ORGANIC LETTERS

1999
Vol. 1, No. 5
749−752

Intramolecular Acid-Catalyzed Amide Isomerization in Aqueous Solution

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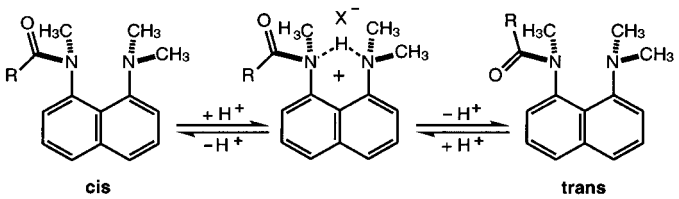
Received June 11, 1999

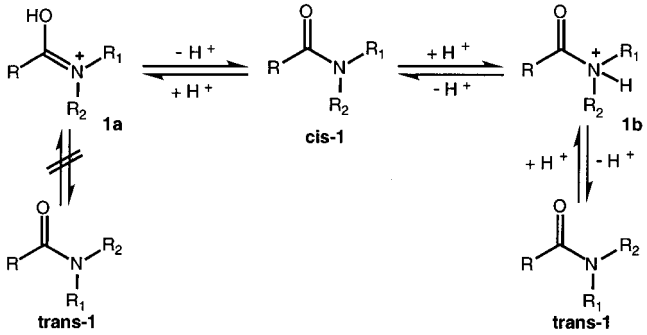
ABSTRACT



We report for the first time that stoichiometric and even catalytic quantities of weak acids in aqueous solution can very efficiently catalyze amide isomerization in a carefully designed system in which a proton donor is situated so that intramolecular hydrogen bonding to the amide nitrogen is highly favored. Our results provide the first experimental verification that hydrogen bond donation to the amide nitrogen by charged proton donors may play a very significant role in the enzymatic catalysis of amide isomerization.

After many years of experimental and theoretical investigations, it is now widely believed that the preferred site of protonation in simple amides is on the carbonyl oxygen.[1] In fact, the ratio of O- to N-protonated species has been calculated to exceed 10^6 for simple amides such as dimethylacetamide (DMA).[2] The resonance theory[3] Pauling advanced many years ago is consistent with this observation and predicts an increase in the double bond-character of the C−N bond upon protonation on oxygen (**1a**; Scheme 1). Perhaps surprisingly, the catalysis of rotation about the C−N bond in amides by strong Brønsted acids is a documented process.[4] For example, the rate of amide isomerization of DMA in water increases by 130-fold when the pH is lowered from 7.0 to 1.8.[4e] This anomaly is rationalized by assuming that although O-protonation is highly favored, a small but kinetically significant amount of N-coordinated species **1b** is present in equilibrium.[2b,5] Despite potential importance in biological systems, the catalysis of amide isomerization in

Scheme 1. Acid-Catalyzed Isomerization of Amides



(1) (a) Fraenkel, G.; Franconti, C. *J. Am. Chem. Soc.* **1960**, *82*, 4478. (b) Homer, R. B.; Johnson, C. D. In *The Chemistry of Amides*; Zabicky, J., Ed.; Wiley: New York, 1970; Chapter 3. (c) Werstiuk, N. H.; Brown, R. S.; Wang, Q. *Can. J. Chem.* **1996**, *74*, 524. (d) Cho, S. J.; Cui, C.; Lee, J. Y.; Park, J. K.; Suh, S. B.; Park, J.; Kim, B. H.; Kim, K. S. *J. Org. Chem.* **1997**, *62*, 4068.

(2) (a) Martin, R. B. *J. Chem. Soc., Chem. Commun.* **1972**, 793. (b) Martin, R. B.; Hutton, W. C. *J. Am. Chem. Soc.* **1973**, *95*, 4752.

(3) Pauling, L. *The Nature of the Chemical Bond*, 3rd ed.; Cornell University Press: Ithaca, NY, 1960; Chapter 1.

(4) (a) Berger, A.; Loewenstein, A.; Meiboom, S. *J. Am. Chem. Soc.* **1959**, *81*, 62. (b) Steinberg, I. Z.; Harrington, W. F.; Berger, A.; Sela, M.; Katchalski, E. *J. Am. Chem. Soc.* **1960**, *82*, 5263. (c) Jackman, L. M.; Kavanagh, T. E.; Haddon, R. C. *Org. Magn. Reson.* **1969**, *1*, 109. (d) Stewart, W. E.; Siddall, T. H., III. *Chem. Rev.* **1970**, *70*, 517. (e) Gerig; J. T. *Biopolymers* **1971**, *10*, 2435. (f) McClelland, R. A.; Reynolds, W. F. *Can J. Chem.* **1979**, *57*, 2896. (g) Redfield, A. G.; Waelder, S. *J. Am. Chem. Soc.* **1979**, *101*, 6153.

