^6–Pro peptide bonds of OT and AVP have been considered to exist completely in the trans conformation.[3,4] Recently, it has been demonstrated using one- and two-dimensional ^1H NMR that some 4–10% of OT and AVP exist in the cis conformation in aqueous and methanol solutions.[5] This is considerably less than is often found for the cis conformation of small proline-containing peptides, e.g., the average abundance of the cis isomer is 27% for a series of pentapeptides with proline at position 2,[2b] which raises the question: What are the factors which favor the trans conformations of OT and AVP, e.g., are the trans conformations stabilized relative to the cis by intramolecular interactions between the macrocyclic hexapeptide rings and the tripeptide tails?

In this paper, we report the results of a ^1H NMR study of the kinetics and thermodynamics of the interconversion between the cis and trans conformations of OT and AVP in aqueous and methanol solutions. The rate of rotation around the Cys^6–Pro imide bond is found to be significantly faster in methanol solution, which is consistent with interconversion by a mechanism which involves a less polar, twisted Cys^6–Pro imide bond in the transition state.[6] The rates of cis ⇌ trans isomerization of OT and AVP are also of interest as models for the dynamics of cis-to-trans and trans-to-cis interconversions of Xaa–Pro peptide bonds in proteins. The majority of the studies of the dynamics of cis ⇌ trans isomerization of Xaa–Pro peptide bonds have involved di- and tripeptides. However, energy minimization studies suggest that the nature of the neighboring atoms may greatly influence the rate of isomerization of prolyl peptide bonds in proteins,[7] and thus it is of interest to characterize the kinetics and thermody-

† Present address: Department of Chemistry, University of Kansas.

(1) Stewart, D. E.; Sarkar, A.; Wampler, J. E. *J. Mol. Biol.* **1990**, *214*, 253–260.

(2) (a) Grathwohl, C.; Wüthrich, K. *Biopolymers* **1976**, *15*, 2025–2041. (b) Dyson, H. J.; Rance, M.; Houghten, R. A.; Lerner, R. A.; Wright, P. E. *J. Mol. Biol.* **1988**, *201*, 161–200.

(3) (a) Hruby, V. J.; Brewster, A. I.; Glasel, J. A. *Proc. Nat. Acad. Sci. U.S.A.* **1971**, *68*, 450–453. (b) Brewster, A. I.; Hruby, V. J.; Spatola, A. F.; Bovey, F. A. *Biochemistry* **1973**, *12*, 1643–1649. (c) Deslauriers, R.; Walter, R.; Smith, I. C. P. *Biochem. Biophys. Res. Commun.* **1972**, *48*, 854–859. (d) Smith, I. C. P.; Deslauriers, R.; Walter, R. In *Chemistry and Biology of Peptides*; Meienhofer, J., Ed.; Ann Arbor Science Publishers, Inc.: Ann Arbor, MI, 1972; pp 29–34.

(4) Hruby, V. J. In *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*; Weinstein, B., Ed.; Marcel Dekker, Inc.: New York, 1974; Vol. 3, pp 1–170.

(5) Larive, C. K.; Guerra, L.; Rabenstein, D. L. *J. Am. Chem. Soc.* **1992**, *114*, 7331–7337.

(6) Harrison, R. K.; Stein, R. L. *J. Am. Chem. Soc.* **1992**, *114*, 3464–3471.

(7) Leavitt, M. *J. Mol. Biol.* **1981**, *145*, 251–263.

0002-7863/93/1515-2833$04.00/0

namics of prolyl peptide bond isomerization in larger peptides as models for proteins.

Experimental Section

Solution Preparation. AVP and OT were obtained as the trifluoroacetate salts from Bachem. OT was also obtained as the acetate salt from Fluka. Solutions of the peptides (5–10 mM) were prepared in 90% H_2O/10% D_2O or in CD_3OH. Solution pH was adjusted to a meter reading of 3.0 with DCl or NaOD in 90% H_2O/10% D_2O or with concentrated DCl or NaOD diluted with CD_3OH. The pH meter readings for the CD_3OH solutions are reported as pH* but are not intended to represent hydrogen ion activity. The pH meter was calibrated with aqueous standard solutions at pH 4.00 and 7.00 (Fisher Scientific).

