Decomposition of Protonated Threonine, Its Stereoisomers, and Its Homologues in the Gas Phase: Evidence for Internal Backside Displacement

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ABSTRACT

Protonated threonine and its allo diastereomer exhibit different proportions of collisionally activated dissociation (CAD) product ions. N-Methylation attenuates these differences. Water loss from protonated allo-threonine gives protonated trans-3-methylaziridinecarboxylic acid via an internal SN2 pathway, rather than protonated vinylglycine.

Protonated amino acids readily expel water in the gas phase. In most cases, loss of water from the carboxylic group is accompanied by CO loss,1 producing an iminium ion,2 as Scheme 1 depicts. In a few instances (notably threonine3), loss of water from the side chain predominates. Protonated amino acids can be differentiated by mass spectrometry. This letter reports the collisionally activated dissociation (CAD) spectra of the conjugate acid ions from threonine, its allo diastereomer, and their N-methyl homologues.

N-Methylthreonine has also been found in peptides,5 but the N-methyl allo diastereomer has yet to be reported. As molecules incorporating this latter isomer may occur in nature, we describe its preparation (starting from the amino acid, using a method previously reported6) and its CAD. The threo and allo isomers exhibit different fragmentation pat-Scheme 1. Dissociation of a Protonated Amino Acid

Threonine has two asymmetric carbons. The diastereomer not involved in protein biosynthesis is found in a variety of naturally occurring cyclic peptides.4 This fact prompts the question as to whether the two diastereomeric amino acids can be differentiated by mass spectrometry. This letter reports the collisionally activated dissociation (CAD) spectra of the conjugate acid ions from threonine, its allo diastereomer, and their N-methyl homologues.

N-Methylthreonine has also been found in peptides,5 but the N-methyl allo diastereomer has yet to be reported. As molecules incorporating this latter isomer may occur in nature, we describe its preparation (starting from the amino acid, using a method previously reported6) and its CAD. The threo and allo isomers exhibit different fragmentation pat-
terns, significantly expanding the list of acyclic stereoisomers that mass spectrometry can distinguish. Three different hypotheses have been explored to account for this difference.

The first hypothesis looks to the two five-membered cyclic transition states drawn in Scheme 2. If they corresponded to the rate-limiting steps for the two most prominent pathways, then competition between them would predict measurable differences between diastereomers. Assuming that the acidic proton resides on the nitrogen to begin with, varying the relative stereochemistry of the two asymmetric centers should affect the likelihood of loss of side chain OH. As Scheme 3 depicts, the transition state for the *allo* puts the two substituents cis to one another in the five-membered ring. For steric reasons, then, the aforementioned hypothesis predicts that loss of a single water ought to be more favorable for the *threo* than for the *allo*.

The experiments described below do not confirm that prediction: the *allo* isomer displays the greater proportion of single water loss. O’Hair and Reid have reported deuterium labeling studies that suggest that the rate-limiting step comes after proton transfer to the side chain oxygen. Scheme 4 illustrates two plausible pathways.

One pathway, originally proposed by O’Hair and Reid, proceeds via internal S$_N$2 displacement to give protonated aziridinecarboxylic acid 1. This mechanism implies that diastereomeric threonines ought to produce isomeric ions: *threo* should yield cis-1, and *allo* should yield trans-1. An alternative pathway would involve internal E2 elimination via five-membered cyclic transition states to yield protonated vinylglycine 2 or protonated α-aminocrotonic acid 3, which MP2 calculations predict to be 1.9 kcal mol$^{-1}$ and 6.2 kcal mol$^{-1}$ ((Z)-isomer), respectively, more stable than trans-1.

The dissociation patterns of the protonated amino acids were studied using an orthogonal quadrupole/time-of-flight mass spectrometer with electrospray sample introduction. Table 1 summarizes the abundance of other neutral losses relative to the dissociation in Scheme 1 (which all R-aminoc acids have in common). The threonine diastereomers show quantitative differences in fragment ion abundances. These patterns vary with the collision energy. The ratios of the two most intense fragment ions are plotted in Figure 1 as a

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**Table 1.** Proportions of Neutrals Lost in MS/MS (12 eV Collision Energy) for Selected Parent Ions (Relative to Loss of H$_2$O + CO)  

<table>
<thead>
<tr>
<th>neutral precursor</th>
<th>m/z of parent</th>
<th>fragment ions from loss of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>H$_2$O</td>
</tr>
<tr>
<td>threonine</td>
<td>120</td>
<td>0.58 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>102</td>
<td>0.85 ± 0.05</td>
</tr>
<tr>
<td>allo-threonine</td>
<td>120</td>
<td>3.19 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>102</td>
<td>2.08 ± 0.22</td>
</tr>
<tr>
<td>N-methylthreonine</td>
<td>134</td>
<td>1.24 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>116</td>
<td>0.49 ± 0.15</td>
</tr>
<tr>
<td>N-methyl-allo-threonine</td>
<td>134</td>
<td>3.05 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>116</td>
<td>0.54 ± 0.14</td>
</tr>
<tr>
<td>vinylglycine</td>
<td>102</td>
<td>0.37 ± 0.07</td>
</tr>
<tr>
<td>1-aminocyclo-propane-1-COOH</td>
<td>102</td>
<td>2.12 ± 0.10</td>
</tr>
<tr>
<td>azetidine-2-COOH</td>
<td>102</td>
<td>0.02 ± 0.00</td>
</tr>
<tr>
<td>homoserine</td>
<td>120</td>
<td>0.49 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>102</td>
<td>1.7 ± 0.5</td>
</tr>
</tbody>
</table>

