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Thermodynamic Origin of Prolyl Peptide Bond Isomers

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Abstract

The thermodynamic 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 folded proteins.[1] Knowing the thermodynamic origin for the relative stability of peptide bond isomers is essential for understanding the thermodynamic basis of protein stability.[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 ^{13}C-labeled proline, and used ^{13}C 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,γ-^{13}C]Pro–OMe (**1**) was synthesized by using standard methods.[6] The *N*-and *C*-termini of **1** were capped so as 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 ^{13}C 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. The entropy difference is, however, less in water [$\Delta S^\circ = -(0.25 \pm 0.11)$ 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 H_2O molecules through hydrogen bonding.[9]

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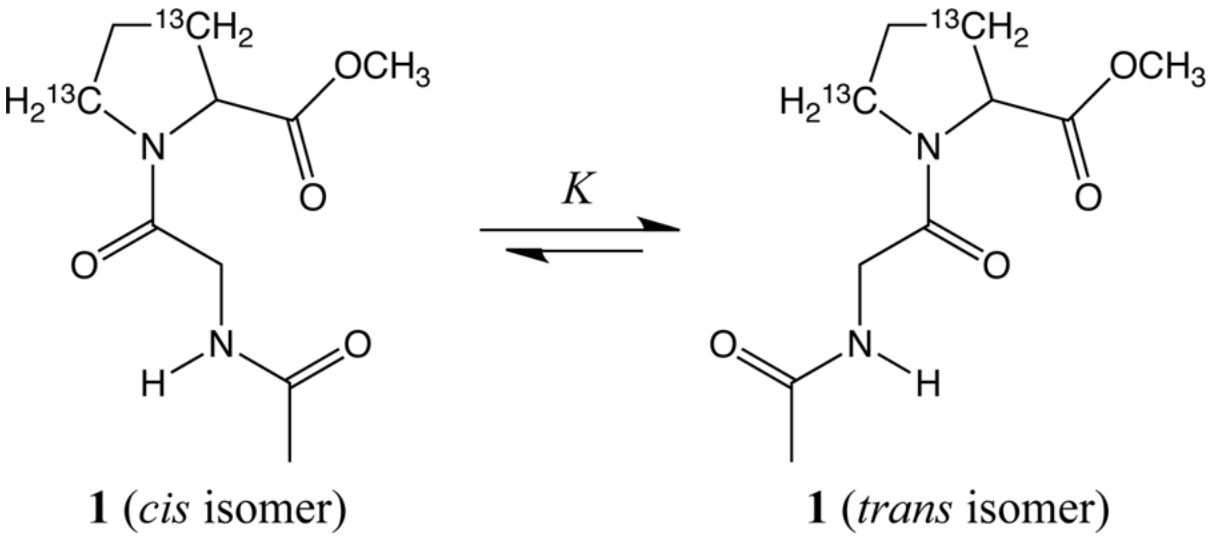

1 (*cis* isomer) **1** (*trans* isomer)

