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# MALDI, AP/MALDI and ESI techniques for the MS detection of amyloid β-peptides

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ABSTRACT

Amyloid β-peptides (Aβs) are involved in several neuropathological conditions such as Alzheimer's disease and considerable experimental evidences have emerged indicating that different proteases play a major role in regulating the accumulation of Aβs in the brain. Particularly, insulin-degrading enzyme (IDE) has been shown to degrade Aβs at different cleavage sites, but the experimental results reported in the literature and obtained by mass spectrometry methods are somehow fragmentary.

The detection of Aβs is often complicated by solubility issues, oxidation artifacts and spontaneous aggregation/cleavage and, in order to rationalize the different reported results, we analyzed Aβs solutions by three different MS approaches: matrix assisted laser desorption ionization-time of flight (MALDI-TOF), atmospheric pressure (AP) MALDI ion trap and electrospray ionization (ESI) ion trap. Differences in the obtained results are discussed and ESI is chosen as the most suitable MS method for Aβs detection. Finally, cleavage sites produced by interaction of Aβs with IDE are identified, two of which had never been reported in the literature.



## 1. Introduction

The pivotal role that amyloid β-peptides (Aβs) play on several neuropathological conditions is universally accepted [1]. Although controversy still exists whether the formation of amyloid deposits is the primary cause of Alzheimer's disease (AD), it is well known that the accumulation of Aβs contributes to its etiology and progression [2]. For this reason, several studies have been focused on the mechanism involved in amyloid fibrillation [3] as well as on the characterization of amyloid deposits [4,5]. Moreover, as experimental evidences [6,7] show that protofilaments formed in the initial self-assembly process of Aβs are more toxic than final fibrils, some experimental attempts to disrupt the fibrillation mechanism by degrading the oligomeric forms of Aβs have been carried out by many independent groups [8,9] and considerable experimental evidences have emerged indicating that several different enzymes play a major role in regulating the accumulation of Aβ in the brain [10]. Furthermore, as hyperinsulinaemia is associated with a high risk of AD [11], it has been proposed that insulin-degrading enzyme (IDE) is particularly important for degrading Aβs [12], playing a critical role in the mechanism associating hyperinsulinaemia and type 2 diabetes with AD. For this reason, since the discovery of IDE cleavage action on Aβs [13], much effort has been put in trying to understand some key questions regarding cleavage sites [14], kinetics of interaction [15], whether IDE is capable of degrading Aβs bearing pathogenic amino acid substitutions [16] and specific features of the IDE-produced amyloid fragments [17]. In this scenario, as reported for the interaction between IDE and insulin molecules [18], a search for a suitable experimental technique able to detect Aβ molecules as well as the fragments produced by IDE cleavage action has been carried out [19] and specific mass spectrometry (MS) methods have been often proposed [14,20,21]. Nevertheless, we found that the reported cleavage sites identified for the action of IDE on Aβs seem to vary dependently to the different experimental approaches used by the investigators [15–17,21]. Moreover, solubility issues [22], oxidation artifacts [23], spontaneous aggregation [24,25] and cleavage [20] of Aβs further contribute to make MS investigations of these peptides a very challenging task.

In this work, we compare the MS results obtained from Aβs aqueous solutions by (i) matrix assisted laser desorption ionization-time of flight (MALDI-TOF), (ii) atmospheric pressure (AP) MALDI ion trap and (iii) electrospray ionization (ESI) ion trap. We show that for both MALDI-TOF and AP MALDI approaches matrix/sample ratios drastically affect Aβs detection. The experimental findings are rationalized and the ESI technique is shown to be more suitable for analyzing amyloid solutions. Experiments involving IDE proteolytic activity are also carried out on Aβ 1–40 and Aβ 1–42 solutions by ESI MS and two unreported cleavage sites are unveiled.

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## 2. Experimental

### 2.1. Materials

IDE, His–Tag, rat, recombinant, from *Spodoptera frugiperda* was purchased from Calbiochem and its activity was verified by carrying out enzymatic digestion of insulin solutions according to the experimental procedure previously reported [18]. Phosphate buffer solution (PBS), $NH_4HCO_3$, α-cyano-4-hydroxycinnamic acid (CHCA), trifluoroacetic acid (TFA), acetonitrile ($C_2H_3N$), ethanol, dimethyl sulfoxide (DMSO) and insulin from bovine pancreas were all purchased from Sigma–Aldrich, while $ZipTip_{SCX}$ pipette tips were from Millipore and Aβ 1–40 and Aβ 1–42 were purchased from Bachem. High-purity water (Milli-Q Element Ultrapure Water) was used for preparation of all the solutions.

