Comparison of dissociation tendencies of meta- & para-trifluorotolylglycine methyl esters and meta- & para-tolylglycine methyl esters in ESI-MS

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Abstract. Peptide fragmentation is an integral part of an evolving field commonly referred to as proteomics. Understanding of peptide fragmentation is vital to continued proteomic research. The framework of this study was the benzylglycine methyl ester with either a methyl or trifluoromethyl constituent in the para- and meta-positions of the benzyl ring. The glycine methyl ester was coupled to the different acids using a PS-carbodiimide resin. The acids used were p-trifluorotoluic acid, m-trifluorotoluic acid, p-toluic acid, and m-toluic acid. Synthesis was confirmed using electrospray ionization mass spectrometry (ESI-MS). Collision induced dissociation (CID) was utilized to observe the dissociation tendencies of the given molecules. The anticipated $b_2^+$ pathway as seen in previous studies was not as readily seen for some of the molecules in this study. Density functional theory calculations were employed for the reaction pathways to determine the thermodynamic characteristics on the pathways to help explain the observed fragmentation pathways.

1. Introduction.

Understanding fundamental peptide fragmentation chemistry is essential to the effective application of tandem mass spectrometry to peptide/protein identification [1,2]. As part of our ongoing effort to study and understand the factors that affect fragmentation, we investigated the collision induced dissociation of variants of hippuric acid methyl ester (benzoic acid-glycine methyl ester). Our specific goal was to determine whether the presence, identity and position of electron donating or withdrawing substituents influence the tendency for the peptides to fragment via two competing pathways by altering the strength of a specific nucleophile important to the “oxazolone” dissociation pathway to $b$-type ions.

2. Experiment, Results, Discussion, and Significance

Variants of hippuric acid methyl ester were synthesized by coupling benzoic acid, meta- or para-toluic acid; or meta- or para-trifluorotoluic acid to glycine methyl ester using a commercially available resin-bound carbodiimide. ESI, CID and tandem MS was performed using a Finnigan LCQDeca ion trap mass spectrometer (ITMS). Double resonance experiments to probe the potential serial dissociation pathways were performed on a modified Bruker Esquire ITMS. DFT calculations at the B3LYP/6-31+G(d,p) were used to determine lowest-energy conformations of all relevant precursor, intermediate and post-reaction species, along with important transition states.

The principal products generated by CID of hippuric acid methyl ester are a formal $b_2$ ion, via elimination of methanol, and phenyl acyllium ion. The relative intensities of the two products are dependent on the presence and position of a ring substituent. For example, the phenyl acyllium ion dominates (9:1 ratio) the spectrum recorded from m-toluic acid-glycine methyl ester, while the product and $b_2$ ion appear at similar relative intensities for p-toluic acid-glycine methyl ester.

Double resonance experimentation allows us to evaluate potential competing pathways. In double resonance experiments, excitation of a parent ion and a potential product ion, is performed simultaneously [3]. Ejection of a particular product ion allows for determination of “parentage” of other products, particularly those that might be formed by a cascade of sequential fragmentation reactions. Double resonance experiments clearly show that the acyllium ions are generated directly from the protonated molecule rather than from the fragmentation of energetic $b_2^+$ ion. The acyllium ion is absent in spectra recorded for the trifluorotoluic versions of the hippuric acid-methyl ester, clearly establishing a dependence of electron donating and withdrawing groups. The experimental
(CID) results are supported by DFT calculations, which show clear differences in the transition state energies for the two fragmentation pathways, and for the relative product energies.

Thermodynamic comparison of preliminary data is inconclusive in that the rate determining step for the formation of $b_2^+$ ion shows a difference of 2 kcal/mol between the methyl and trifluoromethyl substituents. In regards to the acyllium ion the energy difference is about 1 kcal/mol between the two substituents. Single point calculations were conducted at the b3lyp/6-311++G(3d,2p) level of theory exhibiting similar energy trends as the lower level of theory.

3. Conclusions

The effect of different benzene ring substituents is apparent in the fragmentation pathways of the model peptides. The electron donating methyl group in the \textit{para} and \textit{meta} positions favors the acyllium ion from the parent ion. Whereas the electron withdrawing group of the trifluoromethyl group in the same \textit{meta} and \textit{para} positions favors the $b_2^+$ ion from the parent ion.

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