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A new charge derivatization procedure for peptide sequencing

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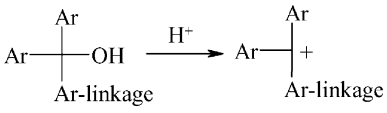
Derivatization of peptides by a trityl cation-containing group and collision-induced dissociation measurements of the cationized peptides results in informative spectra that simplify peptide sequencing.

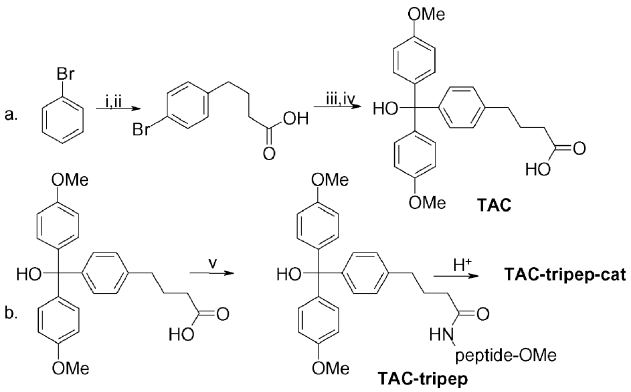
The determination of peptide mixtures has become a crucial step in bioinformatics-related studies and is therefore a major task in modern analytical biochemistry. Mass spectrometry has become the most significant tool for proteomics research and is widely used for the identification and sequencing of peptides.[1] It is a prominent field related, for example, to immunology studies and protein activity.[2] For reliable and confident identification it is desirable to obtain a simple, yet informative, MS/MS spectrum of the charged peptide. Despite other approaches like 'top down'[3] analyses of intact proteins and peptide mass fingerprinting[4] there is still an indubitable interest in tandem mass spectrometry of peptide mixtures using HPLC–MS. The cleavage of a bond in a peptide chain can occur in either of three types of bonds Cα–C, C–N or N–Cα, which yields six types of fragments that are respectively labeled a_n, b_n, c_n when a positive charge is kept by the *N*-terminal side, and x_n, y_n, z_n when the positive charge is kept by the *C*-terminal side.[5] The mass difference between consecutive ions within a series allows the determination of the identity of the consecutive amino acids and thus to deduce the peptide sequence.

Frequently, however, the collision-induced dissociation (CID) spectra of protonated or multiply-protonated peptides show two or more incomplete series of ions that are too complex for interpretation. Consequently, various attempts were made to develop reagents that incorporate a stable charge into the peptide, a charge that directs the fragmentation process, prompting the formation of one informative series of ions.[6] Despite previous work in this field most of the proposed methods form charge-derivatized peptides that are either unstable under electrospray ionization conditions or cleave off during CID. It is also apparent that the proposed methods, for positive ion formation, up to date, are based on ammonium and phosphonium ions while, to the best of our knowledge, no charge derivatization procedure is based on the formation of stable carbenium ions.

Triarylcarbinols give rise to stable trityl cations by the loss of water under acidic conditions (Scheme 1). These cations are easily generated in the electrospray source. Here we report an initial successful attempt to use a new charge derivatization reagent that has been developed for this purpose. The proposed reagent **TAC** (Scheme 2) contains a trityl alcohol that easily forms the desired stable cation upon acidic loss of water, a trimethylene spacer and a carboxylic acid moiety that can be coupled to the peptide by several known methods. The presence of a trimethylene spacer is essential as it has been shown that an analogous reagent that lacks the spacer is less reactive towards the linking step and the CID process that follows.[7]



Scheme 1



Scheme 2 *Reagents and conditions*: i) Succinic anhydride, $AlCl_3$; ii) NH_2NH_2, KOH, triethylene glycol; iii) n-BuLi, THF/hexane; iv) 4,4′-dimethoxybenzophenone; v) NH_2–peptide–OMe, DCC, *N*-hydroxysuccinimide, CH_2Cl_2.[8]

The synthesis of **TAC** follows known procedures as described in Scheme 2a. Reaction of **TAC** with a tripeptide (Ala–Leu–PheOCH$_3$) results in a complete transformation giving rise to the derivatized peptide **TAC–tripep**. Addition of one equivalent of perchloric acid before introduction to the electrospray source results in the formation of the charge-derivatized peptide **TAC-tripep-cat** (Scheme 2b).

