Hydrogen-deuterium exchange as a method facilitating unambiguous assignment of fragment ions in CID spectra of peptides

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Introduction

During the last years mass spectrometry has been proven to be a powerful and sensitive tool for peptide sequencing and is becoming a more and more useful alternative and complementary approach to automated Edman degradation [1, 2]. But sequence determination of an unknown peptide is still a difficult task due to the initially huge number of possible sequences consistent with the molecular weight of peptide among which the correct one must be chosen by using spectral information about fragment ions and (if any) additional data about peptide. Different derivatization procedures are known to be used to facilitate interpretation of mass spectra [3, 4, 5]. Recently we reported about a facile method of derivatizing peptides for sequence analysis of peptides based on hydrogen/deuterium exchange. When a peptide is dissolved in a deuterated solvent containing labile protons (D_2O deuterated methanol etc.) there is exchange of all hydrogens in the peptide attached to oxygen, nitrogen and sulfur. The number of labile protons in the peptide can be easily determined from measurement of the mass difference between the intact peptide and the peptide after hydrogen/deuterium exchange. This information places significant restraints on the number of different amino acid compositions corresponding to the determined molecular weight of the peptide and provides an efficient filter for a list of candidate sequences provided by a computer interpretation program [6]. In an extension of this work, the present paper reports results of collisionally induced dissociation (CID) experiments of peptides before and after hydrogen-deuterium exchange to demonstrate efficiency of our approach to increase accuracy of fragment ions assignment.

Results and Discussion

Twenty different peptides have been analysed before and after hydrogen/deuterium exchange of labile protons of a peptide using a TSQ-700 triple sector quadrupole mass spectrometer (Finnigan MAT, San Jose, CA, U.S.A.) equipped with a standard electrospray ion source; low energy CID spectra were obtained for all peptides studied using Argon collision gas introduced into the octapole collision cell. Deuterium exchanged samples were prepared by dissolving

the peptide samples in 50% D_2O, methanol-D_4. Analysis of data of CID spectra of peptides before and after hydrogen/deuterium exchange has shown that pathways for fragmentation are not significantly affected by deuterium and shifts of masses of fragments after deuterium exchange coincide with calculated values. Thus the CID spectrum of a peptide after hydrogen/deuterium exchange can be used to confirm fragment ion assignments and is especially valuable when there is ambiguity in assignment. For example, one of the main difficulties which arises in sequencing of an unknown peptide is the mass redundancy associated with certain combinations of amino acid residues: for example -Ala, Gly- and -Gln/Lys-; -Gly,Gly- and -Asn-; -Gly,Val- and -Arg-; -Ala,Asp- and -Trp-; -Ser,Val- and -Trp-; -Gly,Leu- and -Ala,Val- etc. Failure to observe an ion resulting from cleavage between amino acid pairs can result in misassignment of a single amino acid residue for what is actually an amino acid pair. For example, the only peptide which was not sequenced correctly in [7] contained -Ala-Gly-. The computer interpretation of the CID spectrum of this peptide gave -Gln-. Similar problems in sequencing an unknown peptide are described in [8] when -Trp- was misassigned in place of -Val-Ser-. The authors of [9] emphasized that this kind of problem is one of the limitations of their approach. In most such instances the number of exchangeable protons in fragment ions which is available from comparison of CID spectra of intact peptide and deuterated one, enables one to resolve ambiguities in assignment of these fragment ions.

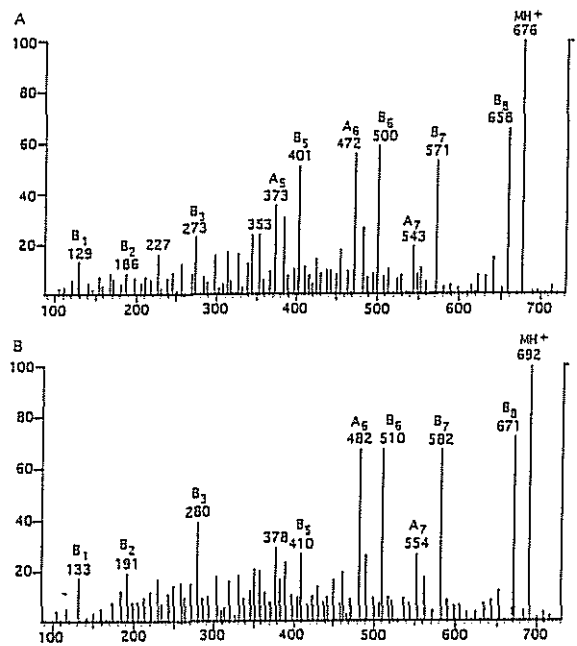

Fig. 1. Collision-induced dissociation fragment-ion spectra of the single protonated peptide KGSGAVAS-amide. Spectrum A is for the ion at m/z 842 formed by electrospray from 20 pm/μl solution in 50% aqueous methanol. Spectrum B is for the hydrogen/deuterium exchanged ion at m/z 864 obtained by electrospray using deuterated water and deuterated methanol.

Figure 1 presents CID spectra of peptide KGSGAVAS-amide with and without deuterium exchange. It is clear from those spectra that no bond cleavage between glycine and alanine observed. However, the shift of B_5 ion from 401 to 410 after deuterium exchange provides the basis to make correct assignment of this fragment to KGSGA (9 labile protons) rather than to KGSQ (10 labile protons).

Comparison of CID spectra of peptides before and after deuterium exchange can be especially valuable when -A,G-; -G,G-; -G,V-; -A,D- etc. are the first two residues of a peptide since very often there is no cleavage between the first and the second residues in CID experiments.

Results presented show that hydrogen/deuterium exchange is a facile technique which has the potential to increase the efficiency of peptides sequencing by mass spectrometry. CID spectra of peptides after hydrogen/deuterium exchange provide important additional information about peptide which can be especially valuable when incorporated in computer interpretation programs.

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