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Ammonium sulfate and MALDI *in-source* decay: a winning combination for sequencing peptides

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Abstract

In previous papers we highlighted the role of ammonium sulfate in increasing peptide fragmentation by *in source decay* (*ISD*). The current work systematically investigated effects of MALDI extraction delay, peptide amino acid composition, matrix and ammonium sulfate concentration on peptides *ISD* fragmentation. The data confirmed that ammonium sulfate increased peptides signal to noise ratio as well as their *in source* fragmentation resulting in complete sequence coverage regardless of the amino acid composition. This method is easy, inexpensive and generates the peptides sequence instantly.

Keywords

Peptide sequencing; *in-source* decay; Ammonium sulfate

Introduction

Sequencing has always played an essential role in the study of proteins. In the seventies, enzymatic digests^{1–6} followed by HPLC separation and Edman degradation^{7,8} were the first brake through which furnished researchers with a solid tool for investigating proteins. In the eighties and nineties, two scientific events, mainly the genome project which resulted in the availability of huge data basis (NCBI and Swiss Prot) where one could practically find the sequence of any protein, and the development of mass spectrometric techniques such as matrix assisted laser desorption ionization (MALDI) and electrospray ionization (ESI) combined with commercial availability of easy to use instruments led to the Proteomics age. On the probe digests using exopeptidases was also used for sequencing purposes.^{2,4,5} However, what made protein sequencing a routine event was collision induced dissociation (CID).

Peptides' MALDI data often contain sequence information generated by *in source* decay but has usually low relative abundance and is mistaken for noise. If these areas are enlarged one can often see a partial or even a total sequence. MALDI instruments are easy to use and have high tolerance for contaminants found in buffered biological samples such as high sodium, potassium and phosphate concentration. In addition, the advent of the reflectron improved MALDI further by increasing its sensitivity. As TOF with CID capabilities are expensive and often unavailable, *in source* decay fragmentation (*ISD*)^{9,10} is a good alternative for structural analysis.

A feature of MALDI sources is their ability to vary their extraction delay (ED).¹¹ The flight time of an ion changes linearly with it. These ions are stored and refocused as a function of

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their m/z ratio in the MALDI source. Consequently, the molecular ions (MH^+) resolution is improved,¹² and analytes fragmentation has time to occur.^{13–16} Chemical parameters such as matrices intrinsic properties can improve *ISD* as they help efficient ionization and desorption of analytes, as well as some energy transfer to the analytes thus inducing their fragmentation. Commonly used matrices for peptides and/or proteins analysis are α -cyano-4-hydroxycinnamic acid (CHCA), 2,5-dihydroxy benzoic acid (DHB), and 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid, SA).

In addition, the physicochemical properties of peptides and proteins do influence their reactivity during the MALDI process.^{17,18} Their amino acid composition influences their *in source* fragmentation. Indeed, charged analytes are ionized and desorbed more efficiently than polar or neutral ones. Valero¹⁷ and others attempted to assign a numerical value, the $\bar{R}_m$ index, to the desorption/ionization ability of individual amino acid residues in a peptide. They recorded signal intensities of peptides which varied by one amino acid out of 15. The data were averaged and normalized to the glycine $\bar{R}_m$ (arbitrarily set at 0) to give each amino acid's $\bar{R}_m$.

Previous work^{4,19–21} demonstrated the usefulness of the addition of either ammonium sulfate or ammonium citrate in enhancing the mass spectral signal of peptides analyzed by MALDI-MS. This effect was also shown for noncovalent complexes of peptides and single or double stranded DNA,²² as well as for lipids.²³ Marzilli *et al.*⁶ applied this technique to observe peptide product ions obtained by *in-source decay* with MALDI-TOF, and demonstrated an increase of the signal to noise ratio of *ISD* and thus of product ions from a peptide by using either ammonium sulfate or ammonium citrate which allowed them to get bradykinin and other peptides whole sequence coverage. The same approach was used to analyze peptides from a biological mixture.

In this work we propose a simple, reproducible and efficient method for sequencing peptides from *ISD* fragments, by adjusting the previously cited parameters and adding ammonium sulfate to our sample preparation (work done in positive ionization mode). As in previous studies^{6,19}, it generated more fragmentation and better resolved peptides' sequence ions.

Material and method

Peptides

SAQESQGNT, SFKRRRSSK and EDDDQSVSED were synthesized at the Peptide Synthesis Core Facility of the Johns Hopkins University School of Medicine, (Baltimore, MD). All peptides were dissolved in deionized water. The stock solution concentration was 1 nmol. μ L⁻¹.

