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Solvent Effects on the Energetics of Prolyl Peptide Bond Isomerization

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Abstract

Racemic Ac-Gly–[β,δ-^{13}C]Pro-OMe was synthesized, and the kinetics and thermodynamics of the isomerization of its prolyl peptide bond were determined in nine solvents by using NMR and IR spectroscopy. The free energy of activation is 1.3 kcal/mol larger in water than in aprotic solvents, and correlates with the ability of a solvent to donate a hydrogen bond but not with solvent polarity. These results are consistent with conventional pictures of amide resonance, which require transfer of charge between oxygen and nitrogen during isomerization. Similar medium effects may modulate the stability of planar peptide bonds in the active site of peptidyl-prolyl cis–trans isomerases (PPIases) and during the folding, function, or lysis of proteins.

The interconversion of cis (*E*) and trans (*Z*) isomers of peptide bonds that include the nitrogen of proline residues can give rise to a slow kinetic phase during protein folding.[1,2] This interconversion is catalyzed by the peptidyl-prolyl cis–trans isomerases (PPIases).[3,4] Two of these enzymes, cyclophilin and FK-506 binding protein (FKBP), have been studied extensively: (1) isotope effects and analyses of mutant enzymes[6] suggest that the prolyl peptide bond does not suffer nucleophilic attack during catalysis, (2) calorimetry shows that binding to FKBP occurs with a large decrease in heat capacity,[7] and (3) structural studies of cyclophilin[8] and FKBP[9] reveal active sites composed of hydrophobic side chains.[10] Consequently, desolvation has been proposed as a significant contributor to catalysis by the PPIases.[11] This proposal is consistent with NMR line shape analyses of simple amides, which suggest that the rate of amide bond isomerization does indeed depend on solvent.[12] To assess the contribution of desolvation to catalysis by the PPIases, we have determined the effect of solvent on the energetics of prolyl peptide bond isomerization (eq 1).

We performed our analyses on the simplest dipeptide that contains a prolyl peptide bond. Racemic Ac–Gly–[β,δ-^{13}C]Pro–OMe (**1**) was synthesized using standard methods.[13] The N- and C-termini of **1** were protected so as to minimize intramolecular electrostatic interactions.[14] Solvent effects on the rate constants for the isomerization of the prolyl peptide bond of **1** were determined using inversion transfer ^{13}C NMR spectroscopy.[15,16] These measurements were performed at temperatures at which the rate constants were in the range detectable by NMR spectroscopy.[17] Solvent effects on the amide I vibrational mode of Ac-Pro-OMe, a model of **1** with only one amide bond, were determined using IR spectroscopy.[18]

The origin of the barrier to the isomerization of amide bonds is commonly attributed to the double-bond character of the C–N bond, which results in net transfer of charge from nitrogen to the carbonyl carbon[19] or oxygen[20] (or both[21]). If the amide group has greater

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Supplementary Material Available: Figures showing ^{13}C NMR spectrum of **1** ($CDCl_3$) and IR spectrum of Ac-Pro-OMe (aqueous), and a table listing activation parameters for isomerization of **1** in all solvents studied (4 pages). Ordering information is given on any current masthead page.

charge separation when planar than when orthogonal, then its isomerization via an orthogonal transition state should be faster in less polar solvents.[22] Further, if the partial charge on oxygen is greater in planar than in orthogonal amides, then protic solvents should restrict isomerization by forming a hydrogen bond to oxygen.[12,23]

Temperature effects on the rate constant for the isomerization of **1** in different solvents are shown as Arrhenius plots in Figure 1. The data in Figure 1 indicate qualitatively that protic solvents restrict isomerization of **1**. The rate constants for the isomerization of **1** do not, however, correlate with solvent dielectric constant or with other measures[22] of solvent polarity. The rate constants do correlate with the ability of a solvent to donate a hydrogen bond. The relationship between the free energy of activation for the isomerization of **1** and the frequency of its amide I absorption band is shown in Figure 2. The amide I vibrational mode, which is primarily a C=O stretch, absorbs at lower frequency with increasing strength of a hydrogen bond to the amide oxygen.[24,25] The data in Figure 2 therefore suggest that the barrier to isomerization ($\Delta G^{\ddagger}$) is proportional to the strength of hydrogen bonds formed to the amide oxygen (given by υ). These results are consistent with conventional pictures of amide resonance (eq 1), which require transfer of charge between oxygen and nitrogen during isomerization.[20,21]