NMR Spectroscopy. ^1H NMR spectra were measured at 500 MHz with a Varian VXR-500S spectrometer. Chemical shifts are reported relative to 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). The variable temperature unit was calibrated using the chemical shifts of neat ethylene glycol and methanol. ^1H NMR measurements were made on pH 3.0 solutions of OT and AVP in 90% H_2O/10% D_2O or pH* 3.0 solutions in CD_3OH so that resonances could be observed for the amide protons. The water resonance in spectra of 90% H_2O/10% D_2O solutions and the hydroxyl resonance in spectra of CD_3OH solutions were suppressed by selective saturation.

ROESY (rotating-frame Overhauser enhancement spectroscopy) spectra were measured by the standard ROESY pulse sequence[8] with elimination of the OH resonance by selective saturation during both the relaxation delay and the mixing period. Spectra were measured with spectral widths of 5000 Hz in both dimensions. A total of 2048 data points were acquired in t_2, and 32 transients were coadded at each of 256 t_1 increments with zero-filling to 2048 points. Phase-sensitive spectra were acquired using the method of States et al.[9] Gaussian apodization was applied in both dimensions.

Rate constants for trans-to-cis interconversion were determined by the inversion-transfer method.[10] The trans resonance of a given cis/trans pair of resonances was inverted with the pulse sequence[11,12] $\pi/2(x) - \tau - \pi/2(x) - t - \pi/2(x,y,-x,-y)$ – acquisition, where τ is a fixed delay of length $^1/_2\Delta$, $\Delta = |\nu_{cis} - \nu_{trans}|$ in hertz, and the carrier is set on the resonance to be inverted. t is a variable delay, the mixing period, during which transfer of magnetization occurs by exchange between the cis and trans forms. t values ranging from 0.0001 s to $>5T_1$ were used; T_1s were estimated by the inversion-recovery method.[13] Typically, in each experiment inversion-transfer spectra were measured at 14–21 t values.

The lifetime of the trans isomer, τ_t, was determined from the dependence of the intensity of the cis resonance on mixing time using Method 4 in ref 14. Rate constants for trans-to-cis interconversion, k_{tc}, were calculated from the lifetimes; rate constants for cis-to-trans interconversion, k_{ct}, were then calculated from k_{tc} and the equilibrium constant $K_{t/c}$ using the relation $K_{t/c} = k_{ct}/k_{tc}$.

$K_{t/c}$ was determined from the relative intensities of the resonances for the trans and cis isomers. Because the fractional populations of the cis isomers are so small, it was found that the best precision was obtained using resonance intensities determined by the cut-and-weight method.

