Quaternary Amine-Induced Peptide Degradation via Cyclization

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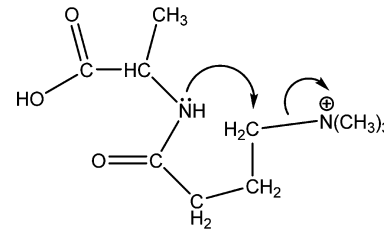
In this study, we investigated intramolecular cyclizations in peptides containing quaternary amines. Two types of cyclization reactions are studied: (a) those involving a trimethylammonium butyric acid (TMAB) charge tag and (b) those involving trimethylated lysine. Both types of reactions result in the release of trimethylamine via an S_N2 mechanism involving a lone pair of electrons on the oxygen or nitrogen. In the case of the TMAB charge tag cyclization, the oxygen attack mechanism leading to a five-membered ring is the preferred pathway. In the trimethylated lysine cyclizations, the preferred pathway involves the nitrogen nucleophile resulting in the formation of a six-membered ring. The similarities and differences between the two reactions are analyzed.

Introduction

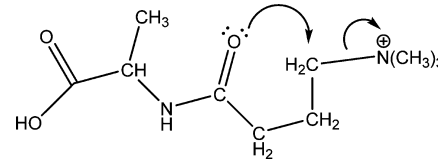
Understanding protein sequence is critical for realizing the goals of genomics and proteomics.[1,2] While the sequence of DNA determines the peptide sequence immediately after translation, post-translational modifications can alter the peptide structure and functionality. For example, it may be necessary to modify a peptide sequence prior to analysis as in the case of adding charge tags for mass spectrometry.[3−5] Another example of post-translational modification commonly encountered in proteins is the methylation of lysine.[6−20] Under standard conditions these modifications are expected to form stable peptides with either new functionality as in the case of methylated lysine or increased detection sensitivity and simplified spectra as in the case of charge tags. A common functional group formed by both modifications is a positively charged quaternary amine. The trimethylation of lysine or the addition of trimethylammonium butyric acid (TMAB) both result in a trimethylamine (TMA) functional group. While one might expect these species to be stable, they have been implicated in peptide degradation to explain puzzling mass spectra.[4] While the generation of the TMA functional groups may have different origins, the resulting species are the same and expected to have similar chemistry. In this article we examine the possibility of amine-induced peptide degradation via cyclization under mass spectrometric conditions.

Charge tags are designed to form stationary local positive charges on peptides to improve the detection limits for certain peptides for mass spectrometry and to simplify the resulting fragmentation patterns. The charge tag is placed on either the N-terminus or the C-terminus of a peptide, resulting in all N- or C-terminal fragment ions, respectively, simplifying the spectra. The TMAB charge tag contains a quaternary amine species with a positively charged nitrogen center. One recent report suggests the positive charge does not remain on the nitrogen.[4] Instead, during the collision-induced dissociation, the charge tag decomposes. The mechanism of decomposition was proposed to follow a cyclization whereby the carbon directly attached to the nitrogen of the charge tag reacts with an oxygen or nitrogen, forming a five-membered heterocyclic ring while dissociating from the charge tag nitrogen. The reaction forms a neutral trimethylamine and a peptide with a charged nitrogen or oxygen species.[4] Minimum energy structures suggest the heterocyclic ring containing oxygen is more stable then the ring incorporating nitrogen.

SCHEME 1



SCHEME 2



In the case of lysine methylation, the terminal amine group of the lysine can be di- or trimethylated.[6,7] Methylation reactions that form tetravalent ammonium species localize a positive charge on the nitrogen center. Recent studies have proposed that the positive charge induces an intramolecular cyclization reaction caused by a nearby lone pair of electrons.[8,9,18] In this reaction the positively charged nitrogen promotes the adjacent carbon to react with either an oxygen or a nitrogen atom of the peptide backbone. This leads to the loss of a neutral amine group at the positively charged nitrogen, leaving behind a cyclic structure with a delocalized positive charge.[8,9,18] Such reactions are particularly likely under the conditions found in mass spectrometry, i.e., molecules in vacuum containing sufficient internal energy to induce fragmentation.