### 2.2. Sample preparation

Predissolution in stock solvents is commonly practiced to overcome the limited solubility of Aβs in physiological buffers used in neurotoxicity assays and, as it is reported in the literature that in pure DMSO Aβs appear to be monomeric [26] and lacking any β-sheet character [22], this solvent was used for the preparation of stock solutions (1 mM). For MS analysis, 1 μl of stock solution was diluted with 99 μl of water or physiological buffers (100 mM $NH_4HCO_3$ or PBS pH 7.4 both gave satisfactory results for enzyme degradation assays) and 10 μl of the resulting solution were either injected into the ESI source (after further dilution with 45 μl of methanol and 45 μl of TFA (1%)) or ZipTipped and spotted onto the MALDI-TOF/AP MALDI plate. CHCA in 30% TFA (0.1%) and 70% $C_2H_3N$ (CHCA was purified by re-crystallization from ethanol solution) was used as matrix in all AP MALDI and MALDI-TOF experiments.

In order to verify IDE cleavage sites on Aβ solutions, ESI was chosen as the most suitable MS approach and an enzymatic degradation was carried out in $NH_4HCO_3$ buffer (pH 7.4) by keeping the reaction mixture containing [IDE] = 36 nM and [Aβ] = 10 μM at 37 °C for 1 h. We used that concentration because it affords an enough mass spectrometric signal keeping Aβ in monomeric form [27]. 45 μl of methanol and 45 μl of TFA (1%) were then added and the resulting solution was directly injected into the ESI source.

### 2.3. MS experimental setup

ESI MS measurements were carried out by using a Finnigan LCQ DECA XP PLUS ion trap spectrometer operating in the positive ion mode and equipped with an orthogonal ESI source (Thermo Electron Corporation, USA). Sample solutions were injected into the ion source at a flow-rate of 10 μl/min, using nitrogen as drying gas. The mass spectrometer operated with a capillary voltage of 46 V and capillary temperature of 200 °C, while the spray voltage was 4.3 kV.

AP MALDI MS measurements were carried out on the same spectrometer which was fitted with a MassTech Inc. (USA) AP MALDI source. The latter consists of a flange containing a computer-controlled *X-Y* positioning stage and a digital camera, and is powered by a control unit that includes a pulsed nitrogen laser (wavelength 337 nm, pulse width 4 ns, pulse energy 300 μJ, repetition rate up to 10 Hz) and a pulsed dynamic focusing (PDF) module that imposes a delay of 25 μs between the laser pulse and the application of the high voltage to the AP MALDI target plate [28]. Laser power was attenuated to about 55%. The target plate voltage was 1.8 kV. The ion trap inlet capillary temperature was 200 °C. Capillary and tube lens offset voltages of 30 and 15 V, respectively, were applied. Other mass spectrometer parameters were as follows: multipole 1 offset at −3.75 V, multipole 2 offset at −9.50 V, multipole RF amplitude 400 V, lens at −24.0 V and entrance lens at −88.0 V. Xcalibur software was used for the elaboration of mass spectra.

MALDI-TOF mass spectra were acquired by a Voyager DE (PerSeptive Biosystem) using an accelerating voltage of 25 kV applied after 50 ns with a potential gradient of 1363 V/mm and a wire voltage of 2.5 V. The instrument is equipped with a nitrogen laser (emission at 337 nm for 3 ns) and a flash AD converter (time base 2 ns). In order to investigate the fragmentation of the analyte, the laser irradiance was varied as described in the next section. Bovine insulin (molecular weight = 5733.5 Da) was used for mass calibration.