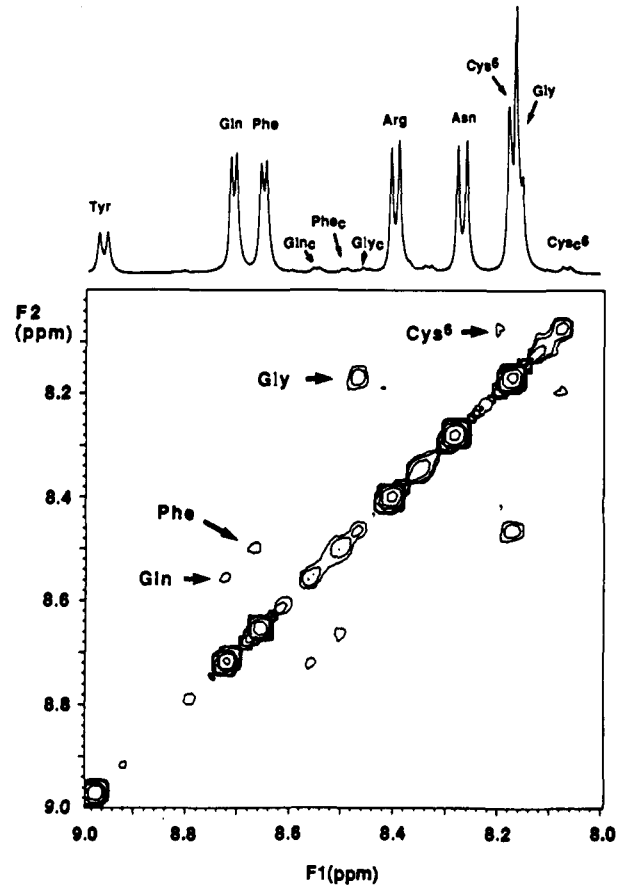


Figure 1. Amide proton region of the phase-sensitive ROESY spectrum (symmetrized) of 10 mM AVP in CD_3OH at pH* 3.0 and 37 °C. A 0.3-s spin-locking period was used. The corresponding region of the one-dimensional spectrum is plotted across the top. Both spectra were measured with suppression of the OH resonance by presaturation. Only positive contours are plotted in the ROESY spectrum. The amide protons giving rise to the exchange cross peaks are identified.

Results

The dynamics of cis ⇌ trans isomerization by rotation around the Cys[6]–Pro peptide bonds of OT and AVP in aqueous and methanol solutions were characterized using amide proton resonances.[15] The assignment of NH resonances to specific amino acids of the cis and trans isomers of OT and AVP in aqueous solution was reported previously.[5] The NH resonances for both the trans and cis isomers of OT and AVP in CD_3OH solution were assigned in this work using methods similar to those used previously.[5] The backbone amide proton region consists of two groups of resonances: the relatively intense resonances for the NH protons of the trans isomer and a smaller number of much less intense resonances for the NH protons of the cis isomer, as illustrated by the one-dimensional spectrum for AVP in CD_3OH solution in Figure 1. Resonances are assigned to specific amino acids of the trans and cis isomers, with assignments for the cis isomers indicated by subscript c.

Rotation around the Cys[6]–Pro peptide bonds of OT and AVP takes place on a time scale which can be characterized by magnetization transfer experiments, as demonstrated by the presence of exchange cross peaks in ROESY spectra of OT and AVP. To illustrate, the phase-sensitive ROESY spectrum of 10 mM AVP in CD_3OH solution at pH* 3.0 and 37 °C is shown in Figure 1. In the phase-sensitive ROESY experiment, cross peaks which result from transfer of magnetization by chemical exchange are the same sign as the diagonal peaks, while those from NOE

(8) (a) Bothner-By, A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811–813. (b) Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *63*, 207–213.

(9) States, D. J.; Haberkorn, R. A.; Ruben, D. J. *J. Magn. Reson.* **1982**, *48*, 286–292.

(10) (a) Dahlquist, F. W.; Longmuir, K. J.; DuVirnt, R. B. *J. Magn. Reson.* **1975**, *17*, 406–410. (b) Alger, J. R.; Prestegard, J. H. *J. Magn. Reson.* **1977**, *27*, 137–141.

(11) Robinson, G.; Kuchel, P. W.; Chapman, B. E.; Doddrell, D. M.; Irving, M. G. *J. Magn. Reson.* **1985**, *63*, 314–319.

(12) The populations of the cis conformations are so small that reliable rate constants cannot be obtained from the changes in intensity of the trans resonances by cis-to-trans interconversion following the selective inversion of resonances for the cis isomers.

(13) Because both the cis and trans conformations are present simultaneously, it was not possible to measure directly actual T_1 values for resonances of the cis and trans conformations in the absence of exchange at the temperatures used in the inversion-transfer experiments. Thus, the T_1 values estimated by the inversion-recovery method are a function of the T_1s of the resonances for each proton in both the cis and trans forms (McLaughlin, A. C.; Leigh, J. S. *J. Magn. Reson.* **1973**, *9*, 296–304).

(14) Mariappan, S. V. S.; Rabenstein, D. L. *J. Magn. Reson.* **1992**, *100*, 183–188.

(15) The majority of resonances for other protons of the cis conformations of OT and AVP are overlapped by trans resonances.[5]

