Intramolecular Catalysis of Amide Isomerization

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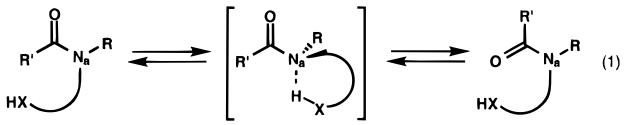
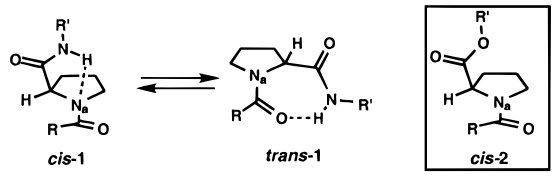
The catalysis of amide bond isomerization (AI) by Brönsted acids is a well-documented reaction that proceeds through a putative N-protonated intermediate.[1] On the other hand, intramolecular general acid-catalyzed AI is a much less-studied but likely biologically-relevant process in which hydrogen bond (H-bond) donation to the amide nitrogen (N_a) through a correctly aligned cyclic intermediate replaces discrete N-protonation (eq 1).[2] As a consequence, the optimal positioning of a donor moiety should permit *direct observation* of the catalytically-active [X−H--N_a] H-bond.



(1)

Intramolecular catalysis of AI is believed to play a key role in the folding of several proteins including dihydrofolate reductase,[3] and Karplus et al. have proposed in a theoretical study that it contributes to cyclophilin and FKBP-promoted folding, whereby the enzyme induces the side chain amide to donate an H-bond to the prolyl-N_a (*cis*-**1**).[4] The authors predicted that the effect should be general and *measurable in model prolines*; however, experimental conformation of these proposals has yet to appear. Rotamase enzymes, including FKBP and cyclophilin, catalyze protein folding through *cis*−*trans* proline isomerization (PI).[1b,5] Details of the mechanisms by which FKBP- and cyclophilin-catalyzed PI occur still remain to be clarified.[4] MO calculations indicate a *cis-to-trans* barrier lowering of 1.4 kcal/mol for the component of FKBP-induced peptide folding due to intramolecular catalysis.[4b] In this Communication, we report the first experimental study of intramolecular catalysis of AI in model systems, including evidence for an H-bond between the side chain and the prolyl N_a in a *cis*-proline peptidomimetic.[6]

We reasoned that small peptides containing the correct structure should show intramolecular catalysis in an organic medium that mimics the desolvated environment[7] of the FKBP enzyme active site, thus permitting clear-cut documentation of the process free from other effects. At first we chose to compare activation barriers for two sterically similar prolines in aqueous and organic media; one contains the requisite N−H general acid in the side chain, the other not, while both side chains are essentially isosteric. Amides **1** and esters **2** fulfill these criteria; in nonpolar solution, we expect the *cis* form of amides **1** to have an H-bonding interaction between the side chain and the prolyl ring N_a;[8] this interaction should be strengthened in the transition state for *cis-to-trans* PI (eq 2). The more stable *trans*



(2)

1a R = 2-fluorophenyl; R' = n-hexyl
1b R = methyl; R' = phenyl
1c R = methyl; R' = 4-methoxyphenyl
1d R = methyl; R' = 4-carbomethoxyphenyl
2a R = 2-fluorophenyl; R' = n-hexyl
2b R = methyl; R' = phenyl
2c R = methyl; R' = 4-methoxyphenyl
2d R = methyl; R' = 4-carbomethoxyphenyl

form contains an H-bond within a seven-membered ring in organic solvents (*trans*-**1**). Thus we define intramolecular catalysis (IC) as $\Delta\Delta G^{\ddagger}$ in the change from aqueous solution to an organic solvent for model amides, subtracted by the comparable $\Delta\Delta G^{\ddagger}$ for model esters (eq 3). We monitored PI