(5) Perrin has also determined that transiently N-protonated amides are intermediates on the pathway of acid-catalyzed proton exchange in some primary and secondary amides; see: Perrin, C. L. *Acc. Chem. Res.* **1989**, *22*, 268 and references therein.

10.1021/ol9901265 CCC: $18.00
Published on Web 08/05/1999

aqueous solution by stoichiometric amounts of acid has not been demonstrated.[6] We report herein that stoichiometric and even catalytic quantities of weak acids in aqueous solution can very efficiently catalyze amide isomerization in a carefully designed system in which a proton donor is situated so that intramolecular hydrogen bonding to the amide nitrogen is highly favored. Our results provide the first experimental verification that hydrogen bond donation to the amide nitrogen by charged proton donors may play a significant role in the enzymatic catalysis of amide isomerization.

Peptidylprolyl isomerases (PPIases) are "rotamase" enzymes that catalyze proline isomerization, a step known to be rate-limiting in the folding of many proteins,[7] both in vitro and in vivo.[8] These enzymes are also the biological targets of immunosupressive drugs used clinically to prevent organ and bone marrow graft rejection.[9] Many unanswered questions remain as to how the PPIases work mechanistically, but the efficiency of acid-catalyzed amide isomerization in nonenzymatic systems has led several authors to suggest that the enzymes use a similar tactic whereby proton donation from an active site donor to the proline nitrogen contributes to the catalysis of isomerization,[10] an interaction termed intramolecular catalysis. In the FKBP rotamase, the substrate is believed to bind as a type VIa proline turn wherein the amide NH adjacent to the proline residue is able to bend back on itself and donate a hydrogen bond to the prolyl amide nitrogen (N_a) (Figure 1A).[11] In contrast, cyclophilin is

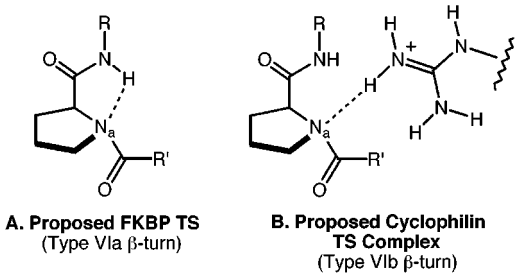
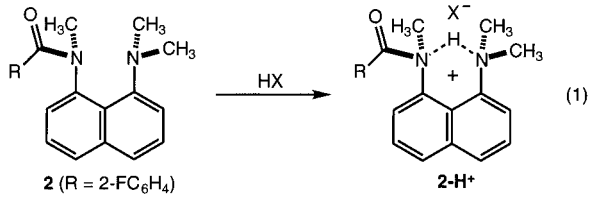


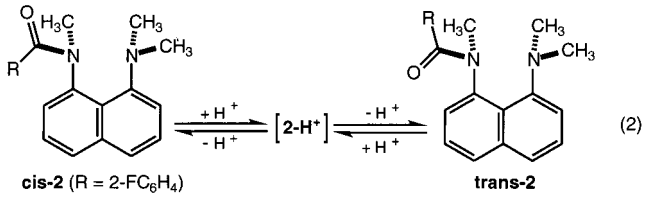
Figure 1. Intramolecular catalysis in the PPIases

believed to bind its substrate in a type VIb proline turn, in which the adjacent amide NH is not properly aligned to induce intramolecular catalysis;[10c] however, Arg^{55} is located within the active site of cyclophilin (but not in the active site of FKBP) and may act as the hydrogen bond donor during catalysis (Figure 1B).[10] Site-directed mutagenesis studies indicate that this residue is involved in enzymatic catalysis.[10b,12] A similar type of intramolecular catalysis of proline isomerization has also been proposed to play a role in the folding of proteins, such as dihydrofolate reductase, into their native forms.[13]

It is known that 1,8-disubstituted naphthalenes containing two peri substituents are ideal for inducing intramolecular interactions.[14] For example, we recently reported that "amide proton sponge" **2** forms a strong intramolecular hydrogen bond between the protonated amino group and the amide nitrogen in organic solvents when treated with acid (eq 1).[15]



The formation of **2**-H^+, characterized by a number of spectroscopic techniques including X-ray diffraction, resulted in unusual reactivity of the amide, such as increased sensitivity to attack by nucleophiles. The formation of **2**-H^+ was also expected to catalyze amide isomerization in aqueous solution (eq 2) by a process analogous to that proposed for the cyclophilin rotamase. The results from our kinetic study of this process are summarized in Table 1.