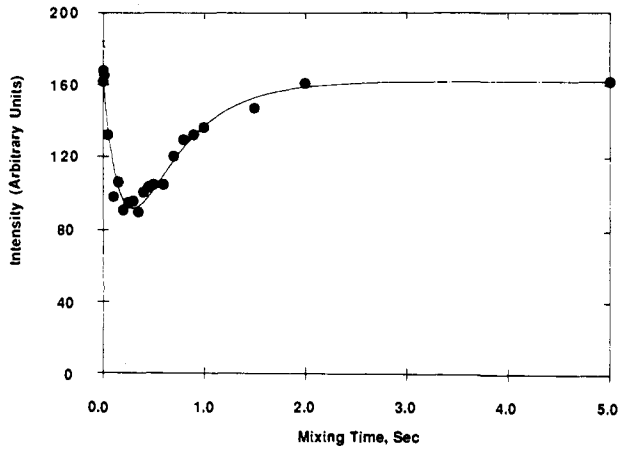


Figure 2. Integrated intensity of the resonance for the Cys⁶-NH proton of the cis isomer of OT in aqueous solution as a function of the mixing time following inversion of the resonance for the Cys⁶-NH proton of the trans isomer in inversion-transfer experiments. The data are for 10 mM oxytocin in 90% H_2O/10% D_2O at pH 3.0 and 65 °C. The smooth curve through the points is the theoretical curve calculated using the parameters obtained by nonlinear least-squares analysis of the data.

transfer are of opposite sign.[16] The positive contours, which comprise the diagonal peaks and the exchange cross peaks, are plotted in Figure 1. As indicated, exchange cross peaks are observed for the amide protons of the Gln, Phe, Gly, and Cys⁶ residues.

The dynamics of cis ⇌ trans isomerization were quantitatively characterized using the one-dimensional inversion-transfer method.[10] The trans resonance of a given cis/trans pair of resonances was selectively inverted, and the transfer of inversion to the cis resonance by rotation around the Cys⁶–Pro peptide bond was monitored.[12] To illustrate, inversion-transfer data for OT in aqueous solution at 65 °C are presented in Figure 2. The integrated intensity of the Cys⁶-NH resonance for the cis isomer is plotted as a function of the mixing (exchange) time following inversion of the Cys⁶-NH resonance for the trans isomer. The smooth curve through the points is the theoretical curve calculated with parameters derived from a nonlinear least-squares analysis of the data,[14] including a lifetime τ_{1c} for the trans isomer of 5.77 s.

Rate constants for trans-to-cis interconversion, $k_{1c} = 1/\tau_{1c}$, were determined at ~5 °C temperature intervals over the temperature range 58–72 °C for OT in aqueous solution, 58–72 °C for AVP in aqueous solution, 21–35 °C for OT in CD_3OH, and 35–49 °C for AVP in CD_3OH. The cis/trans pair of resonances for the Cys⁶-NH proton was used to characterize the cis ⇌ trans isomerization of OT in aqueous and CD_3OH solution. The Phe-NH and Gln-NH resonances were used for AVP in aqueous and CD_3OH solution, respectively.

The activation parameters $\Delta H^{\ddagger}_{1c}$ and $\Delta S^{\ddagger}_{1c}$ for trans-to-cis interconversion were obtained from Eyring plots of $\ln(k_{1c}/T)$ vs $1/T$:[17,18]

$$\ln(k_{1c}/T) = -\Delta H^{\ddagger}_{1c}/RT + \Delta S^{\ddagger}/R + \ln(k_B/h) \qquad (1)$$

where k_B is the Boltzman constant, h is Planck's constant, and R is the gas constant. The results obtained for $\Delta H^{\ddagger}_{1c}$ and $\Delta S^{\ddagger}_{1c}$ and the calculated value of $\Delta G^{\ddagger}_{1c}$ at 25 °C are presented in Table I.

Also listed in Table I are equilibrium constants for the cis ⇌ trans isomerization, $K_{1/c}$ = [trans]/[cis], and values calculated from K_{1c} for the free energy of cis → trans interconversion, ΔG°_{ct}. The rate constants listed in Table I for trans-to-cis interconversion at 25 °C were calculated using eq 1 and the slopes and intercepts of the Eyring plots.