A CID spectrum (Fig. 1) was measured for the derivatized peptide **TAC-tripep-cat** using an LCQDuo ion-trap. The CID of this ion is very informative, giving rise to the expected series of b_i ions accompanied by the a_i satellites. The mass difference between two such b_i ions corresponds to the mass of an amino acid. For comparison, the CID of the corresponding protonated peptide was also measured under the same conditions (Fig. 2). As seen in Fig. 2 the fragmentation product in this case is an abundant y_1 ion. We find that the presence of the cationic moiety increases the sensitivity and facilitates the formation of informative fragmentation products.

An MS/MS spectrum was also recorded for a charge-derivatized octapeptide **TAC-octapep-cat** at *m/z* 1275 that was prepared from an octapeptide with the following sequence AFAFAFAFOMe, using infrared multi-photon photodissociation (IRMPD) and a BioAPEXIII FTICR (Fig. 3). In this case mainly b_i ions are identified in the spectrum. When compared with protonation, an obvious advantage of charge derivatization is that formation of various charge states is avoided. This should significantly increase the sensitivity.

A specific advantage of our reagent is that it has a typical absorption at 500 nm so that the desired modified peptides can be selected from the HPLC chromatogram (see Fig. 4). In conclusion, a new derivatization method has been developed that can provide an exclusive series of b-ions, which makes it very easy to identify the peptide sequence. We find that the FTICR is not the best choice for MS/MS measurements of peptides; however, with short irradiations using maximum laser power better results are obtained. A CID spectrum was also measured for the **TAC-octapep-cat** ion at *m/z* 1275 using an LCQDuo

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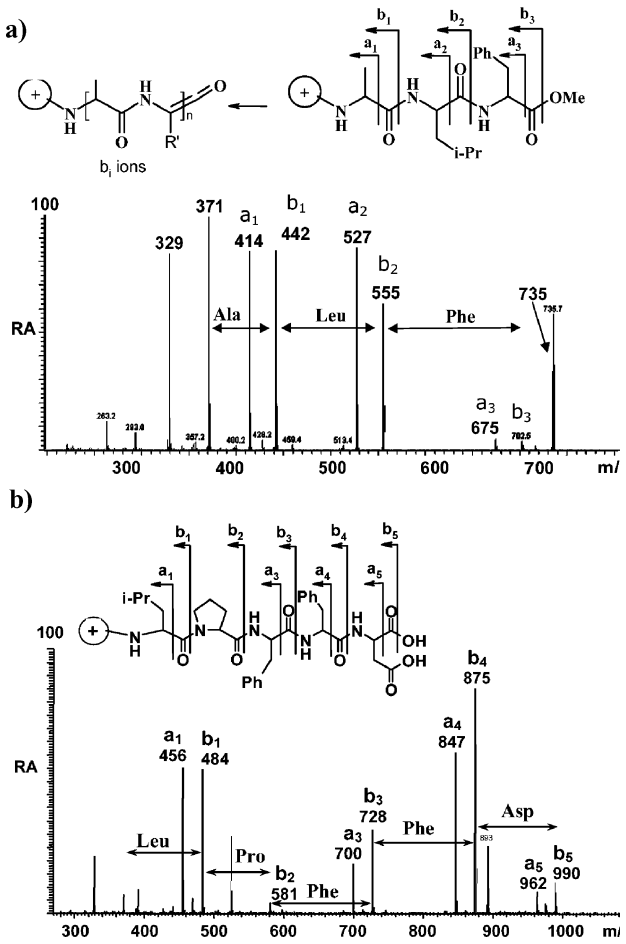

Fig. 1 a) CID spectrum that was measured for the charge-derivatized tripeptide **TAC**–Ala–Leu–PheOCH$_3$ using an LCQDuo Ion-trap; b) CID spectrum that was measured for a free charge-derivatized pentapeptide **TAC**–Leu–Pro–Phe–Phe–AspOH.