It is important to highlight that capillary voltage, capillary temperature, spray voltage (ESI), target plate voltage (AP MALDI), accelerating voltage and wire voltage (MALDI-TOF) did not have any influence on the detection of Aβ fragments as they were widely changed over the whole range of possible values and the only effect on the recorded mass spectra was a change in the signal/noise ratio. The optimization of the latter was in all cases responsible for the chosen reported values of the above mentioned experimental parameters. Because of the isotopic composition, molecular species are detected in the mass spectra as clusters of peaks not always well resolved; to simplify their assignments, reported calculated values refer to average *m*/*z*.

## 3. Results and discussion

Details of the fibrillation mechanism of Aβs are widely reported in the literature, but very often MS investigations have to be combined with hydrogen/deuterium exchange methods [29] as well as enzyme proteolysis [30], due to the difficulties encountered in detecting Aβs fibrils by MS. Moreover, as it was mentioned in the introduction, the initial protofilaments formed in the self-assembly process of Aβs have been shown to be more toxic than the final fibrils [6,7]. For these reasons, the monomeric and oligomeric forms of Aβs represent an important target for MS studies [31,32], but a complete knowledge about the formation of Aβs monomer fragments produced by the action of IDE has yet to be obtained. Specifically, a clear-cut differentiation between Aβs fragments produced by autohydrolysis, IDE, or the fragmentation processes that occur during the MS analysis itself has never been reported. AP MALDI MS has already proven to be a very valuable tool in a number of important applications, including the identification of proteins [33], the characterization of enzymes immobilized on solid support [34] and the detection of intact molecular fragments that are representative of IDE action on insulin molecules [18], due to its ability to detect intact molecular masses up to several thousands Daltons [35]. Nevertheless, depending on the analyzed molecules, AP MALDI MS can induce ion fragmentations more easily than other MS methods, such as ESI [36]. Additionally, as Aβs are prone to spontaneous cleavage [20], the identification of IDE cleavage sites on these peptides has to be carried out with special care. Particularly, we noted that Aβ fragments were formed with solution aging and, although a quantitative correlation between the Aβ fragments intensity and the aging time was not carried out, we noted that the use of buffered solutions rather than water slows the fragmentation process several fold (in $NH_4HCO_3$ or PBS pH 7.4 a significant increase in Aβ fragments intensity occurs after 1 week). However, in order to improve reproducibility, only freshly prepared Aβ solutions from the same commercial lot have been used for the following MS investigations.

In Fig. 1 the mass spectra obtained by AP MALDI (a), MALDI-TOF (b) and ESI (c) from Aβ 1–42 freshly dissolved in water (10 μM) are reported and only peaks attributed to the monomeric form of Aβ are detected in all cases. Although only *m*/*z* values up to 4000 could be sampled by the AP MALDI approach (due to limitation imposed by the ion trap detection range), this does not hinder Aβ detection as the doubly charged molecular peak is easily visible in the mass spectrum (see Fig. 1a). Furthermore, it is important to highlight that

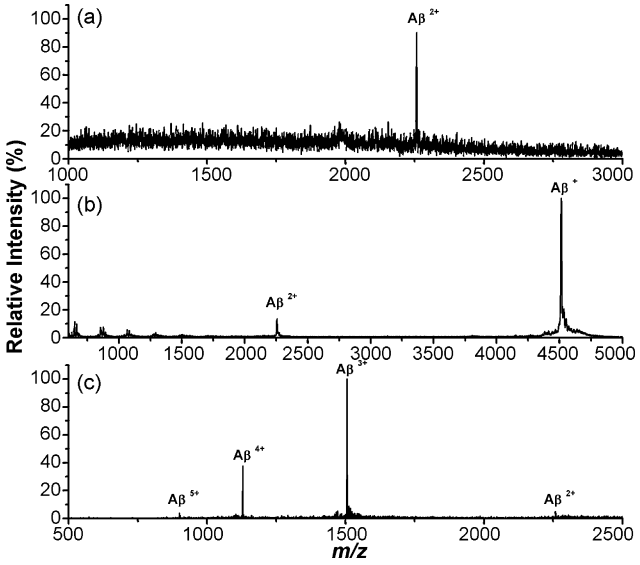


**Fig. 1.** AP MALDI (a), MALDI-TOF (b) and ESI (c) mass spectra obtained from Aβ 1–42 (10 μM) water solution. In (a) and (b) a matrix/sample ratio of 10,000/1 was used and matrix clusters are visible at $m/z < 1500$ (b).

the ion trap detection range is not limiting for the detection of Aβ fragments as they are expected at $m/z$ values <4000.