Table I. Kinetic and Thermodynamic Parameters for cis/trans Isomerizations of Oxytocin and Arginine–Vasopressin in Aqueous and Methanol Solutions

	OT		AVP	
parameter	H_2O	CD_3OH	H_2O	CD_3OH
$\Delta H^{\ddagger}_{tc}$ (kcal/mol)[a]	19.9 ± 1.9	17.0 ± 2.1	17.1 ± 2.0	15.6 ± 1.5
$\Delta S^{\ddagger}_{tc}$ (cal/mol)[a]	−3 ± 5	−7 ± 7	−12 ± 5	−13 ± 5
$\Delta G^{\ddagger}_{tc}$ (kcal/mol)[b]	20.8	18.9	20.6	19.5
ΔG°_{ct} (kcal/mol)	−1.5	−1.8	−1.6	−1.9
$K_{t/c}$	12 ± 2	20 ± 3	15 ± 2	25 ± 4
k_{tc} (s^{-1})[a,b]	(3.5 ± 0.3) × 10^{-3}	(8.1 ± 0.3) × 10^{-2}	(4.6 ± 0.2) × 10^{-3}	(3.0 ± 0.4) × 10^{-2}
k_{ct} (s^{-1})[b]	0.042	1.6	0.067	0.75
$\Delta G^{\ddagger}_{ct}$ (kcal/mol)[b,c]	19.3	17.1	19.0	17.6

[a] Uncertainties calculated using the standard errors of the estimates of the slopes and intercepts obtained from linear least-squares fits of the kinetic data to eq 1. [b] At 25 °C. [c] Calculated using $\Delta G^{\ddagger}_{tc}$ and ΔG°_{tc}.

Table II. Kinetic and Thermodynamic Parameters for cis/trans Isomerization of the Xaa–Pro Peptide Bond in Selected Peptides[a]

	$K_{t/c}$[b]	k_{tc}, s^{-1}	$\Delta G^{\ddagger}_{tc}$, kcal/mol	k_{ct}, s^{-1}	$\Delta G^{\ddagger}_{ct}$, kcal/mol
Gly-Pro[c]	1.7	4.8×10^{-3}	20.6	7.9×10^{-3}	20.3
Gly-Pro[d]	1.7	1.3×10^{-3}	21.3	2.2×10^{-3}	21.0
Gly-4-OH-Pro[c,e]	1.8	3.6×10^{-3}	20.8	6.6×10^{-3}	20.4
Ala-Pro[d]	1.6	1.5×10^{-3}	21.3	2.4×10^{-3}	21.0
Glu-Pro[d]	1.6	8.3×10^{-4}	21.6	1.3×10^{-3}	21.3
His-Pro[d]	2.7	6.3×10^{-4}	21.8	1.7×10^{-3}	21.2
Ala-Ala-Pro[d]	3.0	8.3×10^{-4}	21.7	2.5×10^{-3}	21.0
Phe-Pro-Ala[d]	1.9	2.3×10^{-3}	21.1	4.5×10^{-3}	20.7

[a] In aqueous solution at 25 °C.[20] [b] $K_{t/c}$ = [trans]/[cis]. [c] Reference 19. [d] Reference 29. [e] Glycyl-4-hydroxy proline.

Discussion

The equilibrium constants reported in Table I indicate that the trans conformation across the Cys⁶–Pro peptide bond is strongly favored for both OT and AVP and that the relative abundance of the trans isomer is further increased when the solvent is changed from water to methanol. A comparison of the kinetic and thermodynamic parameters in Table I with similar parameters for other proline-containing peptides provides some insight into factors affecting the cis ⇌ trans equilibrium for OT and AVP. In Table II are listed values for k_{1c}, $\Delta G^{\ddagger}_{1c}$, k_{c1}, and $\Delta G^{\ddagger}_{c1}$ at 25 °C for a group of di- and tripeptides having equilibrium constants $K_{1/c}$ in the range of 1–3.[2a,19,20] The rate constants for trans-to-cis interconversion, k_{1c}, for these peptides are in the range 6.3×10^{-4} to 4.8×10^{-3} s^{-1}, which corresponds to free energies of activation of 20.6–21.8 kcal/mol. Although $K_{1/c}$ is considerably larger for OT and AVP than for the peptides listed in Table II, k_{1c} and $\Delta G^{\ddagger}_{1c}$ for OT and AVP are remarkably similar to k_{1c} and $\Delta G^{\ddagger}_{1c}$ for the smaller peptides. This suggests that the greater abundance of the trans conformation for OT and AVP is not a result of stabilization of the trans conformation by intramolecular interactions, e.g., interactions between the tripeptide tails and the macrocyclic hexapeptide rings. Rather, the rate constants for cis-to-trans interconversion, k_{c1}, indicate that the larger equilibrium constants for OT and AVP are a result of considerably faster rates of cis-to-trans interconversion. It is not apparent