Solvent effects on the equilibrium constant for the isomerization of **1** are small. The value of the equilibrium constant for all solvents studied was $K = k_{EZ}/k_{ZE} = 4.3 \pm 0.9$ at 60°C, as calculated by interpolating the Arrhenius plots of Figure 1.[26] This lack of a solvent effect on K is also evident from the parallel lines in Figure 2. The absence of a significant solvent effect on K is consistent with the behavior observed for other amides.[3d]

Activation parameters indicate that the barrier to isomerization of **1** is almost entirely enthalpic in all solvents studied, as observed with other amides.[12,27] The values of $\Delta G^{\ddagger}$ (Figure 2) for the isomerization of **1** are, however, 1–2 kcal/mol smaller than the analogous values for acyclic tertiary amides.[12a,d] The smaller barriers for prolyl peptide bond isomerization may result from pyramidalization of the prolyl nitrogen, which decreases amide resonance.[28]

The PPIases decrease the free energy of activation for prolyl peptide bond isomerization by 8 kcal/mol.[3d] Desolvation alone can apparently account for 1.3 kcal/mol of this decrease (Figure 2).[29] Similar medium effects may modulate the stability of planar peptide bonds during the folding,[1,2] function,[30] or lysis[28] of proteins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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16. NMR experiments were done on a Brucker AM500 or Varian VXR500 instrument (125.77 MHz). Samples contained 0.1 M **1** in dry solvents that were fully deuterated (except trifluoroethanol:

external deuterium lock) and neat (except water: 100 mM sodium phosphate buffer, pH 7.2, containing 80% (v/v) H_2O). Isomerization rates were not altered by spiking the organic solvents with 0.2 M H_2O or by halving the concentration of **1**.

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18. IR experiments were done on a Nicolet 5PC spectrometer at 25°C using NaCl or CaF_2 plates or a ZnSe crystal. Samples contained 0.01 M Ac-Pro-OMe (Bachem Bioscience, Inc.), except for water and dimethylformamide, which contained 2 M Ac-Pro-OMe. The frequency of the amide I vibrational mode was determined to within 3 cm^{-1}, and was not altered by doubling the concentration of Ac-Pro-OMe or by raising the temperature to 60°C.
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26. The equilibrium population of the cis isomer of **1** at 60°C varies from 14% (in trifluoroethanol) to 24% (in *N*,*N*-dimethylformamide).
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29. Since the free energy of desolvation of a proline residue is 3.0 kcal/mol (Gibbs PR, Radzicka A, Wolfenden R. J Am Chem Soc. 1991; 113:4714–4715.), desolvation destabilizes by 1.7 kcal/mol the transition state for prolyl peptide bond isomerization. Interactions (such as hydrogen bonds) may stabilize by 6.7 kcal/mol an orthogonal transition state in the active sites of the PPIases. For a discussion of the manifestation of binding energy in enzymatic catalysis, see: Hansen DE, Raines RT. J Chem Educ. 1990; 67:483–489.
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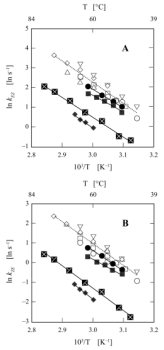


Figure 1.