While both proposed reactions appear plausible, the mechanisms and energetics of the reactions must be analyzed to determine the likelihood of peptide degradation and to understand the nature of the products formed. In particular, the key

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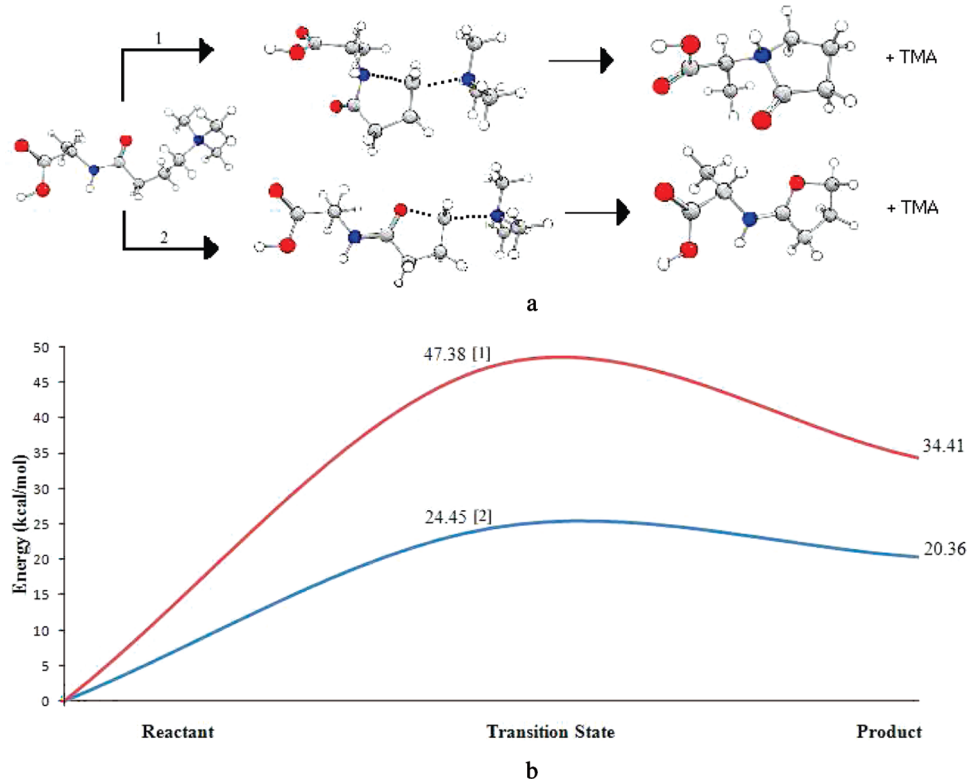


Figure 1. Transition states (a) and reaction profiles (b) for the model peptide TMAB-Ala showing five-membered ring cyclization and trimethylamine elimination. The reaction profile includes the nitrogen attack mechanism (top, red) and oxygen attack mechanism (bottom, blue). A positive charge is located on the quaternary nitrogen in the reactant and on the nitrogen atom of the products.

question is to understand the chemistry of positively charged quaternary amine-induced cyclization. With analogy to an S_N2-type reaction from elementary organic chemistry, all of the cyclization reactions examined require a nucleophile and a leaving group. For both the TMAB charge tag and the trimethylated lysine the leaving group is TMA. The TMA functionality is known to be an excellent leaving group, easily displaced by a nearby nucleophile. Another commonality in both cyclization reactions is the identity of the possible nucleophiles. In either case a lone electron pair on an oxygen or a nitrogen from the peptide backbone can act as a nucleophile driving the final reaction. The main difference between TMAB charge tag cyclization and trimethylated lysine is the size of the ring formed in the product. For TMAB both nitrogen and oxygen lead to the formation of a five-membered ring. For trimethylated lysine two possibilities exist. If the attacking nucleophile is nitrogen, the product can be a six-membered ring or a seven-membered ring. If the attacking nucleophile is oxygen, the product is a seven-membered ring. In this study, we fully characterize the reactions of peptide degradation induced by positively charged quaternary amine groups for TMAB charge tags and methylated lysine.