We used ^{19}F saturation transfer (ST) NMR to measure the rate of amide isomerization[16] and derived Eyring plots to determine the activation parameters for this process under various conditions in a 50/50 mixture of D_2O/EtOH. Due to

Table 1. Saturation Transfer Results on **2** and **3** in 50/50 D_2O/EtOH[a]

entry	substrate	additive	$\Delta G^{\ddagger}$ [b]	$\Delta H^{\ddagger}$ [c]	$\Delta S^{\ddagger}$ [d]
1	**2**		20.9	20.0	−3
2	**2**	0.5 equiv CAA	15.9	12.9	−10
3	**2**	1 equiv BA	17.2	14.2	−10
4	**3**		18.7	18.4	−1
5	**3**	10 equiv CAA	18.4	17.4	−3

[a] Performed on solutions of 5 mg/mL concentration; see the Supporting Information for experimental details and error analysis. Barriers reported are cis to trans. Abbreviations: CAA = chloroacetic acid; BA = benzoic acid. [b] At 25 °C; ±0.2 kcal/mol. [c] ±0.3 kcal/mol. [d] ±4 cal/(mol·K).

(6) In a recent report, researchers claim to have observed intramolecular catalysis of proline isomerization in mildly acidic aqueous solution for small peptides in which a His residue directly precedes the Pro in linear sequence; however, the increase in rate was very modest (<10-fold). See: Reimer, U.; Mokdad, N. E.; Schutkowski, M.; Fischer, G. *Biochemistry* **1997**, *36*, 13802.

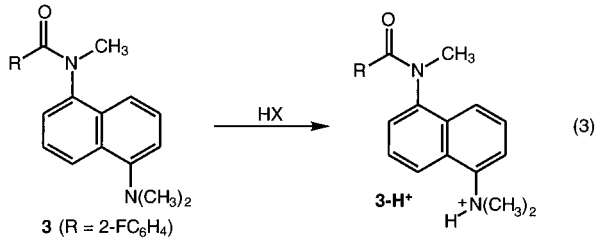
(7) (a) Brandts, J. F.; Halvorson, H. R.; Brennan, M. *Biochemistry* **1975**, *14*, 4953. (b) Schmid, F. X. In *Protein Folding*; Creighton, T. E., Ed.; Freeman: New York, 1992. (c) Reference 8b.

(8) (a) Stein, R. L. *Adv. Protein Chem.* **1993**, *44*, 1. (b) Schmid, F. X.; Mayr, L. M.; Mücke, M.; Schönbrunner, E. R. *Adv. Protein Chem.* **1993**, *44*, 25. (c) Fischer, G. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1415. For a description of the third, recently discovered class of PPIases termed the parvulins, see: (d) Rahfeld, J.-U.; Rücknagel, K. P.; Schelbert, B.; Ludwig, B.; Hacker, J.; Mann, K.; Fischer, G. *FEBS Lett.* **1994**, *352*, 180. (e) Scholz, C.; Rahfeld, J.; Fischer, G.; Schmid, F. X. *J. Mol. Biol.* **1997**, *273*, 752.

(9) (a) Fischer, G.; Wittmann-Liebold, B.; Lang, K.; Kiefhaber, T.; Schmid, F. X. *Nature* **1989**, *337*, 476−478. (b) Schreiber, S. L.; Crabtree, G. R. *Immunol. Today* **1992**, *13*, 136−142. (c) Fruman, D. A.; Burakoff, S. J.; Biere, B. E. *FASEB J.* **1994**, *8*, 391.