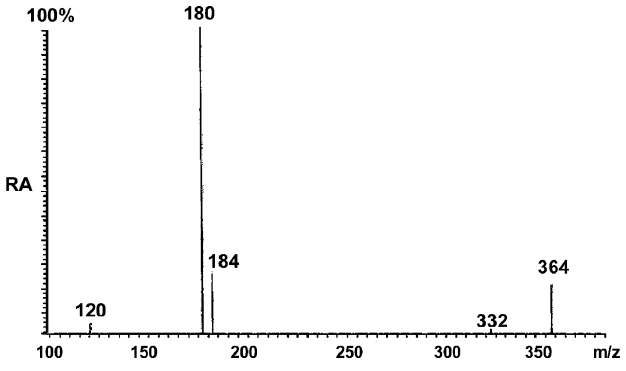

Fig. 2 CID spectrum that was measured for the MH$^+$ ion of Ala–Leu–PheOCH$_3$ using an LCQDuo Ion-trap.

(not shown) and a full b_i ion series was readily identified. CID spectra were also measured for derivatized peptides with a free *C*-terminus giving rise to similar results as shown above (Fig. 1b). Peptides with up to 12 amino acids were examined. No experiments were carried out on peptides longer than 12 amino acids. Presently our new reagent is being tested with peptides that contain different mixtures of all amino acids. In order to use this procedure for a digest mixture it has to be further optimized for small quantities. However, it is concluded that charge derivatization with the proposed reagent, followed by MS/MS measurements contributes to peptide characterization by introducing informative data that are sometimes lacking in the MS/MS spectra of protonated peptides.

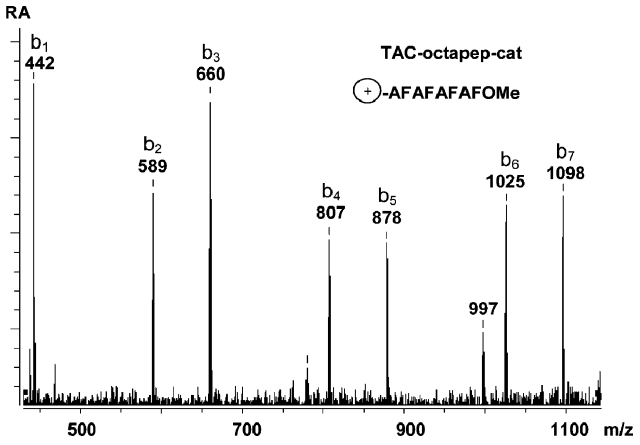

Fig. 3 IRMPD spectrum that was measured for **TAC-octapep-cat** using a BioAPEXIII FTICR.

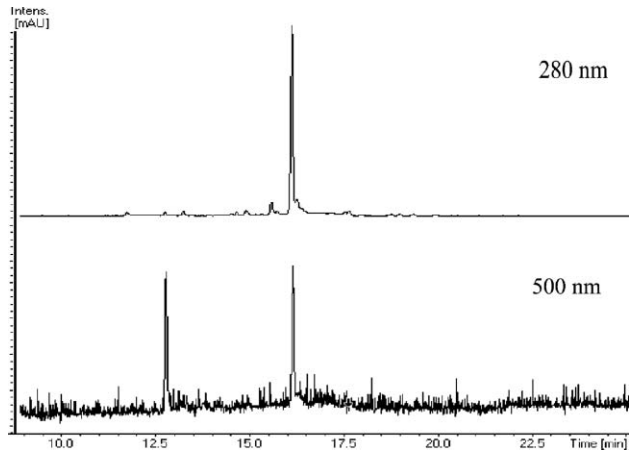

Fig. 4 HPLC chromatogram that was measured for derivatized **TAC-octapep-cat** at 280 and 500 nm. The first peak in the bottom chromatogram corresponds to the excess of the derivatizing agent **TAC**.

Notes and references

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8 The peptide was dissolved in water (1 mg mL^{-1}) and 50 μL of the solution were introduced into an ephendorph tube with 1 mL of acetonitrile. 50 equiv. of diisopropylethylamine were added to the solution, followed by addition of 1–3 equiv. of the activated ester 1 (a stock solution was prepared in CH_2Cl_2, 10 mg mL^{-1}). The reaction takes 2–6 h depending on the peptide and the quantities. The yield of the derivatization was 50–100% depending on the peptide and the quantities. The minimum quantity of peptide used for derivatization was 50 μg. No purification is needed before analysis by mass spectrometry.