Matrices

Saturated solution of 2,6-dihydroxy-acetophenone matrix (DHA), 2,5-dihydroxy benzoic acid (DHB), α -cyano-4-hydroxycinnamic acid (CHCA) and trihydroxy-acetophenone (THA) were prepared in 50% ethanol. All matrices were purchased from Fluka, Buchs, Switzerland.

Salts

The following concentration of ammonium sulfate solutions (J.T. Baker, Phillipsburg, PA) 250mM, 1M, 3M and 5M were tested.

MALDI TOF-TOF

Spectra were acquired in positive ion mode, on a MALDI TOF-TOF (4700 Proteomics Analyzer, Applied Biosystems, Framingham MA) mass spectrometer used in reflectron mode.

The laser power was optimized for each experiment (Nd:YAG, 355 nm, repetition rate of 200 Hz). Ion acceleration and grid voltages were set at 20 kV and at 14 kV, respectively. The extraction delay (DE) was optimized depending on the experiments.

Samples preparation

Samples were prepared directly on the MALDI plate: to 0.3 μL of 1 nmol. μL^{-1} peptide was added 0.3 μL of ammonium sulfate or not (for the blank), followed by 0.3 μL of saturated matrix (DHA, DHB, CHCA or THA).

Results

Instrumental parameter variation effects: extraction delay variation

Mass spectra of SAQESQGNT with THA matrix were acquired using fixed laser fluency and various extraction delay times (900ns in light grey, 1350ns in dark grey and 1800ns in black). Figure 1 displays the relative intensities (*I*) of the peaks of interest plotted as a function of ion labels from low to high *m/z*. For each spectrum, the MH^+ with the highest relative intensity value was set at 100 %. Other intensities were calculated relative to the latter.

When the extraction delay was increased to 1800ns (black in Figure 1), two additional sequence ions (b_4 at *m/z* 416, b_6 at *m/z* 631) were observed as compared to the lower delay. However, the number of peaks due to noise as well as the peak width increased, thus increasing ambiguity about peak assignment. On the other hand, when delayed extraction was decreased, the mass spectral sensitivity for the peptide sequence ions of interest decreased. As an example, y_4 at *m/z* 419 is not observed at 900ns (light grey in Figure 1). Moreover, for short delayed extraction, more matrix ions are observed and interfere with peptide signals.

Effects of the variation of chemical parameters

Matrix effect on the number and nature of observed product ions in the absence of Ammonium Salts—Four matrices were used to analyze the peptides of interest: CHCA, DHA, DHB and THA. The data in Table 1 show several differences in the composition and number of generated product ions depending on the nature of the matrix employed. In the case of the polar weakly charged SAQESQGNT peptide, DHA gave the worst results, i.e., the smallest number of product ions (2 versus about ten for other matrices). However, no differences were observed between the three other matrices CHCA, DHB, and THA which generated the same peptide sequence profile. SFKRRRSSK fragmentation was more difficult due to the presence of numerous adjacent basic residues. The best results were obtained with $\text{CHCA} > \text{DHB} = \text{THA}$ (67% of the sequence). In the case of the acidic peptide EDDQSVSED, none of the matrices gave the peptide whole sequence. The best results were obtained with $\text{DHB} = \text{DHA} \approx \text{THA}$ ($\approx 45\%$ of the sequence).

No simple correlation was found between *ISD* mass spectra enhancement and the pH of [matrix + peptide] or hydrophobicity¹⁷ or matrix proton affinity. The rest of the parameters reported in this paper are those obtained using THA for matrix.

Product ions generated as a function of peptides amino acid composition in the absence of Ammonium Salts—A qualitative display of peptide ionization efficiency as a function of their amino acid composition (THA matrix used) is seen in figure 2. The efficiency is reported as the ratio of the molecular ion intensity MH^+ over the intensity of a known ion at *m/z* 390 which comes from THA ionization. Ten mass spectra were acquired per peptide. The more basic the peptide, the better the ionization: $\text{SFKRRRSSK} > \text{SAQESQGNT} > \text{EDDQSVSED}$. Error bars indicate the irreproducibility of MALDI mass spectra but a reproducible trend in peptides ionization/desorption as a function of their sequence is observed.

