Intramolecular Catalysis of Amide Isomerization

Christopher Cox,† Victor G. Young, Jr.,‡ and Thomas Lectka*§

Department of Chemistry
Johns Hopkins University
Baltimore, Maryland, 21218
X-Ray Crystallographic Laboratory
University of Minnesota
Minneapolis, Minnesota 55455

Received November 20, 1996

The catalysis of amide bond isomerization (AI) by Brønsted acids is a well-documented reaction that proceeds through a putative N-protonated intermediate. 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) through a correctly aligned cyclic intermediate replaces discrete N-protonation (eq 1). As a consequence, the optimal positioning of a donor moiety should permit direct observation of the catalytically-active [X=H=N] H-bond.

Intramolecular catalysis of AI is believed to play a key role in the folding of several proteins including dihydrofolate reductase, and Karplus et al. have proposed in a theoretical study that it contributes to cyclophilin and FKBP-promoted peptide folding due to intramolecular catalysis. In this Communication, we report the first experimental study of trans-to-cis proline isomerization (PI). Details of the mechanisms by which FKBP- and cyclophilin-catalyzed PI occur still remain to be clarified. 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. 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 in a cis-proline peptidomimetic.

We reasoned that small peptides containing the correct structure should show intramolecular catalysis in an organic medium that mimics the desolvated environment 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, this interaction should be strengthened in the transition state for cis-to-trans PI (eq 2). The more stable trans form contains an H-bond within a seven-membered ring in organic solvents (trans-1). Thus we define intramolecular catalysis (IC) as ∆∆G‡ in the change from aqueous solution to an organic solvent for model amides, subtracted by the comparable ∆∆G‡ for model esters (eq 3). We monitored PI in prolines by 19F (1a–2a) and 1H (1b–d, 2b–d) saturation transfer (ST) NMR. Full kinetic and thermodynamic profiles of cis−trans isomerization of prolinamide 1a and proline ester 2a were constructed from Eyring plots. For example, in 1:1 H2O/acetonitrile, the barriers to rotation (AG‡S'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 H2O, so that IC is not observed. In CDCl3 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), 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 ∆S‡ values were found in all

medium that mimics the desolvated environment 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, this interaction should be strengthened in the transition state for cis-to-trans PI (eq 2). The more stable trans form contains an H-bond within a seven-membered ring in organic solvents (trans-1). Thus we define intramolecular catalysis (IC) as ∆∆G‡ in the change from aqueous solution to an organic solvent for model amides, subtracted by the comparable ∆∆G‡ for model esters (eq 3). We monitored PI

$IC = [\Delta G^\ddagger_{\text{amide(aqueous)}} - \Delta G^\ddagger_{\text{amide(organic)}}] - \left[\Delta G^\ddagger_{\text{ester(aqueous)}} - \Delta G^\ddagger_{\text{ester(organic)}}\right]$ (3)

† Johns Hopkins University.
‡ University of Minnesota.
(8) Prolines prefer to place the side chain pseudoaxially, with the carbonyl group exo to the proline ring: Thomas, L. M.; Ramanasubbu, 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 19F ST NMR to take advantage of the broad chemical shift range and generally favorable peak separations of the 19F 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 (H2O/acetonitrile) 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.
Table 1. Kinetic and Thermodynamic Parameters for Prolines 1 and 2

<table>
<thead>
<tr>
<th>proline</th>
<th>solvent</th>
<th>$\Delta G^a$ cis</th>
<th>$\Delta G^b$ cis</th>
<th>$\Delta G^a$ trans</th>
<th>$\Delta G^b$ trans</th>
<th>$\Delta H^a$</th>
<th>$K^e$</th>
<th>IC $^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>H$_2$O/acetone</td>
<td>18.8 ± 0.3</td>
<td>18.7</td>
<td>−3.3 ± 1.0</td>
<td>17.8 ± 0.2</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>CDCl$_3$</td>
<td>16.8 ± 0.1</td>
<td>15.5</td>
<td>−3.0 ± 1.0</td>
<td>16.0 ± 0.3</td>
<td>9.8</td>
<td></td>
<td>2.4/1.3</td>
</tr>
<tr>
<td>2a</td>
<td>H$_2$O/acetone</td>
<td>18.9 ± 0.3</td>
<td>18.5</td>
<td>−1.1 ± 0.9</td>
<td>18.5 ± 0.2</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td>CDCl$_3$</td>
<td>18.2 ± 0.1</td>
<td>17.7</td>
<td>−1.1 ± 1.0</td>
<td>17.9 ± 0.3</td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Trans-to-cis isomerization, kcal/mol. $^b$ Cis-to-trans isomerization, kcal/mol. $^c$ 25 °C. $^d$ cal/mol K. $^e$ $K = [\text{trans}]/[\text{cis}].$ $^f$ 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, so that catalysis quantities defined in terms of either $\Delta H^e$ or $\Delta G^e$ 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$_2$Cl$_2$. 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-COOCH$_3$) 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). 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$^{-1}$. 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]$_q$ interaction. Additional evidence for an [N-H-...N]$_q$ H-bond comes from X-ray crystallography of 3, which reveals a distance from the side chain proton (H(10A)) to the ring $N_a$ of 2.35 Å, 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]$_q$ interaction is weakened somewhat over that observed in solution by intermolecular H-bonding.

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

Acknowledgment. T.L. thanks the American Cancer Society for a New Faculty Grant and the NIH for a First Award (R29 GM54348), and C.C. thanks JHU for a Marks Fellowship. The authors thank Professor Steve Scheiner for helpful comments.

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