Although it is well known that different sample-matrix preparation procedures greatly influence the quality of the MALDI mass spectra of peptides and proteins [37], we also found that certain matrix/sample ratios induce Aβ peptide fragments detection. Particularly, AP MALDI and MALDI-TOF experiments were conducted on a 10 μM Aβ 1–42 solution by systematically changing the matrix/sample ratio from 10,000/1 to 10/1 (11 values obtained by consecutively halving the matrix amount). In Fig. 2 the mass spectra obtained by the two MS approaches at the experimental conditions indicated are reported. Particularly, by choosing a matrix/sample ratio = 2500/1, AP MALDI MS detects the Aβ 1–42 fragments reported in Table 1 together with the doubly charged molecular peak (see Fig. 2a). At the same experimental conditions, MALDI-TOF detects only the molecular peaks (data not shown), while the Aβ 1–42 fragments reported in Table 2 could be detected

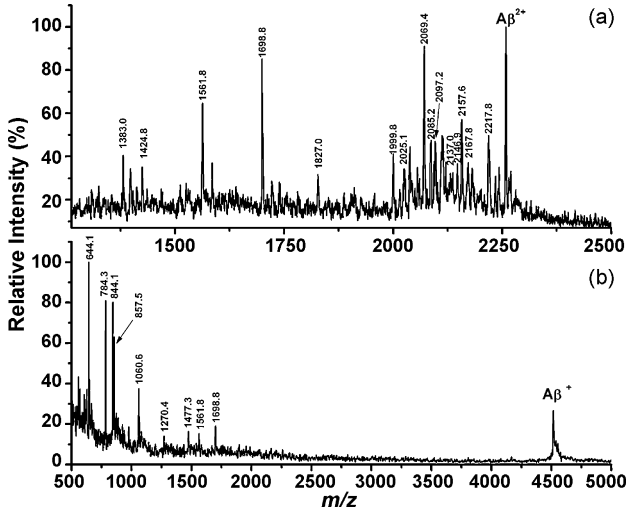


**Fig. 2.** AP MALDI (a) and MALDI-TOF (b) mass spectra of Aβ 1–42 (10 μM) water solution obtained by using a matrix/sample ratio of 2500/1 (a) and 312/1 (b). It is possible to see that peaks assigned to peptide fragments are also detected together with the molecular peaks (see Table 1 for assignment and Fig. 1S for MS/MS experiments).

**Table 1**
Assignment of the Aβ 1–42 peptide fragments detected by AP MALDI MS using a matrix/sample ratio of 2500/1.

| Aβ 1–42 fragments | Residues number | Calculated mass ($m/z$) | Experimental mass ($m/z$) |
|---|---|---|---|
| **FRHDSGYEVHH** | 4–14 | 1383.4 | 1383.0 |
| **DAEFRHDSGYEV** | 1–12 | 1424.4 | 1424.8 |
| **DAEFRHDSGYEVH** | 1–13 | 1561.6 | 1561.8 |
| **DAEFRHDSGYEVHH** | 1–14 | 1698.7 | 1698.8 |
| DAEFRHDSGYEVHHQ | 1–15 | 1826.8 | 1827.0 |
| **FRHDSGYEVHHQKLVF** | 4–19 | 1999.2 | 1999.8 |
| VFFAEDVGSNKGAIIGLMVG | 18–37 | 2024.3 | 2025.1 |
| RHDSGYEVHHQKLVFFA | 5–21 | 2070.3 | 2069.4 |
| EVHHQKLVFFAEDVGSNK | 11–28 | 2084.3 | 2085.2 |
| HHQKLVFFAEDVGSNKGAI | 13–31 | 2097.3 | 2097.2 |
| LVFFAEDVGSNKGAIIGLMVG | 17–37 | 2137.5 | 2137.0 |
| **FRHDSGYEVHHQKLVFF** | 4–20 | 2146.4 | 2146.9 |
| HDSGYEVHHQKLVFFAED | 6–23 | 2158.3 | 2157.6 |
| **DAEFRHDSGYEVHHQKLV** | 1–18 | 2167.3 | 2167.8 |
| FRHDSGYEVHHQKLVFFA | 4–21 | 2217.4 | 2217.8 |