Moreover, differences are detected between fragmentation pathways of both the polar weakly charged (SAQESQGNT) and the acidic (EDDDQSVSED) versus the basic (SFKRRRSSK) peptide in the *in-source* decay process (Table 1). In this study, only sequence ions will be discussed. SAQESQGNT and EDDDDQSVSED *in source* fragmentation gave mostly complementary y_n and b_n series. SFKRRRSSK *in source* decay spectra also display mostly y_n and b_n ions as well as c_n ions. In addition, fragment ions show loss of water, and some internal fragments as $[\text{QESQG-H}_2\text{O-NH}_3 + \text{H}]^+$ at m/z 439.21 (Figure 1a) are observed. Sodium and potassium adducts were seen. *ISD* also provided other minor ion series depending on the studied peptides.¹⁰ The matrix's nature also affects peptides ionization and *ISD* fragmentation.

Ammonium sulfate effect—To confirm previous results^{6,19,24}, we did a systematic investigation of the effect of ammonium sulfate concentration (250mM, 1M, 3M and 5M) on inducing *ISD* in all 3 peptides (EDDDQSVSED, SAQESQGNT and SFKRRRSSK). Each of these ammonium sulfate concentrations enhanced all peptides' *ISD*. However, the highest salt concentration required higher laser fluency. Results obtained with the 3M concentration will be discussed.

Ammonium sulfate addition enhanced peptides ionization (Figure 2 and 3) independently of the peptide's make up and the matrix used. In addition, the more basic the peptide, the better it ionized, as previously noted, but with a magnified effect due to ammonium sulfate addition: SFKRRRSSK > SAQESQGNT > EDDDDQSVSED. An increase in the number of peptide product ions formed by *ISD* was also observed (Figure 2 and 3). Use of THA in these conditions gave the best results and seemed to improve the efficiency of the fragmentation of all peptides. Indeed, the complete sequence of the three different peptides (Table 2) was obtained. Use of DHB was adequate in obtaining the sequence of the polar weakly charged peptide, SAQESQGNT, and of the acidic peptide, EDDDDQSVSED, but only a partial sequence of the basic one, SFKRRRSSK. With CHCA, the sequence of SAQESQGNT was obtained with or without ammonium sulfate. DHA did not improve the number of product ion from peptides' *ISD*.

No simple correlation was found between *ISD* mass spectra enhancement and [matrix + peptide + 3M salt solution] pH²⁹ or hydrophobicity or matrix proton affinity.

Discussion

Instrumental parameter variation effects: extraction delay variation

Optimization of extraction delay times resulted in an increase in the number of product ions and their relative abundance and resolution. The extra time added before ions' acceleration from the MALDI source into the analyzer is necessary to optimize either metastable ion fragmentation^{10,13–16} or prompt ion fragmentation¹⁵, as demonstrated in previous papers. The resolution of MH^+ generated by *ISD* is usually degraded by the use of high laser fluency, which determines the time needed to dissipate the cloud of material obtained. This phenomenon can be counterbalanced by increasing the extraction delay value. It allows focusing ions in time in the field free region of the MALDI source.^{12,25,26} However, it should be optimized for the m/z range, as a short delay time favors ions of lower m/z , while a longer one favors ions of higher m/z .

Effect of the variation of chemical parameters

Type of product ions generated as a function of peptides amino acid composition—Peptide amino acid composition influences peptides ionization by MALDI. Several authors^{17,18} highlighted the role of amino acids proton affinity on molecular ion

yielded in MALDI. Valero *et al.*¹⁷ obtained normalized mean desorption index, $\bar{R}_m$, for 17 amino acids out of 20 studying peptides in positive ion mode. They chose glycine G as their amino acid of reference and set its $\bar{R}_m$ at 0. Their results show that arginine's presence highly favors peptides ionization, compared to G and that acidic residues (D and E) are unfavorable to peptides ionization. As far as neutral polar amino acids presence is concerned, the authors observed heterogeneous effects on peptides ionization. Surprisingly, they found that K was less favorable to peptides' ionization than G. A peptide $\bar{R}_m$ was obtained in our study by summing the $\bar{R}_m$ of its amino acids. SFKRRRSSK $\bar{R}_m$ equals 33.8 ± 1.6 ; SAQESQGNT $\bar{R}_m$ equals 2.5 ± 1.6 ; and EDDQSVSED $\bar{R}_m$ equals 0.2 ± 1.6 . The peptides ionization efficiency order is in agreement with what was observed in the present study. However, there is no linear correlation between a peptide $\bar{R}_m$ and its ionization when comparing Valero's *et al.* peptides $\bar{R}_m$ and their respective ionization. Indeed, a part of Valero's *et al.*¹⁷ observations were confirmed by Baumgart *et al.*¹⁸ Nevertheless, the latter noticed that the impact of amino acids on peptides intensity is affected by the amino acids nearby. Efforts put to describe and predict peptides ionization/desorption need to take into account other parameters such as the matrix to describe the MALDI process as correctly as possible. This type of experiments should be pursued as they will generate a better understanding of the MALDI process as well as the need to improve sample preparation.