$$\mathrm{IC} = [\Delta G^{\ddagger}_{\mathrm{amide(aqueous)}} - \Delta G^{\ddagger}_{\mathrm{amide(organic)}}] - [\Delta G^{\ddagger}_{\mathrm{ester(aqueous)}} - \Delta G^{\ddagger}_{\mathrm{ester(organic)}}] \quad (3)$$

in prolines by ^{19}F (**1a**−**2a**) and ^{1}H (**1b**−**d**, **2b**−**d**) saturation transfer (ST) NMR.[9,10] Full kinetic and thermodynamic profiles of *cis*−*trans* isomerization of prolinamide **1a** and proline ester **2a** were constructed from Eyring plots.[11] For example, in 1:1 H_2O/acetone,[12] the barriers to rotation ($\Delta G^{\ddagger}$'s) of amide **1a** and ester **2a** were found to be identical within experimental error at 25 °C. Equilibrium constants K ([*trans*]/[*cis*]) were also roughly equivalent. Under these conditions the effects of intramolecular H-bonding on PI are "washed out" by H_2O, so that IC is not observed. In $CDCl_3$ however, the barrier to rotation in amide **1a** dropped by 2.0 kcal/mol for the *trans-to-cis* isomerization and 3.2 kcal/mol for the *cis-to-trans*, whereas in ester **2a** the respective barrier lowerings were 0.7 and 0.8 kcal/mol (in line with a solvent effect),[13] leaving a difference of 1.3 kcal/mol (*trans-to-cis*) and 2.4 kcal/mol (*cis-to-trans*) that we ascribe to IC (Table 1). Slightly negative $\Delta S^{\ddagger}$ values were found in all

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(1) (a) Somayaji, V.; Brown, R. S. *J. Org. Chem.* **1986**, 51, 2676. (b) Stein, R. L. *Adv. Protein Chem.* **1993**, *44*, 1. Perrin investigated the mechanism of acid-catalyzed proton exchange in N-methyl amides: (c) Perrin, C. L.; Arrhenius, G. M. L. *J. Am. Chem. Soc.* **1982**, *104*, 6693.

(2) However, intermolecular hydrogen bonding to the carbonyl oxygen has a barrier-raising effect on AI: Scheiner, S.; Kern, C. W. *J. Am. Chem. Soc.* **1977**, *99*, 7042.

(3) In this case intramolecular catalysis applies to groups proximate in tertiary structure: Texter, F. L.; Spencer, D. B.; Rosenstein, R.; Matthews, C. R. *Biochemistry* **1992**, *31*, 5687.

(4) (a) Fischer, S.; Michnick, S.; Karplus, M. *Biochemistry* **1993**, *32*, 13830. (b) Fischer, S.; Dunbrack, Jr., R. L.; Karplus, M. *J. Am. Chem. Soc.* **1994**, *116*, 11931.

(5) (a) Harrison, R. K.; Stein, R. L. *Biochemistry* **1990**, *29*, 1684. (b) Schreiber, S. L. *Science* **1991**, *251*, 283. (c) Schmid, F. X. In *Protein Folding*; Creighton, T. E., Ed.; Freeman: New York, 1992; p 197.

(6) For earlier discussions of [N−H−N_a] interactions, see: (a) Gieren, A.; Dederer, B.; Schanda, F. *Z. Naturforsch.* **1980**, *35c*, 741. (b) Scarsdale, J. N.; Van Alsenoy, C.; Klimkowski, V. J.; Schäfer, L; Momany, F. A. *J. Am. Chem. Soc.* **1983**, *105*, 3438.

(7) Liang, G.-B.; Rito, C. J.; Gellman, S. H. *J. Am. Chem. Soc.* **1992**, *114*, 4440.

(8) Prolines prefer to place the side chain psuedoaxially, with the carbonyl group *exo* to the proline ring: Thomas, L. M.; Ramasubbu, N.; Bhandary, K. K. *Int. J. Peptide Protein Res.* **1994**, *44*, 207.

(9) Many proline derivatives show poor *cis*−*trans* ratios in nonpolar solvents. Our test substrates were chosen in part because sufficient *cis* form could be detected in chlorocarbon solvents to facilitate NMR analysis.