Confirmation of the reported assignment was obtained by MS-MS experiments (see for an example Fig. 1S of the supporting information). The fragments are produced by photo-degradation processes and are not pre-existent in the solution. Peptide fragments that would be expected from IDE cleaving action are indicated in bold. For discussion/explanation see text.

if a matrix/sample ratio was as low as 312/1 (see Fig. 2b). From Fig. 2 it is possible to see that by using the reported matrix/sample ratios the two molecular peaks at $m/z$ 4515.0 ($MH^+$) and 2258.0 ($M+2H^+$) decreased their intensities while the Aβ fragments reported in Tables 1 and 2 were detected respectively by AP MALDI and MALDI-TOF MS. This experimental finding can only be explained by assuming that the peptide fragmentation is generated by photo-degradation and the peptide fragments were not preexistent in the solution. It is well known that the matrix plays a key role in MALDI by absorbing the laser light energy and causing laser desorption of the target molecules. According to our results, if the matrix/sample ratio is lowered under a certain threshold (for Aβ solutions 2500/1 and 312/1 respectively in AP MALDI and MALDI-TOF), Aβ peptides are photo-degraded as they are exposed to the direct laser power and they are detected as fragments. Such described photo-induced fragmentation occurs in source and it is also dependent on the laser fluency as reported in Fig. 3. The intensity of the molecular peak at $m/z$ 4515.0 ($MH^+$) decreases at higher laser fluencies, while Aβ fragments are always detected for such a low value of matrix/sample ratio (312/1). We have observed that at high matrix/sample ratios (>2500/1) Aβ fragments are not detected even at the highest possible laser fluency (data not shown), confirming the matrix key role of absorbing the laser light energy and causing the substrate to vaporize without being photo-degraded.

**Table 2**
Assignment of the Aβ 1–42 peptide fragments detected by MALDI-TOF MS using a matrix/sample ratio of 312/1.

| Aβ 1–42 fragments | Residues number | Calculated mass ($m/z$) | Experimental mass ($m/z$) |
|---|---|---|---|
| IIGLMV | 31–36 | 644.9 | 644.1 |
| NKGAIIGL | 27–34 | 784.9 | 784.3 |
| GLMVGGVVI/IGLMVGGVV | 33–41/32–40 | 844.1 | 844.1 |
| LMVGGVVIA | 34–42 | 858.1 | 857.5 |
| GSNKGAIIGLM | 25–35 | 1060.3 | 1060.6 |
| GAIIGLMVGGVVIA | 29–42 | 1269.6 | 1270.4 |
| HQKLVFFAEDVGS | 14–26 | 1476.6 | 1477.3 |
| **DAEFRHDSGYEVH** | 1–13 | 1561.6 | 1561.8 |
| **DAEFRHDSGYEVHH** | 1–14 | 1698.7 | 1698.8 |

The reported fragments are produced by photo-degradation processes and are not pre-existent in the solution. Peptide fragments that would be expected from IDE cleaving action are indicated in bold. For discussion/explanation see text.

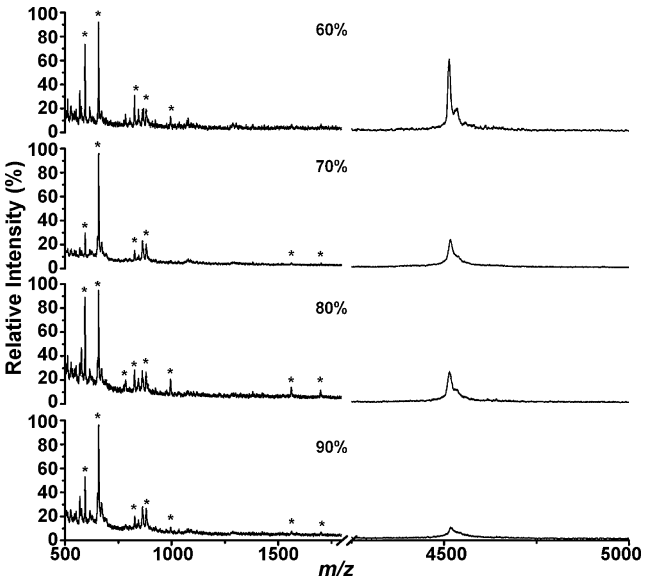


**Fig. 3.** MALDI-TOF mass spectra of Aβ 1–42 (10 μM) water solution obtained by using a matrix/sample ratio of 312/1 and the laser fluency indicated as a percentage of the maximum value (4.5 kJ/cm$^2$). Note the decrease of the intensity of the molecular peak at *m/z* 4515.0 at higher laser fluencies. Aβ fragments are indicated with a (*). For the assignment see Fig. 2 and Table 1.