(16) Neuhaus, D.; Williamson, M. *The Nuclear Overhauser Effect in Structural and Conformational Analysis*; VCH Publishers, Inc.: New York, 1989; p 212.
(17) Eyring, H. *J. Chem. Phys.* **1935**, *3*, 107–115.
(18) Maskill, H. The Physical Basis of Organic Chemistry; Oxford University Press: Oxford, 1985; p 247.
(19) Mariappan, S. V. S.; Rabenstein, D. L. *J. Org. Chem.* **1992**, *57*, 6675–6678.
(20) The values listed in Table II for $\Delta G^{\ddagger}_{ct}$ for peptides from ref 2a were recalculated using k_{ct} and the relation $k_{ct} = (k_B T/h) \exp(-\Delta G^{\ddagger}_{ct}/RT)$. k_{tc} was calculated from k_{ct} and $K_{t/c}$, and $\Delta G^{\ddagger}_{tc}$ was calculated from k_{tc}.

what structural features destabilize the cis conformations. However, it will be of interest to elucidate these features, particularly since the results of energy minimization studies suggest that the rates of cis ⇌ trans isomerization of proline peptide bonds in proteins are greatly influenced by the protein matrix.[7]

The rate and equilibrium parameters for cis ⇌ trans isomerization are significantly different in methanol solution for both OT and AVP: $K_{t/c}$ increases, as do both k_{tc} and k_{ct}, with corresponding decreases in $\Delta G^{\ddagger}_{tc}$ and $\Delta G^{\ddagger}_{ct}$.[21] The increases in k_{tc} and k_{ct} are approximately a factor of 4 larger for OT than for AVP. Also, for both OT and AVP, k_{ct} is increased by a larger factor than is k_{tc}, indicating an additional destabilization of the cis conformations relative to the trans in methanol solution. A mechanism proposed recently for cis ⇌ trans interconversion by rotation around the Xaa–Pro peptide bond involves a twisted amide bond in the transition state, with no nucleophilic participation by the solvent.[6] In the twisted transition state, polar resonance structures are no longer possible. Thus, according to this mechanism, the rate of cis ⇌ trans interconversion is expected to be faster in nonaqueous solvents. This has been observed for rotation around the C–N bond of simple amides[23] and the imide bond of the simple peptide Ac-Gly-Pro-OMe.[22] The finding in the present study that the rates of cis ⇌ trans interconversion are significantly larger for OT and AVP in methanol solution suggests that rotation around the Cys[6]–Pro imide bonds of OT and AVP also proceeds via the twisted transition-state mechanism. However, the different effect of solvent on k_{ct} and k_{tc} for both OT and AVP indicates that additional factors are involved, e.g., changes in conformation which further destabilize the cis isomers in methanol solution.

Acknowledgment. This research was supported in part by National Institutes of Heath Grant GM 37000 and by the University of California, Riverside, Committee on Research. An American Chemical Society Analytical Division Fellowship, sponsored by Proctor and Gamble Co. (C.K.L.) and a Dissertation Fellowship from UC Riverside (C.K.L.) are gratefully acknowledged.

(21) In contrast, the equilibrium constant for the dipeptide Ac-Gly-Pro-OMe is independent of solvent in water, *N*,*N*-dimethylformamide, acetonitrile, trifluoroethanol, ethanol, isopropyl alcohol, toluene, benzene, and dioxane.[22]

(22) Eberhardt, E. S.; Loh, S. N.; Hinck, A. P.; Raines, R. T. *J. Am. Chem. Soc.* **1992**, *114*, 5437–5439.

(23) (a) Neuman, R. C., Jr.; Woolfenden, W. R.; Jonas, V. *J. Phys. Chem.* **1969**, *73*, 3177–3180. (b) Neuman, R. C.; Jonas, V.; Anderson, K.; Barry, R. *Biochem. Biophys. Res. Commun.* **1971**, *44*, 1156–1161. (c) Drakenberg, T.; Forsén, S. *Chem. Commun.* **1971**, 1404–1405. (d) Drakenberg, T.; Dahlquist, K.-I.; Forsén, S. *J. Phys. Chem.* **1972**, *76*, 2178–2183.