Ammonium sulfate effect—The parameter that most effectively enhanced peptides ionization or *ISD* was the use of ammonium sulfate. Classically, salts are extensively used to induce protein precipitation in many biochemical methods for protein purification. In the sulfate salt case, this anion is highly charged and is known as the kosmotrope leading the Hofmeister lyotropic series.^{27,28, 29} SO_4^{2-} anion in a solution of proteins and/or peptides is one of the most efficient anion to increase the solvent surface tension and thus the most efficient to decrease proteins and peptides solubility.²⁹ By building strong interactions with water molecules in the solvent, it disrupts peptide/water interaction, allowing peptides and proteins precipitation. So it is no surprise that this salt is traditionally used in X-ray crystallography to precipitate proteins.³⁰ consequently in the presence of sulfate, peptides (and proteins) are less solvated and thus easily co-crystallize with matrix. (Addition of ammonium sulfate salt to sample has a directly observable effect on sample crystallization. See figure 6 from a previous paper¹⁹ which compare spots of sample + matrix +/- ammonium sulfate. Spots with ammonium sulfate present a more opaque color). They are more easily ionizable during the MALDI process, although its effect on protein crystallization does not seem to be yet completely understood.²⁹

Ammonium sulfate addition has also a strong effect on the number of peptide sequence ions generated. Its presence seems to make the energy applied in the source more available for peptide fragmentation, as the salt does decrease the amount of solvent around peptides.

The third effect observed is the decrease of salt adducts in the presence of ammonium sulfate. SO_4^{2-} anion can bind cations usually forming adducts with peptides, thus decreasing possibilities for these cations to bind peptides.

Biological samples—We demonstrated the usefulness of ammonium sulfate among the parameters able to improve *ISD* on peptides. In fact, ammonium sulfate and other salts already proved to be efficient in complex matrices. Woods *et al.* used ammonium salts since 1994 to improve peptides ionization/desorption and *ISD* from cell lysates. They first reported ammonium salts use to isolate and sequence a tap-dependent leader peptide⁴ from an HPLC fraction. Then, they used the same approach to identify and sequence MHC class one peptides with on the probe exopeptidases digestion^{19,21} of a ganglioside recognition domain of tetanus toxin purified by SDS-PAGE⁵. These previous results and our current study show the breadth and depth of this technique, as it is not just helpful in the case of synthetic peptides, it also

contributes to resolving the structure of peptides present in femtomoles quantity in biological samples. We believe that with any type of sample if improvement of ionization/desorption and/or *in source* fragmentation is needed, one should always repeat the analysis using ammonium salts.

Conclusion

Peptide sequencing can be done easily by *ISD* mass spectrometry if certain parameters are taken into account and optimized. An extraction delay needs to be applied as well as optimized as a function of the *m/z* interval of interest. An efficient matrix needs to be chosen. In this study, THA gave the best results. A peptide precipitating agent (3M ammonium sulfate) added to the sample tremendously improved peptide's *ISD*. This method is easy, inexpensive and efficient.

Although our samples were pure peptides, it was also proven useful in complex biological samples such as those of MHC class one peptides^{21,23} and others.^{19,5} More peptides as well as proteins will be studied to optimize the parameters and determine the limits of this method: longer peptides, different amino acid compositions, peptides with post-translational modifications.