Theoretical Methods

All calculations were performed at the B3LYP/6-31+G(d,p)[21−25] model chemistry using the Gaussian suite of programs.[26] Analytical evaluation of the second derivatives was used to verify the nature of the minima and transition states. In particular, all transition states have one and only one imaginary frequency, and the corresponding normal coordinates were used to confirm the nature of the reactions. Zero-point-corrected energies were computed in all cases and used in the following discussion.

Results and Discussion

Although TMAB charge tag cyclization involving nitrogen or oxygen both result in five-membered rings, the reaction pathways differ significantly in their quantitative details. Schemes 1 and 2 show the key mechanistic step leading to cyclization in the case of nitrogen and oxygen attack, respectively. The computed reaction pathways do agree with the expectations from a cyclic S_N2 mechanism. The leaving group in both cases is TMA. The nature of the product formed is determined by the nucleophilic atom, either oxygen or nitrogen. The nitrogen (Figure 1a (path 1)) attacks the carbon bound to the TMA, displacing the nitrogen, while the oxygen attack (Figure 1a (path 2)) follows a similar pathway. Our calculations show (Figure 1b (path 2)) the oxygen attack has a barrier 23 kcal/mol lower than the nitrogen attack. A way of characterizing the difference in transition-state energies is to consider Hammond's postulate. The oxygen attack transition state is closer to the structure of the product than the nitrogen transition state and products. Therefore, the energy when reorganizing from the transition state to the products is much smaller for oxygen. The lower barrier height for the oxygen attack is due to this low reorganization energy, making it a later transition state. This transition state results from a polarization of the C═O double bond by the adjacent nitrogen and the repulsive steric interactions of the bulky group attached to the nitrogen. The product containing the heterocyclic oxygen ring is also thermodynamically favored as previously seen.[4] The increased stability is due to the delocalization of the positive charge in the product and fewer repulsive interactions between adjacent groups. The product that forms in the oxygen attack reaction has more positive charge delocalization, and the C−O bond is more stable than the C−N bond. Thus, the oxygen attack product is favored

