Structure of anionic peptides and their CID fragments

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While peptide sequencing by collision induced dissociation tandem mass spectrometry (CID MS) has found wide application in biochemistry, the underlying reaction chemistry remains an issue of lively debate. In recent years, the application of infrared (IR) spectroscopy to CID fragment ions has provided important new information on the molecular structures of CID fragment ions, for which several isomeric forms had been suggested.

Peptide sequencing via tandem MS in negative ion mode (*i.e.* on the deprotonated peptide) is not nearly as popular as its positive ion mode counterpart, although additional information from the deprotonated peptide could obviously increase the sequence coverage. For instance, ***c***-type ions are commonly observed in CID of peptide anions [1]. However, the dissociation modes of deprotonated peptides appear to be more complex and have often been reported to be more residue-specific.

In this contribution, we investigate the structure of selected anionic peptides and their CID fragments obtained through IR spectroscopy using the free electron laser FELIX in combination with an ESI FTICR mass spectrometer. This method has recently been applied to identify the deprotonation site in amino acids [2], which had been under much debate for tyrosine and cysteine in particular. Though different structures have been hypothesized, peptides lacking acidic residues have generally been assumed to deprotonate on the C-terminus. Our IR spectra of the conjugate base of tri-alanine, compared to calculated spectra at the DFT level, indeed provide strong support for a carboxylate structure.

More intriguing questions relate to the charge site in CID fragments. While carboxylate structures are lowest in energy for C-terminal fragments, the location of the negative charge in anionic N-terminal fragments remains more of a mystery to date. Using IR spectroscopy, we show that deprotonation on an amide N-atom forming an amidate anion is the most likely structure for anionic *a*-type peptide fragments lacking acidic residues [3]. Amidate structures are substantially more stable than tautomeric enolate structures as is further verified by spectra of the conjugate base of N-metylacetamide.

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[3] J. Oomens, J.D. Steill, The structure of deprotonated tri-alanine and its a_3^- fragment anion by IR spectroscopy. *J. Am. Soc. Mass Spectrom.* **2010**, *21,* 698-706