

Comparing the Fragmentation Reactions of $[M+H]^+$, $[M+2H]^{2+}$ and $[M]^{\bullet+}$ ions of 20 Tripeptides GXR

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Introduction:

Peptide radicals play an important role in biochemistry and biomedicine, peptide damage, enzymes etc [1]. Electrospray ionization coupled with multistage mass spectrometry techniques (ESI/MSⁿ) provides a unique way of examining fundamental unimolecular and bimolecular behaviour of these species in the absence of solvent and counterions. An intriguing recent paper has demonstrated an unprecedented method of producing molecular radical cations of peptides in the gas phase through the use of copper complexes $[Cu^{II}(\text{ligand})M]^{2+}$ (where ligand = tpy, Me₅dien and dien; M = oligopeptides) [2]. These peptide radical cations demonstrate a rich radical-induced fragmentation chemistry. Here we report a systematic study on the fragmentation of a series of GXR peptide radical cations, where X = all the 20 amino acids. The fragmentation reactions of these species will also be compared with the fragmentation reactions of their singly and doubly protonated counterparts.

Methods and Instrumentation:

We use electrospray ionization (ESI) of a methanolic mixture of $[Cu^{II}(\text{tpy})(NO_3)_2]$ and peptides, GXR, to generate cations in which the peptides are bound to the metal complex $[Cu^{II}(\text{tpy})(\text{GXR})]^{2+}$. Using multi-stage mass spectrometry experiments in an ion trap (LCQ), these cations are subject to CID. The radical cations of peptides formed are mass selected and subject to a further stage of CID. $[M+H]^+$ and $[M+2H]^{2+}$ are formed in the gas phase by electrospray ionizing a 1:9 H₂O/MeOH solution of peptide GXR. $[M+H]^+$ and $[M+2H]^{2+}$ formed are mass selected and subject to a further stage of CID.

Results:

The CID reactions of $[M+H]^+$, $[M+2H]^{2+}$ and $[M]^{\bullet+}$ of peptides have been examined. $[M]^{\bullet+}$ fragment quite differently to their protonated counterparts and demonstrate rich radical-induced fragmentation chemistry. Side chain losses which uniquely identify residues such as leucine and isoleucine are also observed [3].

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 - [3] Wee, S.; O'Hair, R. A. J.; McFadyen, W.D., "Side Chain Radical Losses from Radical Cations Allows Distinction of Leucine and Isoleucine Residues in the Isomeric Peptides Gly-XXX-Arg." *Rapid Communications in Mass Spectrometry*, **2002**, *16*, 884-890.
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