limited solubility, NMR studies could not be performed in pure water; however, we generally find that barriers to isomerization for water-soluble amides are similar in pure water and in water/polar organic solvent mixtures.[11b] As illustrated in entry 1, amide **2** had a free energy of activation ($\Delta G^{\ddagger}$) of 20.9 kcal/mol[17] that was totally enthalpic, in line with previous studies on amide isomerization.[18] Upon the addition of 1 equiv of triflic acid (HOTf), the isomerization was so rapid that no kinetic data could be obtained at accessible temperatures. The use of a catalytic amount of HOTf was prohibited by the fact that the proton underwent slow exchange on the NMR time scale, an effect that has been observed in the parent proton sponges.[19] After some experimentation, we found that the weaker chloroacetic acid (CAA) allowed for fast exchange, but was still strong enough to exhibit a large degree of catalysis. Upon the addition of 1 equiv of CAA, the cis and trans fluorine resonances shifted slightly upfield and broadened to some extent but, like HOTf, catalyzed the reaction too well to analyze kinetically by saturation transfer. However, 0.5 equiv of CAA produced a substantial but easily measured catalytic effect, with $\Delta G^{\ddagger} =$ 15.9 kcal/mol (entry 2). In contrast to **2** without acid catalysis, there is a measurably negative entropy of activation ($\Delta S^{\ddagger} = -10$ cal/(mol·K)) that corresponds to a greater degree of organization in the transition state for amide isomerization, consistent with the proposed intramolecular hydrogen bond. The use of 1 equiv of the weaker benzoic acid (BA) also shows a sizable catalytic effect: $\Delta G^{\ddagger} =$ 17.2 kcal/mol; $\Delta S^{\ddagger} = -10$ cal/(mol·K) (entry 3).

We also examined the isomeric 1,5-disubstituted naphthalene **3** as a control compound to factor out any through-bond effects due to the electron-withdrawing character of the protonated amino nitrogen (eq 3). The kinetics of amide



(3)

isomerization were studied under conditions where there was no acid (entry 4) or enough acid present to protonate >90% of the substrate (entry 5). This system can be viewed as a conservative control, because under the conditions employed for **2** in entries 2 and 3 in Table 1, much less than 90% of **2** is protonated.[20] As expected, there was a small amount of catalysis in **3-H**$^+$ due to the inductive effect of a protonated amine on the naphthalene system ($\Delta(\Delta G^{\ddagger} = 0.3$ kcal/mol),[21] but not nearly as much as in the case of **2**, where 0.5 equiv of CAA resulted in $\Delta(\Delta G^{\ddagger}) = 5.0$ kcal/mol and 1 equiv of benzoic acid resulted in $\Delta(\Delta G^{\ddagger}) = 3.7$ kcal/mol. After factoring out the small amount of catalysis observed in the control, we estimate 4.7 and 3.4 kcal/mol of intramolecular acid-catalyzed isomerization in these systems to be reasonable values. The catalytic effect of 4.7 kcal/mol represents an approximately 2500-fold rate acceleration of amide isomerization at 25 °C, by far the greatest amount of intramolecular catalysis yet observed.[22]

The PPIases have been determined to catalyze amide isomerization by 6−8 kcal/mol.[10a] With this perspective in mind, the numbers we found represent a very substantial amount of catalysis, especially considering that only a small fraction of **2** is protonated at any one time. The results presented herein and in our previous studies support the validity of intramolecular catalysis of amide isomerization in model systems, and current work in our laboratories is aimed at providing experimental evidence for the existence of this mechanistic scheme in the active site of the enzymes.

(10) (a) Kofron, J. L.; Kuzmic, P.; Kishore, V.; Colón-Bonilla, E.; Rich, D. H. *Biochemistry* **1991**, *30*, 6127. (b) Zydowsky, L. D.; Etzkorn, F. A.; Chang, H. Y.; Ferguson, S. B.; Stolz, L. A.; Ho, S. I.; Walsh, C. T. *Protein Sci.* **1992**, *1*, 1092. (c) Fischer, S.; Michnick, S.; Karplus, M. *Biochemistry* **1993**, *32*, 13830. (d) Wiederrecht, G.; Etzkorn, F. *Perspect. Drug Discovery Des.* **1994**, *2*, 57. (e) Zhao, Y.; Ke, H. *Biochemistry* **1996**, *35*, 7356.

(11) This hydrogen bond has been estimated to generate 1.4 kcal/mol of catalysis by ab initio calculations,[10c] and we have recently demonstrated up to 3.3 kcal/mol of catalysis from an analogous interaction in model dipeptides in organic solution; see: (a) Cox, C.; Young, V. G., Jr.; Lectka, T. *J. Am. Chem. Soc.* **1997**, *119*, 2307. (b) Cox, C.; Lectka, T. *J. Am. Chem. Soc.* **1998**, *120*, 10660.

