

Thermodynamic Origin of Prolyl Peptide Bond Isomers

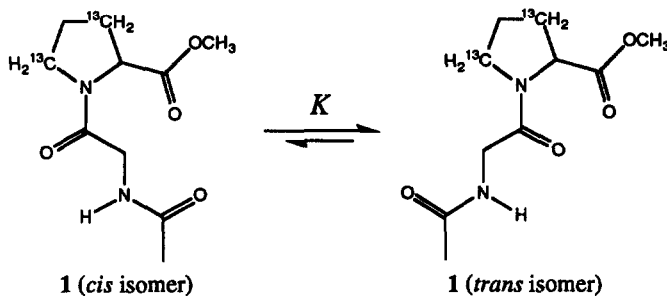
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Abstract: The preference for the *trans* isomer of prolyl peptide bonds arises almost entirely from enthalpy in aqueous buffer and in toluene.

The *trans* (*Z*) isomer of a typical peptide bond is favored greatly over the *cis* (*E*) isomer. In contrast, a *trans* bond involving the nitrogen atom of a proline residue is favored only slightly, and both isomers are common in peptides and folded proteins.¹ Knowing the thermodynamic origin for the relative stability of X-Pro bond isomers is essential for understanding the conformation of peptides and proteins containing such bonds.² The difference in enthalpy for the *cis* and *trans* isomers of X-Pro bonds in aqueous solution has been reported to be zero for model peptides,³ or small (ca. 1.2 kcal/mol) for poly(Pro-Gly).⁴ The difference in free energy for the *cis* and *trans* isomers of amides has been calculated with the 6-31G** basis set of the Gaussian 82 *ab initio* program to be largely enthalpic in the gas phase.⁵ We have synthesized a peptide containing ¹³C-labeled proline, and used ¹³C NMR spectroscopy to determine the precise difference in enthalpy and entropy between the X-Pro bond isomers in protic and aprotic solvents.

Racemic Ac-Gly-[(β,δ-¹³C)Pro-OMe] (1) was synthesized by using standard methods.⁶ The *N*- and *C*-



termini of 1 were capped to minimize intramolecular electrostatic interactions, which have been shown to alter the relative stability of the *cis* and *trans* isomers of X-Pro bonds.⁷ The equilibrium constant (*K*) for the isomerization of 1 was determined by integration of the

C_{β} resonances observed with ¹³C NMR spectroscopy at temperatures relevant for the study of protein stability.⁸

The effect of temperature on the value of *K* in aqueous buffer and in toluene is shown in Fig. 1. Van't Hoff analysis of these results (assuming $\Delta C_p^{\circ} = 0$) indicates that the difference in free energy for the X-Pro isomers of 1 originates almost entirely from enthalpic differences between these isomers. Further, the similarity of the enthalpies determined in aqueous buffer [$\Delta H^{\circ} = -(1.27 \pm 0.04)$ kcal/mol] and in toluene [$\Delta H^{\circ} = -(1.27 \pm 0.06)$ kcal/mol] suggests that the enthalpic forces that differentiate the *cis* and *trans* isomers of prolyl peptide bonds are similar in protic and aprotic environments. Differences in entropy, though

small, favor the *cis* isomer in both aqueous buffer and toluene. This entropic preference is, however, less in aqueous buffer [$\Delta S^\circ = -(0.25 \pm 0.11)$ cal-mol/K] than in toluene [$\Delta S^\circ = -(0.71 \pm 0.18)$ cal-mol/K]. This result is consistent with the lower solvent accessibility of the amide C=O group in the *trans* isomer of **1**, which diminishes the ability of this group to restrict H₂O molecules through hydrogen bonding.⁹

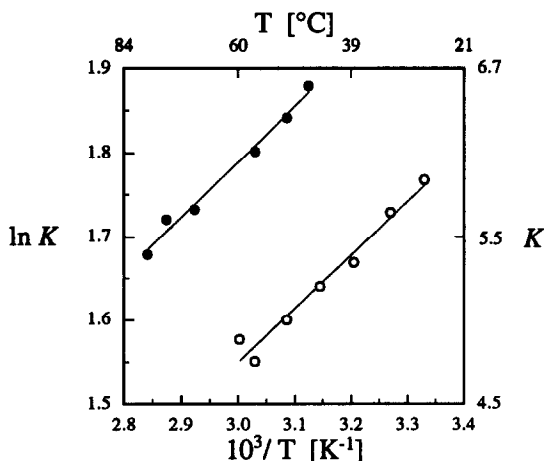


Fig. 1. Van't Hoff plot for the *cis* to *trans* isomerization of **1**.

●, aqueous buffer:

$$\Delta H^\circ = -(1.27 \pm 0.04) \text{ kcal/mol}$$

$$\Delta S^\circ = -(0.25 \pm 0.11) \text{ cal-mol/K}$$

○, toluene:

$$\Delta H^\circ = -(1.27 \pm 0.06) \text{ kcal/mol}$$

$$\Delta S^\circ = -(0.71 \pm 0.18) \text{ cal-mol/K}$$

At 25°C in aqueous buffer:

$$\Delta G^\circ = -(1.34 \pm 0.05) \text{ kcal/mol}$$

At 25°C in toluene:

$$\Delta G^\circ = -(1.48 \pm 0.08) \text{ kcal/mol}$$

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- NMR experiments were performed on a Bruker AM500 instrument. Samples contained 0.1 M **1** in 100 mM sodium phosphate buffer, pH 7.2, containing 20% (v/v) D₂O, or in dry toluene-*d*₆. ¹³C NMR of **1** (125.77 MHz, CDCl₃, 25 °C) δ 29.01 (C_β, *trans*), 31.26 (C_β, *cis*), 45.96 (C_γ, *trans*), 46.61 (C_γ, *cis*). δ was essentially independent of solvent or temperature.
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