(10) For applications of ST to AI, see: Perrin, C. L.; Thoburn, J. D.; Kresge, J. *J. Am. Chem. Soc.* **1992**, *114*, 8800. We used ^{19}F ST NMR to take advantage of the broad chemical shift range and generally favorable peak separations of the ^{19}F nucleus, see: Cox, C.; Ferraris, D.; Murthy, N. N.; Lectka, T. *J. Am. Chem. Soc.* **1996**, *118*, 5332.

(11) ST measurements on all substrates were made at 15 mM in the solvent of choice. We found the degree of catalysis to be fairly insensitive to concentration. For a discussion of activation parameters, see: Carpenter, B. *Determination of Organic Reaction Mechanisms*; John Wiley: New York, 1984; p 123.

(12) A mixed solvent system (H_2O/acetone) affords excellent NMR peak separations; in general we find that the barriers to rotation in pure water are not significantly different.

(13) Solvent effects on PI have been measured: Eberhardt, E. S.; Loh, S. H.; Hinck, A. P.; Raines, R. T. *J. Am. Chem. Soc.* **1992**, *114*, 5437.

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Table 1. Kinetic and Thermodynamic Parameters for Prolines **1** and **2**

proline	solvent	$\Delta G^{\ddagger}$ [a,c]	$\Delta G^{\ddagger}$ [b,c]	$\Delta S^{\ddagger}$ [d]	$\Delta H^{\ddagger}$ [a]	K[e]	IC [f]
1a	H_2O/acetone	18.8 ± 0.3	18.7	−3.3 ± 1.0	17.8 ± 0.2	1.3	
1a	$CDCl_3$	16.8 ± 0.1	15.5	−3.0 ± 1.0	16.0 ± 0.3	9.8	2.4/1.3
2a	H_2O/acetone	18.9 ± 0.3	18.5	−1.1 ± 0.9	18.5 ± 0.2	2.0	
2a	$CDCl_3$	18.2 ± 0.1	17.7	−1.1 ± 1.0	17.9 ± 0.3	2.5	

[a] *Trans-to-cis* isomerization, kcal/mol. [b] *Cis-to-trans* isomerization, kcal/mol. [c] 25 °C. [d] cal/mol K. [e] $K = [trans]/[cis]$. [f] IC = degree of intramolecular catalysis, kcal/mol, first number is for the *cis-to-trans* isomerization, second is for *trans-to-cis*.

cases, consistent with other amides,[14] so that catalysis quantities defined in terms of either $\Delta H^{\ddagger}$ or $\Delta G^{\ddagger}$ are similar at 25 °C.

The degree of catalysis should correlate with the acidity of the side chain amide proton. For example, amide **1b**, with an anilide side chain, affords a 2.6 kcal/mol (*cis-to-trans*) barrier lowering at 25 °C in CD_2Cl_2. A remote electron donating substituent (**1c**, *p*-OMe) placed on the aryl group affords less catalysis (2.1 kcal/mol, *cis-to-trans*), whereas an electron withdrawing substituent (**1d**, *p*-COOMe) affords the greatest degree of catalysis (3.1 kcal/mol, *cis-to-trans*), representing a 188-fold rate enhancement. In order to better characterize what we believed would be an intramolecular H-bond in the *cis* isomer between the prolyl N_a and the side chain N−H, we made proline peptidomimetic **3** (R = 4-bromophenyl) that is locked in the *cis* conformation (eq 4).[15] It was our belief that **3** should faithfully model the H-bonding of actual *cis* proline substrates without interference from the *trans* isomer. The IR spectrum of **3** in $CHCl_3$ (3 mM) shows a band of a weakly H-bound N−H stretch at 3382 cm^{-1}.[16] At concentrations above 15 mM, a new band at 3300 cm^{-1} appears for **3** due to intermolecular H-bonding. To calibrate, control amide **4**, which cannot engage in intramolecular H-bonding, shows an N−H stretch at 3418 cm^{-1}. Given the locked geometry of **3**, the weak intramolecular H-bond must be between N−H and the prolyl ring N_a. Collectively, the data indicate a red shift of ca 36 cm^{-1} upon formation of an [N−H--N_a] interaction. Additional evidence for an [N−H--N_a] H-bond comes from X-ray crystallography of **3**,[17] which reveals a distance from the side chain proton (H[10A]) to the ring N_a of 2.35 Å,[18] and an N−N distance of 3.01 Å (Figure 1). H[10A] was refined positionally, and the asymmetric unit consists of two enantiomorphs of **3** and one-half molecule of benzene. Presumably the [N−H--N_a] interaction is weakened somewhat over that observed in solution by intermolecular H-bonding.

