Intramolecular Acid-Catalyzed Amide Isomerization in Aqueous Solution

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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. In fact, the ratio of O- to N-protonated species has been calculated to exceed $10^6$ for simple amides such as dimethylacetamide (DMA). The resonance theory 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.

We now 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 of the importance of hydrogen bond donation to the amide nitrogen by charged proton donors 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. In fact, the ratio of O- to N-protonated species has been calculated to exceed $10^6$ for simple amides such as dimethylacetamide (DMA). The resonance theory 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. 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. This anomaly is rationalized by assuming that although O-protonation is highly favored, a small but kinetically significant amount of N-coordinated species is present in equilibrium. Despite potential importance in biological systems, the catalysis of amide isomerization in

(5) Perrin has also determined that transiently N-protonated amides are intermediates in 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.
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 (eq 1) may play a significant role in the enzymatic catalysis of amide isomerization.\(^6\)

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 immunosuppressive 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 (\(N_p\)) (Figure 1A).\(^11\) In contrast, cyclophilin is 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 \(^{2-}\text{H}^+\), 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 \(^{2-}\text{H}^+\) 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.

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\(2-\text{H}^+\) forms a strong intramolecular hydrogen bond between the protonated amino group and the amide nitrogen in organic solvents when treated with acid (eq 1).

Figure 1. Intramolecular catalysis in the PPIases

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### Table 1. Saturation Transfer Results on 2 and 3 in 50/50 \(\text{D}_2\text{O/EtOH}\)

<table>
<thead>
<tr>
<th>entry</th>
<th>substrate</th>
<th>additive</th>
<th>(\Delta G^b) (\text{kcal/mol})</th>
<th>(\Delta H^c) (\text{kcal/mol})</th>
<th>(\Delta S^d) (\text{cal/(mol*K)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>0.5 equiv CAA</td>
<td>20.9</td>
<td>15.9</td>
<td>-10</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1 equiv BA</td>
<td>17.2</td>
<td>14.2</td>
<td>-10</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>1 equiv CAA</td>
<td>18.4</td>
<td>14.4</td>
<td>-10</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>10 equiv CAA</td>
<td>18.4</td>
<td>14.7</td>
<td>-3</td>
</tr>
</tbody>
</table>

\(\Delta G\) and \(\Delta H\) values were obtained from the Supporting Information for experimental details and error analysis. Barriers reported are cis to trans. Abbreviations: CAA = chloroacetic acid; BA = benzoic acid.\(^6\) At 25 °C; ±0.2 kcal/mol. ±0.3 kcal/mol. ±0.4 kcal/mol.±0.4 cal/(mol*K).
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.\textsuperscript{11b} As illustrated in entry 1, amide 2 had a free energy of activation ($\Delta G^\ddagger$) of 20.9 kcal/mol\textsuperscript{11} that was totally enthalpic, in line with previous studies on amide isomerization.\textsuperscript{18} Upon the addition of 1 equiv of triflic acid (HOTf), the isomerization catalyzed the reaction too well to analyze kinetically by NMR.\textsuperscript{20} HOTf is slightly upfield and broadened to some extent but, like HOTf, isomerization for water-soluble amides are similar in pure water and in water/polar organic solvent mixtures.\textsuperscript{11b} Upon the addition of (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 NMR.\textsuperscript{20} 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 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.\textsuperscript{20} As expected, there was a small amount of catalysis in $3^{\text{H}^+}$ due to the inductive effect of a protonated amine on the naphthalene system ($\Delta G^\ddagger = 0.3$ kcal/mol),\textsuperscript{21} but not nearly as much as in the case of 2, where 0.5 equiv of CAA resulted in $\Delta G^\ddagger = 5.0$ kcal/mol and 1 equiv of benzoic acid resulted in $\Delta G^\ddagger = 3.7$ 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.\textsuperscript{22}

The PPlases have been determined to catalyze amide isomerization by 6–8 kcal/mol.\textsuperscript{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.\textsuperscript{11b}


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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.

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