(12) However, a recent study has suggested that this active site mutant was indeed catalytically active for proline isomerization, but was unstable to the conditions of the kinetic assay; see: Dolinski, K.; Scholz, C.; Muir, R. S.; Rospert, S.; Schmid, F. X.; Cardenas, M. E.; Heitman, J. *Mol. Biol. Cell* **1997**, *8*, 2267.

(13) Texter, F. L.; Spencer, D. B.; Rosenstein, R.; Matthews, C. R. *Biochemistry* **1992**, *31*, 5687.

(14) (a) Alder, R. A. *Chem. Rev.* **1989**, *89*, 1215. (b) Kirby, A. J. *Acc. Chem. Res.* **1997**, *30*, 290.

(15) Cox, C.; Wack, H.; Lectka, T. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 798.

(16) For the use of ST NMR in the investigation of amide isomerization, see: (a) Perrin, C. L.; Thoburn, J. D.; Kresge, J. *J. Am. Chem. Soc.* **1992**, *114*, 8800. (b) Cox, C.; Ferraris, D.; Murthy, N. N.; Lectka, T. *J. Am. Chem. Soc.* **1996**, *118*, 5332. (c) Reference 11b.

(17) We believe that *cis*-**2** is the thermodynamically favored isomer, in which case the barriers reported herein represent the cis-to-trans isomerization of the amide residue. This conclusion is based on several pieces of data. (1) Previous experimental (Itai, A.; Toriumi, Y.; Saito, S.; Kagechika, H.; Shudo, K. *J. Am. Chem. Soc.* **1992**, *114*, 10649) and theoretical (Saito, S.; Toriumi, Y.; Tomioka, N.; Itai, A. *J. Org. Chem.* **1995**, *60*, 4715) results indicate that *N*-methylanilides are more stable in the cis conformation. (2) Single-crystal X-ray structures of **2** and **2-H**$^+$ indicate that cis is the favored form in the solid state.[15] (3) We observed a 3% NOE between a phenyl ring proton and the methyl group attached to the amide nitrogen in the least thermodynamically stable conformation at low temperature.

(18) (a) Drakenberg, T.; Dahlqvist, K.-I.; Forsen, S. *J. Phys. Chem.* **1972**, *76*, 2178. (b) Albers, M. W.; Walsh, C. T.; Schreiber, S. L. *J. Org. Chem.* **1990**, *55*, 4984. (c) Eberhardt, E. S.; Loh, S. N.; Hinck, A. P.; Raines, R. T. *J. Am. Chem. Soc.* **1992**, *114*, 5437.

(19) Staab, H. A.; Saupe, T. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 865.

(20) Although the state of protonation of **2** and **3** can be easily determined in organic solvents by UV−vis and IR,[15] these techniques are adversely affected in aqueous solution. Neither technique is able to identify protonation of control **3**, so we assume here that **3** exhibits similar basicity similar to that of *N*,*N*-dimethylaniline, a compound easily characterized in H_2O/EtOH by UV−visible spectroscopy at 290 nm. UV−visible analysis indicates that *N*,*N*-dimethylaniline is 90% protonated by 10 equiv of CAA in 50/50 H_2O/EtOH. IR analysis is able to detect the state of protonation of **2** in D_2O/EtOH because the amide carbonyl shifts from 1610 to 1661 cm^{-1} upon protonation due to the formation of the hydrogen bond indicated in **2-H**$^+$.[15] As estimated from integration of these two peaks, 1 equiv of HOTf results in about 75% protonation of the amino nitrogen. With 0.5 equiv of chloroacetic acid or 1 equiv of benzoic acid, the conditions employed in Table 1, there is <10% protonation.

(21) We define $\Delta(\Delta G^{\ddagger})$ as the difference in activation energy between the catalyzed and uncatalyzed reactions.

(22) The greatest amount of intramolecular catalysis previously observed was a 260-fold rate increase in a model dipeptide in organic solution.[11b]

Acknowledgment. T.L. thanks the NIH (Grant No. R29 GM54348), the NSF Career Program, DuPont, Eli Lilly for a Young Faculty Grantee Award, and the Dreyfus Foundation for a Teacher-Scholar Award. C.C. thanks the Organic Division of the ACS for a Graduate Fellowship sponsored by Organic Reactions, Inc. (1997−1998), and JHU for a Kilpatrick Fellowship (1998−1999).

Supporting Information Available: Experimental procedures, including the synthesis and characterization of **2** and **3**, and details of saturation transfer experiments and Eyring analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

OL9901265