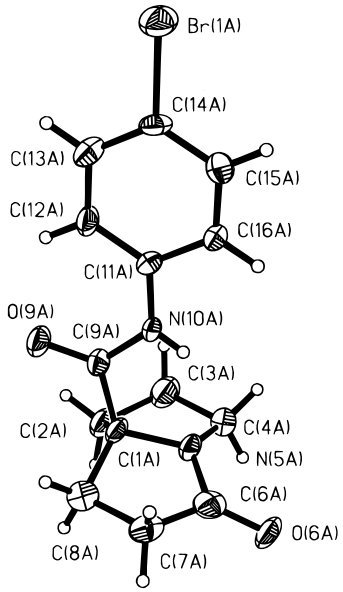
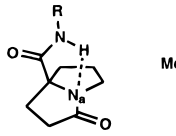
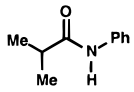


Figure 1. Crystal structure of **3** (50% ellipsoids). Selected bond distances (Å): H(10A)−N(5A) 2.35 (5); H(10A)−N(10A) 0.74 (5); N(5A)−N(10A) 3.01 (6); Selected bond angle (deg): N(10A)−H(10A)−N(5) 153.0.

Related studies on the catalysis of AI are underway and will be reported in due course.



3 (R = 4-bromophenyl)



4

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Supporting Information Available: Full X-ray parameters and spectroscopic data for **3**, an Eyring plot and ST measurement, and tabulated barriers to rotation for amides **1b**−**d** and esters **2b**−**d** (19 pages). See any current masthead page for ordering and Internet access instructions.

JA964017M

(14) Albers, M. W.; Walsh, C. T.; Schreiber, S. L. *J. Org. Chem.* **1990**, *55*, 4984.

(15) A discussion of proline peptidomimetics can be found in: Giannis, A.; Kolter, T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244.

(16) Thorough work by Gellman has established strong hydrogen bound vs nonhydrogen bound amide N−H IR frequencies; for example: Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1994**, *116*, 1054. Gardner, R. R.; Liang, G.-B.; Gellman, S. H. *J. Am. Chem. Soc.* **1995**, *117*, 3280.

(17) Crystals of *rac*-**3** were grown from a benzene/hexane solution. One antipode is depicted for simplicity. Crystal data for **3**: $a = 11.8158(2)$ Å, $b = 20.2329(3)$ Å, $c = 13.2733(3)$ Å, $\alpha = 90.00$, $\beta = 104.44\ (10)°$, $\gamma = 90.00$, space group = $P2_1/c$, R1 = 0.0558, $Z = 8$, GOF = 0.926.

(18) Based on sums of van der Waals radii (N and N, 3.40 Å; N and H, 2.70 Å; from Bondi, A. *J. Phys. Chem.* **1964**, *68*, 441), the [N−H--N_a] interaction of **3** in the crystal qualifies as a weak H-bond. Using Etter's criteria for bent [N−H--O] H-bonding in nitroaniline crystals, the bond of **3** (with a [N−H--N_a] bond angle of 112°) also qualifies: Panunto, T. W.; Urbáncyk-Lipkowska, Z.; Johnson, R.; Etter, M. C. *J. Am. Chem. Soc.* **1987**, *109*, 7786.