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function of collision energy for all four stereoisomers. Figure 2 displays the same type of plot for all four stereoisomers of N-methylthreonine. Given the pronounced increase of single-water loss in the allo relative to threo, the first question concerns whether that dissociation represents elimination only from the side chain. This was assessed by labeling the allo with both carboxylic oxygens replaced by 18 O (in the same manner as doubly 18 O-labeled threo has been prepared). The CAD spectrum of labeled allo shows that loss of a single water comes exclusively from the side chain, while loss of H2O plus CO comes exclusively from the carboxyl group (consistent with Scheme 1).

As will be discussed below, additional experimental data corroborate the SN2 mechanism shown in Scheme 4. Before presenting that evidence, though, an alternative hypothesis should be weighed; namely, that the difference between diastereomers does not result from competing transition states but instead reflects the rotameric distribution of the protonated amino acids prior to dissociation. The effect of methylation tests this second hypothesis. Schemes 5 and 6 portray these equilibria, including ab initio enthalpy differences. The most favored structures have the NH3+ hydrogen-bonded to both the side chain oxygen and the carbonyl oxygen. Only side-chain water loss can take place from this geometry. Less favored rotamers have the NH3+ hydrogen-bonded to the side-chain oxygen and to the carboxylic OH group. Loss of water from either position can occur from this geometry.

N-Methylation increases the enthalpy difference (ΔΔH) between allo and threo isomers. The calculated ΔΔH for the amino acids is 1.37 kcal mol⁻¹ (the difference between ΔE^{MP2} + ΔZPE in Scheme 6 and ΔE^{MP2} + ΔZPE in Scheme 5). The corresponding difference for the N-methyl amino acids is ΔΔH = 1.66 kcal mol⁻¹. If the rotameric equilibrium by itself determined the dissociation pattern, one would have expected N-methylation to increase the proportion of allo side-chain water loss relative to threo. Comparison of Figures 1 and 2 demonstrates that the exact opposite result is obtained.

The observed consequences of N-methylation do agree with expectation, if the rate-limiting step for side-chain water loss corresponds to the pathways depicted in Scheme 4. The question then arises whether that reaction operates via the SN2 pathway or via the E2. O'Hair and Reid have argued in favor of the former, on the basis of their deuterium labeling experiments. MS/MS of the ions from skimmer-induced side-chain water loss substantiates their interpretation.

As Table 1 summarizes, CAD of the ions from water loss (m/z 102 from the threonines; m/z 116 from the N-methyl derivatives) shows significant differences between the ions.

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**Scheme 5.** Rotamers of Protonated Threonine and Their Dissociation Products

**Scheme 6.** Rotamers of Protonated allo-Threonine and Dissociation Products

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of side-chain water from N-methylthreonine can produce the two protonated aziridines 5a and 5b. The latter ion has all substituents cis and is therefore likely to be formed much less often than is 5a. By contrast, both of the protonated aziridines from N-methyl-allo-threonine, 5c and 5d, have one cis and two trans substituents on the ring, as does ion 5a.

The difference between protonated threonine and protonated allo-threonine can be ascribed to the steric hindrance introduced by placing two substituents cis on the developing ring during displacement of side-chain water. In the N-methyl homologues, 5a, 5c, and 5d all have nearly the same degree of steric hindrance. While the torsional strain in the aziridine rings does not fully develop in the S_n2 transition state, it does tend to diminish the difference between threo and allo isomers. If ions 5a, 5c, and 5d all form at the same rate, then (on the basis of a naïve statistical argument) side-chain water loss in the N-methyl-allo should be twice as abundant as in the N-methyl-threo. The ratio of the two curves in Figure 2 ranges from 2.3 (at low collision energies) to 2.9, not far from this prediction.

The distinctions described above hinge on the stereochimistry of backside displacement, thus enlarging the repertoire of stereospecific dissociations by which diastereomers can be differentiated using MS/MS. Methods previously reported from this laboratory for discriminating acyclic diastereomers have relied on syn eliminations via four-membered transition states. Are there other mechanisms by which MS/MS can tell acyclic stereoisomers apart? Preliminary negative ion data suggest there might be.

Anions from threonine display the same CAD patterns for both diastereomers. By contrast, MS/MS of negative ions from isoleucine do exhibit a statistically significant difference. The isoleucine and allo-isoleucine M+1 anions display different extents of dissociation via CO + H_2O loss, relative to the parent ion (although the M+1 positive ions show no differences). No variations are to be found among the competing fragmentation pathways themselves.

The example of isoleucine illustrates a less robust way to differentiate between diastereomers than the competition among pathways described above for the M+1 positive ions of the threonines. Current efforts seek methodologies for dipeptides and other modified amino acids.

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Supporting Information Available: Synthesis and spectra of N-methylthreonines and of ester 4; tabulated DFT and ab initio energies. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) Geometries were optimized at the B3LYP and MP2(FC) levels using the 6-31G** basis. Computed stabilities include DFT zero-point energies.