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References

1. Caprioli RM, Fan T. Peptide sequence analysis using exopeptidases with molecular analysis of the truncated polypeptides by mass spectrometry. *Anal. Biochem* 1986;154:596–603. [PubMed: 2425658]
2. Welsh AR, Woods AS, McNally LM, Cotter RJ, Gibson WA. Herpesvirus maturational proteinase, assemblin: identification of its gene, putative active site domain, and cleavage site. *Proc. Nat. Acad. Sci* 1991;88:10792–10796. [PubMed: 1961747]
3. Woods AS, Yoshioka M, Bullesbach E, Schwabe C, Cotter RJ. Enzymatic digestion on the sample foil as a method for sequence determination by plasma desorption mass spectrometry: the primary structure of porpoise relaxin. *Int. J. Mass Spectrom. Ion Processes* 1991;111:77–88.
4. Aldrich CJ, DeCloux A, Woods AS, Cotter RJ, Soloski MJ, Forman J. Identification of a Tap-Dependant Leader Peptide Recognized by alloreactive T Cells Specific for a Class Ib Antigen. *Cell* 1994;79:649–658. [PubMed: 7525079]
5. Shapiro RE, Specht CD, Collins BE, Woods AS, Cotter RJ, Schnaar RL. Identification of a Ganglioside Recognition Domain of Tetanus Toxin Using a Novel Ganglioside Photoaffinity Ligand. *J. Biol. Chem* 1997;272:30380–30386. [PubMed: 9374528]
6. Marzilli LA, Golden TR, Cotter RJ, Woods AS. Peptide sequence information derived by pronase digestion and ammonium sulfate in-source decay matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *J. Am. Soc. Mass Spectrom* 2000;11:1000–1008. [PubMed: 11073263]
7. Edman P, Begg G. A protein sequenator. *Eur. J. Biochem* 1967;1:80–91. [PubMed: 6059350]
8. Hewick RM, Hunkapiller MW, Hood LE, Dreyer WJ. A gas-liquid solid phase peptide and protein sequenator. *J. Biol. Chem* 1981;256:7990–7997. [PubMed: 7263636]
9. Kaufmann R, Spengler B, Lützenkirchen F. Mass spectrometric sequencing of linear peptides by product-ion analysis in a reflectron time-of-flight mass spectrometer using matrix-assisted laser desorption ionization. *Rapid. Commun. Mass Spectrom* 1993;10:902–910. [PubMed: 8219321]
10. Hardouin J. Protein sequence information by Matrix Assisted Laser Desorption/Ionization *in-source decay* mass spectrometry. *Mass Spec. Rev* 2007;26:672–682.

11. Wiley WC, MacLaren IH. Time-of-flight mass spectrometer with improved resolution. *Rev. Sci. Instr* 1955;26:1150–1157.
12. Vestal ML. Delayed extraction matrix-assisted laser desorption time-of-flight mass spectrometry. *Rapid. Commun. Mass Spectrom* 1995;9:1044–1050.
13. Brown RS, Lennon JJ. Sequence-specific fragmentation of Matrix-Assisted Laser-Desorbed Protein/peptide ions. *Anal. Chem* 1995;67:3990–3999. [PubMed: 8633762]
14. Brown RS, Carr LB, Lennon JJ. Factors that influence the observed fast fragmentation of peptides in Matrix-Assisted Laser Desorption. *J. Am. Soc. Mass Spectrom* 1996;7:225–232.
15. Brown RS, Feng J, Reiber DC. Further studies of in-source fragmentation of peptides in matrix-assisted laser desorption-ionization. *Int. J. Mass Spectrom. Ion Processes* 1997;169–170. 1–18.
16. Katta V, Chow DT, Rohde MF. Applications of in-source fragmentation of protein ions for direct sequence analysis by delayed extraction MALDI-TOF mass spectrometry. *Anal. Chem* 1998;70:4410–4416. [PubMed: 9796424]
17. Valero ML, Giralt E, Andreu D. An investigation of residue-specific contributions to peptide desorption in MALDI-TOF mass spectrometry. *Letters in Peptide Science* 1999;6:109.
18. Baumgart S, Lindner Y, Kühne R, Oberemm A, Wenschuh H, Krause E. The contributions of specific amino acid side chains to signal intensities of peptides in matrix-assisted laser desorption/ionization mass spectrometry. *Rapid Commun. Mass Spectrom* 2004;18:863–868. [PubMed: 15095354]
19. Woods AS, Huang AYC, Cotter RJ, Pasternack GR, Pardoll DM, Jaffee EM. Simplified high-sensitivity of a major histocompatibility complex class I-associated immunoreactive peptide using matrix-assisted laser desorption/ionization mass spectrometry. *Anal. Biochem* 1995;226:15–25. [PubMed: 7785766]
20. Plafker SM, Woods AS, Gibson W. Phosphorylation of simian cytomegalovirus assembly protein precursor (pAPNG.5): multiple attachment sites identified, including two adjacent serines in a casein kinase II consensus sequence. *J. Virol* 1999;73:9053–9062. [PubMed: 10516011]
21. Lo W-F, Woods AS, DeCloux A, Cotter RJ, Metcalf ES, Soloski MJ. Molecular mimicry mediated by MHC class Ib molecules after infection with Gram-negative pathogens. *Nature Medicine* 2000;6:215–218.
22. Lin S, Cotter RJ, Woods AS. Detection of non-covalent interaction of single and double stranded DNA with peptides by MALDI-TOF. *Proteins Structure, Function and Genetics* 1998;2:12–21.
23. Joyce S, Woods AS, Yewdell JW, Bennink JR, De Silva AD, Boesteanu A, Balk SP, Cotter RJ, Brutkiewicz RR. A major natural ligand of mouse CD1d1: is cellular glycosylphosphatidylinositol. *Science* 1998;279:1541–1544. [PubMed: 9488653]
24. Pedneault, F.; Vrandeć, M.; Arsenault, A.; Pimenov, A. The importance of adducts selection on selectivity and sensitivity for determination of calcitriol in human plasma by LC/MSMS. *Proc. Of the 50th ASAM Conference on Mass Spectrometry and Allied Topics*; June 2–6 2002; Orlando, Florida.
25. Juhasz P, Vestal ML, Martin SA. On the initial velocity of ions generated by matrix-assisted laser desorption/ionization and its effect on the calibration of delayed extraction time-of-flight mass spectra. *J. Am. Soc. Mass Spectrom* 1997;8:209–217.
26. Brown RS, Lennon JJ. Mass resolution improvement by incorporation of pulsed ion extraction in a matrix-assisted laser desorption-ionization linear time-of-flight mass spectrometer. *Anal. Chem* 1995;67:1998–2003. [PubMed: 8694246]
27. von Hippel, PH.; Schleicht, T. *Structure and Stability of Biological Macromolecules*. New York: Dekker; 1969. The effects of neutral salts on the structure and conformational stability of macromolecules in solution; p. 417-574.
28. Dennison C, Lovrien R. Three phase partitioning: concentration and purification of proteins. *Protein expression and purification* 1997;11:149–161. [PubMed: 9367811]
29. Curtis RA, Prausnitz JM, Blanch HW. Protein-protein and protein-salt interactions in aqueous protein solutions containing concentrated electrolytes. *Biotechnology and Bioengineering* 1998;57:1, 11–20. [PubMed: 10099172]
30. Mcpherson A. A comparison of salts for the crystallization of macromolecules. *Protein Science* 2001;10:418–422. [PubMed: 11266627]