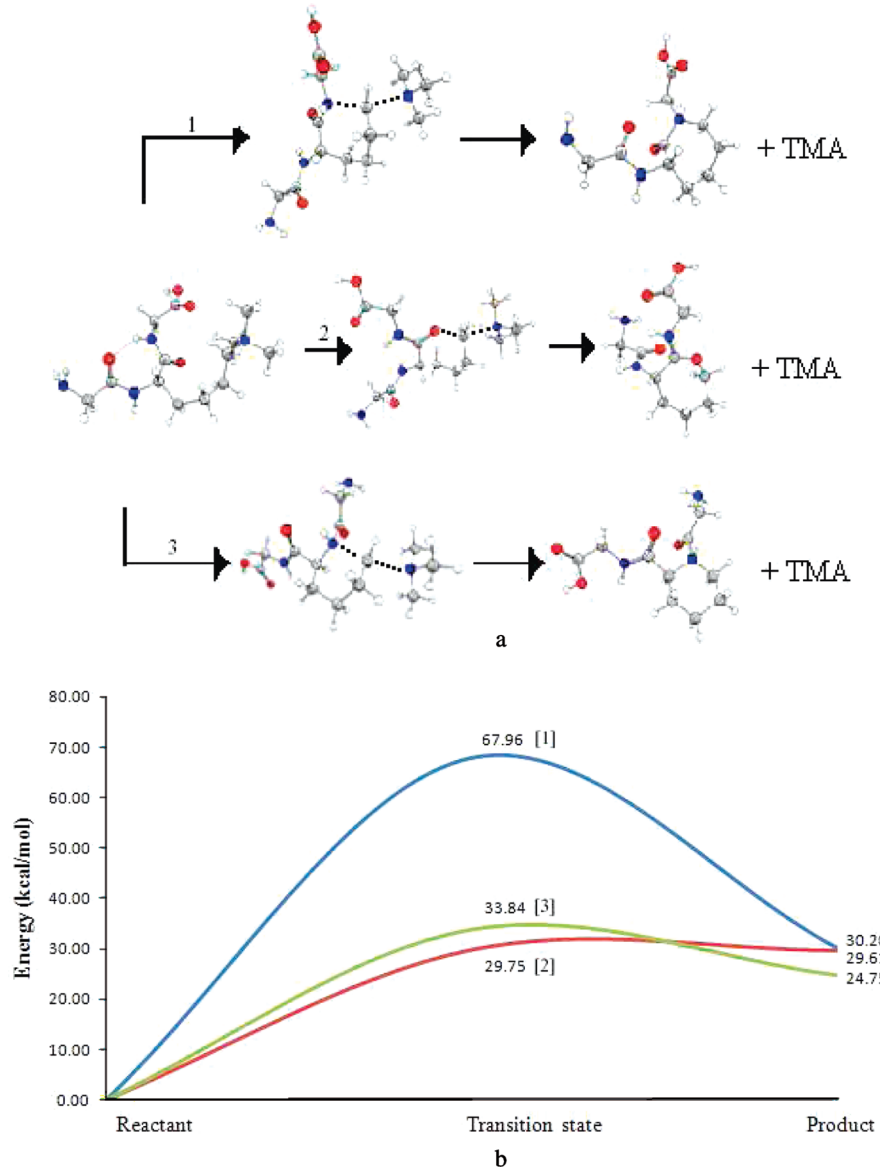


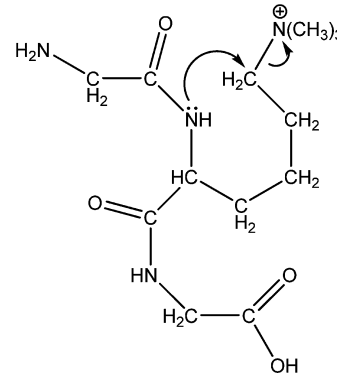
Figure 2. Transition states (a) and reaction profiles (b) involving the six- and seven-membered ring cyclizations for the model peptide trimethylated Ala-Lys-Ala. The reaction profiles show the seven-membered ring formation via nitrogen attack (blue), seven-membered ring formation via oxygen attack (green), and six-membered ring formation (red). A positive charge is located on the quaternary nitrogens of the arginine in the reactant and on the nitrogen on either the hetrocyclic ring for the reactions 1 and 3 or the ring carbon bound to both oxygen and nitrogen (path 2) atoms.

both thermodynamically and kinetically and expected to be the only product experimentally observed.

As seen in Figure 1b, the products are significantly endothermic, 20 kcal/mol for the ring containing oxygen and 34 kcal/mol for the ring containing nitrogen. Such endothermic reactions would not normally be expected to occur after functionalization. They are only likely under mass spectrometric conditions. In particular, under the conditions of collision-induced fragmentation or matrix-assisted laser desorption, these reactions should occur readily.

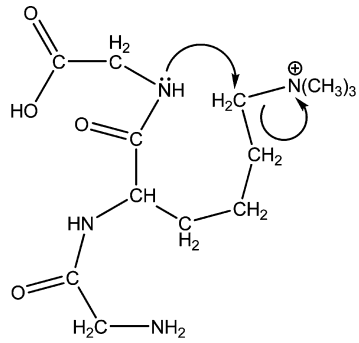
The cyclization reaction involving the model trimethylated Ala-Lys-Ala tripeptide is similar to that involving the TMAB charge tags, Figure 2a.[1−3] However, in this case there are two possibilities for nitrogen attack leading to the formation of a seven- (Figure 2a (path 1)) or six-membered ring (Figure 2a (path 3)) depending on the backbone nitrogen involved. Scheme 3 shows the formation of the six-membered ring via nitrogen attack, while Scheme 4 shows the corresponding seven-membered ring formation. On the basis of the expected stabilities of the rings, the pathway involving the six-membered ring