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8. 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) D_2O, or in dry toluene-d_8. ^{13}C NMR of **1** (125.77 MHz, $CDCl_3$, 25 °C) δ 29.01 (C_β, *trans*), 31.26 (C_β, *cis*), 45.96 (C_δ, *trans*), 46.61 (C_δ, *cis*). δ was essentially independent of solvent or temperature.
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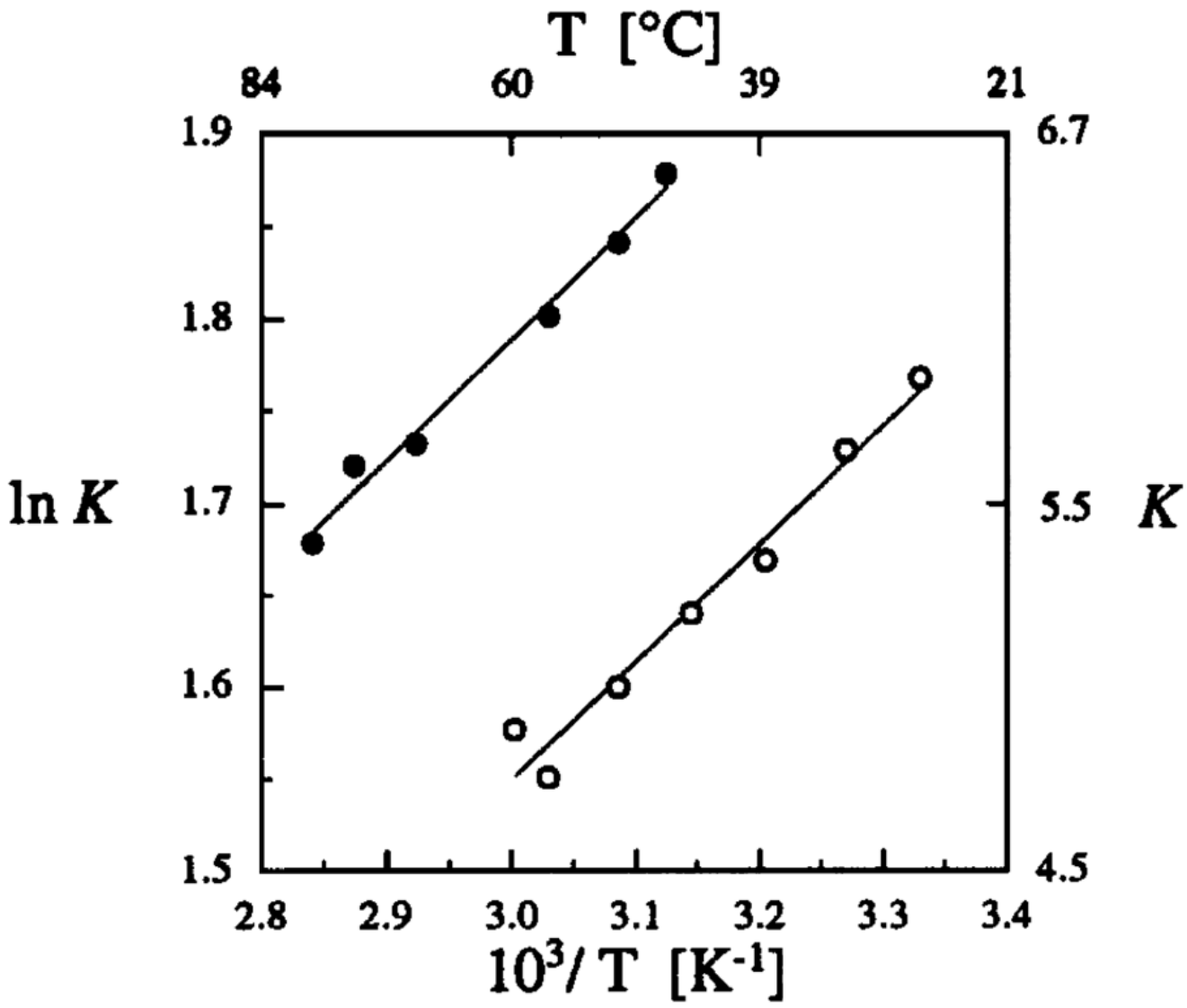

Fig. 1.

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

●, aqueous buffer:

$\Delta H° = -(1.27 \pm 0.04)$ kcal/mol

$\Delta S° = -(0.25 \pm 0.11)$ cal·mol/K

○, toluene:

$\Delta H° = -(1.27 \pm 0.06)$ kcal/mol

$\Delta S° = -(0.71 \pm 0.18)$ cal·mol/K

At 25°C in aqueous buffer:

$\Delta G° = -(1.34 \pm 0.05)$ kcal/mol

At 25°C in toluene:

$\Delta G° = -(1.48 \pm 0.08)$ kcal/mol