Figure 1.

ISD spectra obtained from SAQESQGNT using THA as a matrix, at fixed laser fluency and different extraction delay times. For each ED, the molecular ion MH^+ relative intensity (I) was set at 100 %. Other intensities were calculated relative to this one.

Figure 2.

Qualitative peptide ionization efficiency as a function of their make up and addition or not of $(\text{NH}_4)_2\text{SO}_4$ 3mol.l^{-1} . The efficiency is reported as the ratio of the molecular ion intensity I $[\text{MH}^+]$ over the intensity of m/z 390, a known ion from THA. Ten mass spectra were acquired per peptide.

Figure 3.

ISD spectra of SAQESQGNT a) without and b) with addition of $(\text{NH}_4)_2\text{SO}_4$ 3mol.l^{-1} and THA, of SFKRRRSSK c) without and d) with addition of $(\text{NH}_4)_2\text{SO}_4$ 3mol.l^{-1} and THA, and of EDDDQSVSED e) without and f) with addition of $(\text{NH}_4)_2\text{SO}_4$ 3mol.l^{-1} and THA. Triangles indicate y_n series and related ions. circles indicate b_n series and related ions. Squares indicate c_n series and related ions.

Table 1

Percentage of sequence recovered by *ISD* as a function of peptide make up and matrix used. The percentage was calculated by dividing the number of recognized amino acid (through sequence ions assignment) by amino acid number present in the peptide of interest.

	DHA	THA	CHCA	DHB	sequence
SAQ E SQGN T	b y	b c y	b c y	b c y	0% 100%
SF K RRRRSS K	b	c y	b c y	b c y	
E DD D QSV S E D	b c y	b y	b	b c y	

Table 2

Percentage of sequence recovered by *ISD* as a function of peptide make up, matrix used and addition of $(\text{NH}_4)_2\text{SO}_4$ 3 mol.l^{-1} . The percentage was calculated by dividing the number of recognized amino acid (through sequence ions assignment) by amino acid number present in the peptide of interest.

	DHA	THA	CHCA	DHB	sequence
SAQESQGN^T	b y	b c y	b y	b c y	
SFKRRRSSK	c y	b c y	b c y	c y	
EDDDQSVSE^D	b c y	b c y	b c y	b c y	0% 100%