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Letter to the Editor

To the Editor-in-Chief
Sir,

The metastable decomposition of a peptide containing oxidized methionine(s) in matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

Peptide-mass fingerprinting of proteins, in conjunction with database searching, has become a routine method. It is quite useful for the rapid identification of various types of protein samples, such as those obtained from two-dimensional gel electrophoresis. In that case proteins, even at the subpicomole level, can be treated in-gel with an enzyme, chemical reagent, etc., and then be analyzed by high-sensitivity matrix-assisted laser desorption ionization (MALDI) time-of-flight mass spectrometry (TOFMS). However, proteins are often modified, a situation which complicates database searching or the assignment of signals to a sequence based on observed molecular masses of peptide fragments. For the case of an unknown modification, collision-induced dissociation (CID) or post-source decay (PSD) techniques are required to reveal the modification site as well as the chemical structure since the molecular mass alone might not permit a valid structure elucidation. Some specific signal(s) are uniquely derived from a known modification, for example, those observed for a phosphorylated amino acid (pSer, pThr, pTyr)[1] or an oxidized Met.[2–4] When these specific signals are observed in a mass spectrum, the interpretation is quite definite on peptide-mass fingerprinting or database searching.

Met oxidation is one of the most common modifications which spontaneously occur on a protein or a peptide during purification, enzymatic digestion, sample preparation for mass measurements, etc. This affords either completely oxidized proteins or a range of molecular ions with 16 Da intervals as the result of partial oxidation. While the satellite signal at 16 Da higher than the original ion can serve as an indicator of the presence of Met in a sequence, a peptide which contains completely oxidized Met residues (and thus no 16 Da spacing) does not fit any part of a sequence without prior knowledge of this event, and occasionally interferes with the assignment of the signal to a sequence or database search based only on the mass values.[5] Jiang *et al.*[2] and Lagerwerf *et al.*[3] have reported characteristic fragmentation data for methionine sulfoxide (Met(O)) based on low- and high-energy CID, respectively, which involves the elimination of CH_3SOH (64 Da) from the precursor and backbone-fragmented ions containing Met(O). In subsequent studies Qin and Chait,[4] using a MALDI ion trap mass spectrometer, also reported Met(O)-specific fragmentation and commented on its utility for the identification of a Met-containing peptide. In this letter, we wish to report that this unique fragmentation occurs via unimolecular decomposition in both the ion source and field-free region of a TOF mass spectrometer, providing in-source or metastable fragment ions, respectively. These ions are useful for the rapid and unambiguous identification of a Met-containing peptide in its oxidized form, even in a mixture.

Mass spectrometric measurements were performed using a Voyager Elite XL time-of-flight mass spectrometer equipped with a delayed-extraction system (PE Biosystems, Framingham, MA, USA) with flight paths of 4.2 and 6.5 meters for the linear mode and reflector or PSD mode, respectively. Peptide solutions (1 μL) containing 1–5 pmol, obtained by the enzymatic treatment of a protein, were mixed with a matrix solution consisting of the supernatant of a 50% acetonitrile/water solution saturated with α-cyano-4-hydroxycinnamic acid (CHCA), or 2,5-dihydroxybenzoic acid (DHB) solution (20 mg/ml) in 20% acetonitrile/water. This mixture was then air dried on the flat surface of a stainless steel plate. Other measurement conditions were as described previously.[6]

A Met(O)-containing peptide isolated from a lysylendopeptidase digest of *Penicillium minioluteum* dextranase (EC 3.2.1.11),[7] was subjected to MALDI-TOFMS (Fig. 1). In the linear-mode measurement, the peptide gave a signal at *m/z* 2670.4, which is in agreement with the theoretical mass of the peptide containing Met(O) (Fig. 1(a)). A fragment ion was observed at 64 Da lower than the precursor ion by irradiating a sample spot with a relatively higher laser intensity. This fragment ion was assumed to arise from in-source fragmentation (Fig. 1(b)). In the reflector mode using the same laser intensity as in Fig. 1(a), an intense signal separated by 63 Da from the precursor signal was observed (Fig. 1(c)), which was determined by PSD measurement (Fig. 1(e)) to be a fragment ion with 64 Da less mass than the precursor, from which it was formed by metastable decomposition. This discrepancy in mass between the reflector and PSD measurements can be accounted for by the fact that the mass calibration in the reflector mode is accurate only for the case of ions with full acceleration energy, which is not the case for metastable fragment ions, which have less kinetic energy than their precursor ion. Annan and Carr[1] reported a similar observation for the use of reflector-mode TOFMS for a peptide containing a phosphorylated amino acid, which underwent metastable decomposition during flight in a field-free region via the β-elimination of a phosphate group on a pSer residue. It is noteworthy that the yield of the present metastable ion was apparently lowered when DHB was used as the matrix (Fig. 1(d)). The latter can be considered as a 'cool matrix', in comparison with the intensity of the corresponding ion obtained with CHCA (Fig. 1(c)), which is classified as 'hot matrix' in terms of effective energy transfer from matrix to samples.[8] It is also clear, as demonstrated by measurements of Met(O)-containing peptides ranging from 500 to 6000 Da, that larger peptides undergo more intense fragmentation (64 Da loss) than smaller ones (data not shown).

The above results indicate that Met(O), when present in a peptide, can be easily probed by observing either an in-source fragment ion in the linear mode (with high laser intensity) or a metastable fragment ion in the reflector or PSD mode, though the PSD method allows for a more definitive identification of Met(O). The evidence for Met(O) in a sequence could be effectively applied for database searching.

