Free Radical–Induced Site-Specific Peptide Cleavage in the Gas Phase: Low-Energy Collision-Induced Dissociation in ESI- and MALDI Mass Spectrometry

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Protein identification is routinely accomplished by peptide sequencing using mass spectrometry (MS) after enzymatic digestion. Site-specific chemical modification may improve peptide ionization efficiency or sequence coverage in mass spectrometry. We report herein that amino group of lysine residue in peptides can be selectively modified by reaction with a peroxycarbonate and the resulting lysine peroxycarbamates undergo homolytic fragmentation under conditions of low-energy collision-induced dissociation (CID) in electrospray ionization (ESI) and matrix-assisted laser desorption and ionization (MALDI) MS. Selective modification of lysine residue in peptides by our strategy can induce specific peptide cleavage at or near the lysine site. Studies using deuterated analogues of modified lysine indicate that fragmentation of the modified peptides involves apparent free-radical processes that lead to peptide chain fragmentation and side-chain loss. The formation of a-, c-, or z-types of ions in MS is reminiscent of the proposed free-radical mechanisms in low-energy electron capture dissociation (ECD) processes that may have better sequence coverage than that of the conventional CID method. This site-specific cleavage of peptides by free radical– promoted processes is feasible and such strategies may aid the protein sequencing analysis and have potential applications in top-down proteomics. (J Am Soc Mass Spectrom 2007, 18, 807–816) © 2007 American Society for Mass Spectrometry

Mass spectrometry (MS) plays an indispensable role in identification of proteins in complex mixtures by providing sequences for peptides after enzymatic digestions [1, 2]. The sequencing is often accomplished using tandem mass spectrometry (MS/MS) by collision-induced dissociation (CID) or electron capture dissociation (ECD) of protonated species [3, 4]. Chemical modification of peptides or proteins has also provided strategies that are helpful in assignment of peptide sequence, enhancement of the MS sensitivity [5], and, importantly, in protein quantification [6]. Even though database searches for protein identification are primarily based on b- and y-ions observed in MS/MS spectra, complex fragmentation patterns can result from the ECD process and improve the sequence coverage in protein identification [7, 8]. The typical a-, c-, and z-fragments in ECD are postulated to arise from free-radical intermediates. The nomenclature of the fragments as proposed by Roestorff is shown in Scheme **1** [9].

In a recent communication we reported a method to generate radicals in a site-specific manner on peptides in the gas phase, after modification of lysine residues as peroxycarbamates [10]. Fragmentation appears to result from initial free-radical dissociation of the peroxide bond followed by decarboxylation. The chemistry can

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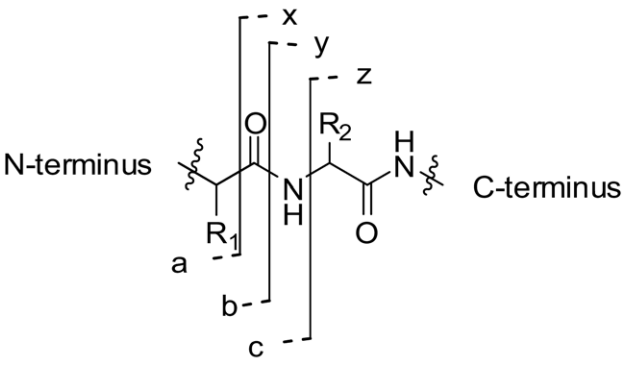

Scheme 1

also be used for N-terminus amino acid identification based on the neutral loss of the side chain [11]. The "digestion" of peptides or proteins in the gas phase after chemical derivatization may provide an alternative to enzyme digestion and could potentially increase the throughput of proteomics analysis. Here we report a detailed study of the free-radical mechanisms and fragmentation pathways generated in the gas phase of MS after lysine modification by peroxycarbamate chemistry. The free radical–induced peptide fragmentation results from the selective modification of lysine residues, which may help to assign the peptide/protein sequences in a top-down approach of proteomics [12].