Arrhenius plots for the cis to trans (A) and trans to cis (B) isomerizations of **1** in different solvents. Solvents (dielectric constant at 25°C) were as follows: ◇, dioxane (2.21); ○, benzene (2.27); ▽, toluene (2.38); ●, isopropyl alcohol (19.92); ■, ethanol (24.55); ◆, trifluoroethanol (26.14); □, acetonitrile (35.94); △, *N*,*N*-dimethylformamide (36.71); and ⊠, water (78.30). Linear regression analysis is shown for each protic solvent (—) and all aprotic solvents (---).

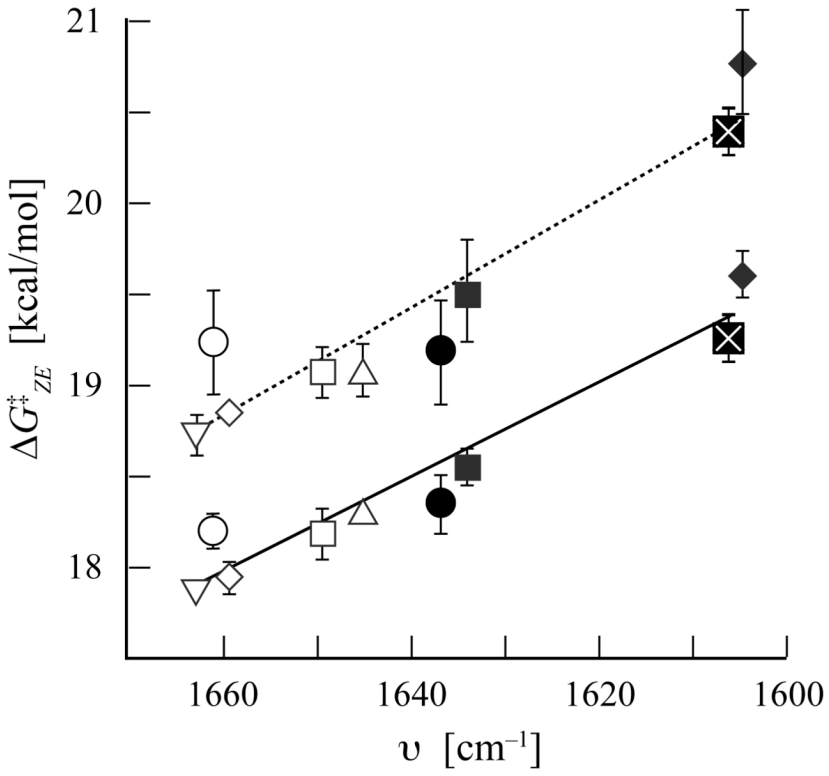
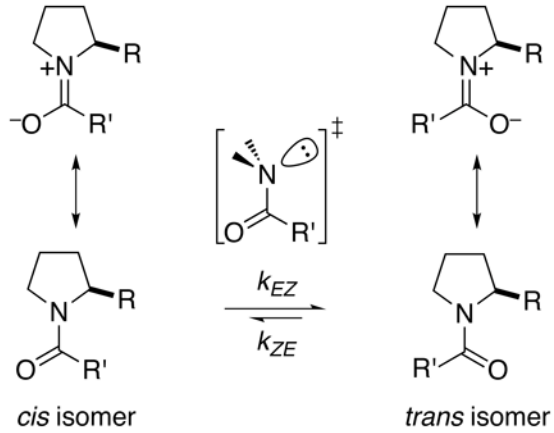


Figure 2.

Plots of $\Delta G^{\ddagger}$ for isomerization of **1** vs υ of amide I vibrational mode of Ac-Pro-OMe in different solvents. Symbols are as in Figure 1. Values of $\Delta G^{\ddagger}$ were calculated by interpolating the Arrhenius plots of Figure 1 at 60°C. Weighted linear regression analysis is shown for cis to trans [—, slope = − 0.025 ± 0.003 kcal·cm/mol] and trans to cis [---, slope = − 0.029 ± 0.002 kcal·cm/mol]. $\Delta G^{\ddagger}_{\text{aprotic}} - \Delta G^{\ddagger}_{\text{water}} = 1.3 \pm 0.2$ kcal/mol.



Equation 1.