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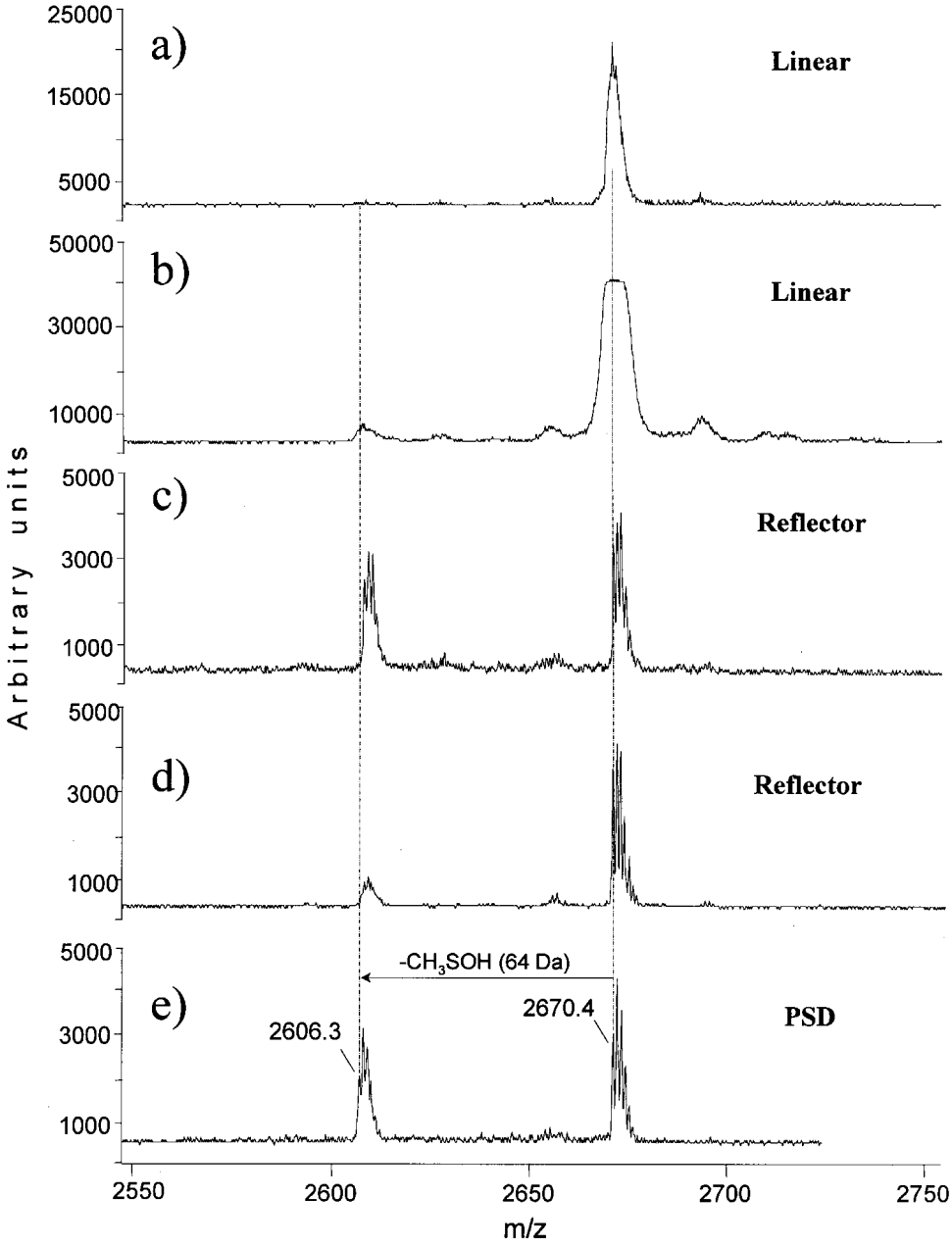

Figure 1. MALDI-TOF mass spectra of an Met(O)-containing peptide (SETVVPSAIIGASPFYASGM (O)SPDSRK, $MH^+_{calc.}$: 2670.3) derived from dextranase. The spectra were obtained using CHCA (a–c, e) or DHB (d) as a matrix by linear-mode (a, b), reflector-mode (c, d), and PSD-mode (e) measurements. The metastable ion observed in (c) and (d) was less well characterized in mass than the precursor ion (*m/z* 2670.4) in the reflector TOFMS experiments, but gave the correct mass at *m/z* 2606.3 in the PSD-mode measurement (e) (see text).

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REFERENCES

1. R. S. Annan and S. A. Carr, *Anal. Chem.* **68**, 3413 (1996).
2. X. Jiang, J. B. Smith and E. C. Abraham, *J. Mass Spectrom.* **31**, 1309 (1996).
3. F. M. Lagerwerf, M. van-de-Weert, W. Heerma and J. Haverkamp, *Rapid Commun. Mass Spectrom.* **10**, 1905 (1996).
4. J. Qin and B. T. Chait, *Anal. Chem.* **69**, 4002 (1997).
5. C. W. Sutton, K. S. Pemberton, J. S. Cottrell, J. M. Corbett, C. H. Wheeler, M. J. Dunn and D. J. Pappin, *Electrophoresis* **16**, 308 (1995).
6. W. Mo, T. Takao and Y. Shimonishi, *Rapid Commun. Mass Spectrom.* **11**, 1829 (1997).
7. B. Garcia, E. Margolles, H. Roca, D. Mateu, M. Raices, M. E. Gonzales, L. Herrera and J. Delgado, *FEMS Microbiol. Lett.* **143**, 175 (1996).
8. W. Mo, T. Takao, H. Sakamoto and Y. Shimonishi, *Anal. Chem.* **70**, 4520 (1998).

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