Experimental

N-Acetyl lysine was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA) as methyl ester derivatives in their hydrochloride salt forms. Peptides were purchased from American Peptide Company (Sunnyvale, CA, USA). Solvents were HPLC grade, obtained from Fisher Scientific (Pittsburgh, PA, USA). All other reagents were purchased from Aldrich Chemical. *t*-Butyl *p*-nitrophenyl peroxycarbonate and its deuterated analogues were synthesized according to a previously published procedure [10]. Acetate buffers (10 mM) of various pH values were prepared from ammonium acetate titrated to pH 5.2, 5.5, and 5.8 with acetic acid. Tris buffers were 0.1 M solutions titrated to pH 7.5 and pH 8, respectively. Ammonium bicarbonate buffer was a 0.1 M solution at pH 8.6. Buffer systems used to modify peptides and amino acids consisted of a 1:1 mixture of the appropriate buffer and acetonitrile.

MS analyses of modified single amino acid derivatives and peptides were performed on a Thermo Electron Corporation (San Jose, CA, USA) TSQ 7000 or ThermoFinnigan TSQ Quantum 1.0 SR 1 mass spectrometer triple-quadrupole instrument, equipped with an electrospray ionization (ESI) source. The capillary temperature was kept at 200 °C. The electrospray needle voltage was 4.5 kV, and the tube-lens voltage was maintained between 70 and 100 V. The sheath and auxiliary gases (N_2) were adjusted to maximize the signal. Samples were introduced into the ESI source as either lithium chloride solutions or acidic solutions at a rate of 10 to 20 μL/min in direct liquid infusion experiments. For LC-MS analyses of peptides, a Grace Vydac (Hesperia, CA, USA) C18 column (10 cm $\times$ 1 mm) was used. The solvent conditions consisted of a 20-min gradient from 5 to 50% solvent B (95% acetonitrile, 5% water, 0.05% TFA) into solvent A (95% water, 5% acetonitrile, 0.05% TFA). MALDI spectra were obtained on an Applied Biosystems 4700 Proteomics Analyzer using α-cyano-4-hydroxycinnamic acid as the matrix. The MS fragments are assigned based on the calculation of a web-based software MS-Products from UCSF (http://prospector.ucsf.edu/ucsfhtml4.0/msprod.htm).

Results

The selective modification of lysine or N-terminus of a peptide is based on the peroxycarbamate chemistry reported recently [10, 11]. Modification can be achieved by reaction of the peptide solution in ammonium bicarbonate buffer with *t*-butyl *p*-nitrophenyl peroxycarbonate. The appearance of an immediate yellow color indicates the formation of *p*-nitrophenoxide. Even though N-terminus and lysine residues can be modified using the reagent, experimental conditions, especially the pH of the reaction medium, can be optimized to primarily

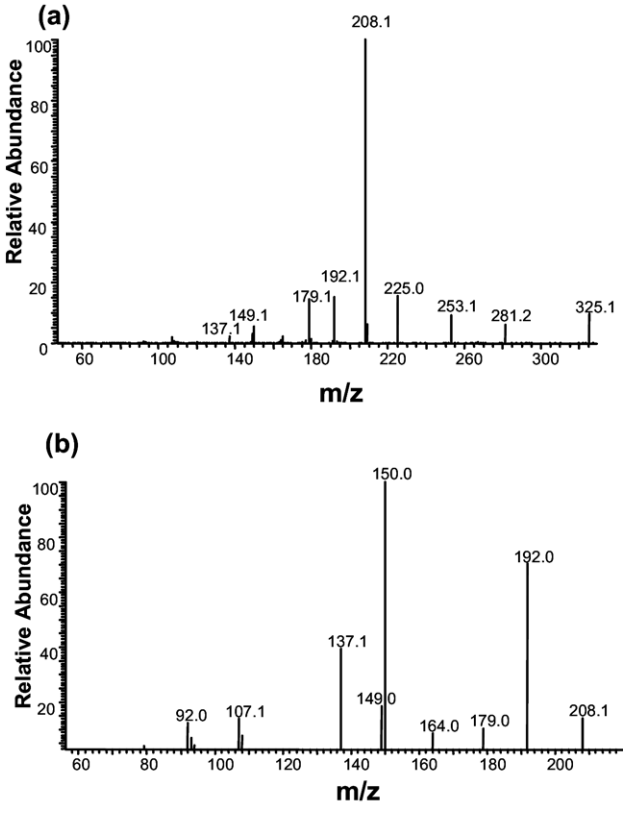

Figure 1. (**a**) CID spectrum of lithium adduct of peroxycarbonate-modified N-acetyl lysine methyl ester (*m/z* 325); (**b**) CID of putative aminyl radical *m/z* 208.