SCHEME 3

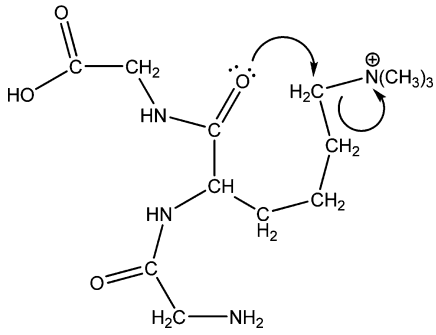


(Figure 2a (path 3)) is expected to be more favorable. Interestingly, in the case of oxygen attack, only the formation of a seven-membered ring (Figure 2a (path 2)) is possible. This key step is illustrated in Scheme 5. The additional stability from oxygen (relative to nitrogen) is likely to be offset by the expected

SCHEME 4



SCHEME 5



lesser stability of the seven-membered ring (relative to the six-membered ring). These simple expectations are borne out by detailed calculations.

Details of the three possible transition states (TS) for cyclization of trimethylated lysine are shown in Figure 2. The nitrogen attack resulting in a six-membered ring (Figure 2a (path 3)) proceeds by the nitrogen nucleophile attacking the δ-carbon of the lysine, forming a N−C bond and breaking the C−N bond of the carbon with the TMA. The positive charge is shifted from the nitrogen of the TMA to the nitrogen on the peptide backbone, which acts as a nucleophile during the reaction. A seven-membered ring forms when a nucleophilic oxygen or nitrogen atom from the peptide backbone attacks the carbon bound to the nitrogen atom of the TMA functional group (Figure 2a). Both reaction paths are similar to that of the six-membered ring formation.

The reaction profiles for the different pathways are shown in Figure 2b. As expected and shown in the reaction coordinate, the nitrogen attack forming the seven-membered ring has the highest activation barrier (68 kcal/mol). Due to the high activation barrier, the formation of the seven-membered ring via nitrogen attack is unlikely. Among the other two more competitive pathways, the oxygen attack pathway is kinetically favored by 4 kcal/mol over nitrogen attack forming the six-membered ring. The origin of the lower barrier is in the formation of the stronger C−O bond (~86 kcal/mol) versus C−N bond (~73 kcal/mol) and the nature of the TS. The more stable C−O-bonded product combined with the later transition state begets a lower barrier height. Additionally, the orientation of the TMA in the TS involving oxygen has less steric hindrance than the nitrogen attack TS. However, the ordering of the product stabilities is different. The stability of the six-membered ring product is lower than the seven-membered ring produced by the oxygen attack by 5 kcal/mol. Also, it is lower than the seven-membered ring produced by the nitrogen attack by 6 kcal/mol. The energy difference between oxygen attack TS and product is a meager 0.14 kcal/mol. This tiny energy difference indicates the seven-membered ring may not be stable as a product complex. However, the six-membered ring product from nitrogen attack is 9 kcal/mol more stable than its TS. The energy difference is due to the greater stability of the six-membered ring and positive charge centered on the nitrogen. Since the formation of the six-membered ring is more stable and the reaction is not reversible, we argue the observed product is the six-membered ring formed via nitrogen attack. The seven-membered ring products will be formed in quantities too small to give a significant signature in mass spectrometric measurements. This conclusion is consistent with the qualitative mechanism proposed in ref 6.

Conclusions

We have shown the mechanisms of peptide cyclization for TMAB charge tags and trimethylated lysine. In both cases the presence of a TMA group induces peptide degradation via cyclization with the loss of the TMA group. Furthermore, the cyclization reactions shift the charge from the lysine or charge tag to the adjacent atoms in the ring via an S_N2 reaction mechanism. The reactions involving oxygen attack have lower barriers than those involving nitrogen. However, prediction of the product stabilities is more difficult. As the reactions we have chosen show, product formation is determined by the quality of the nucleophile, steric interactions in the transition state, and the stability of the product. In the case of the trimethylated lysine, the product of the oxygen attack is a seven-membered ring which is less stable than the six-membered ring formed from nitrogen attack. In the reaction involving the TMAB charge tag, the oxygen attack pathway forming the five-membered ring is more likely to occur, while in the trimethylated lysine species the nitrogen attack forming a six-membered ring is more likely.

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