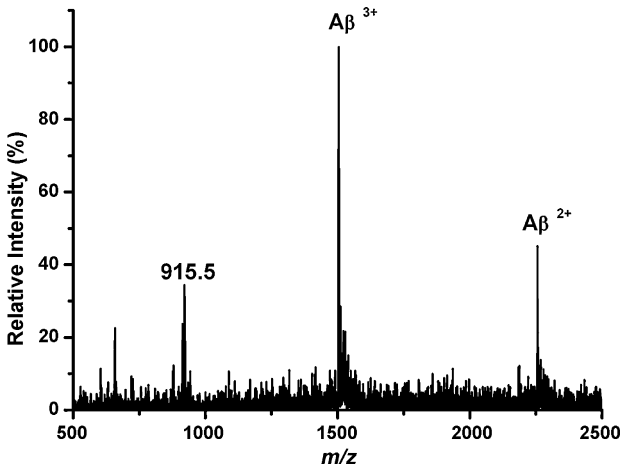


**Fig. 4.** ESI mass spectrum obtained from Aβ 1–42 (10 μM) water solution after 3 days aging. Only one Aβ fragment is detected at *m/z* 915.5; MS/MS experiment confirmed the sequence GLMVGGVVIA (see Fig. 2S of the supporting information), excluding the other two possibilities (VGSNKGAIIG and NKGAIIGLM). The expected Aβ aggregation occurring because of the aging process produces a drastic decrease in the intensity of the peptide molecular peaks. For this reason, other peaks originating from impurities commonly detected in diluted DMSO solutions are in this case also visible in the mass spectrum at a relative intensity just below 20% and have been neglected. For discussion/explanation see text.

From Fig. 2 and Tables 1 and 2 it is possible to note that the most intense Aβ fragments generated by a low matrix/sample ratio involve aromatic residues (F, H, W, and Y). It is well known that the latter have a significant absorption in the UV region [38] and therefore it is not surprising that at low matrix/sample ratios photo-fragmentation occurs during the ionization process. Moreover, although MALDI was initially believed to be a nondestructive ionization technique [39], it became clear that a significant degree of fragmentation is associated with the ionization event, particularly in the case of peptides detection [40]. The latter phenomenon can be considered an advantage if a specific investigation of peptide structures has to be carried out [41]. In our case it was proven to be very difficult to identify experimental parameters other than the matrix/sample ratio influencing the formation of particular Aβ fragments (mass analyzer, MALDI plate, etc. might also play a role), due to loss of sensitivity occurring at different experimental setups. However, our results show that special care has to be applied if these two MS approaches are to be used for the detection of Aβ fragments produced by the action of an enzyme such as IDE, as photo-degradation could be mistaken for specific enzyme cleaving action. Moreover, it was observed that MALDI produces photo-induced fragments even at commonly used matrix/sample ratios (AP MALDI is more sensitive in this respect, see Fig. 2). Therefore it is important to exercise great care (due to the spot inhomogeneity, local low matrix/sample ratios can normally occur) in performing and interpreting the MALDI analysis of biological samples, especially if cleavage sites identification has to be carried out. In the case of Aβ solutions this aspect is ever more important as some of the fragments generated at low matrix/sample ratios because of photo-degradation are also expected to be produced by IDE cleaving action (Tables 1 and 2, bold). It is therefore important to highlight that, according to the above reported results, identification of IDE cleavage sites on Aβ peptides by the MALDI approach is meaningful only if the matrix/sample ratios are larger than 2500/1 throughout the desorbing spot.

On the contrary, ESI MS does not require the use of any matrix and therefore the injection of freshly prepared Aβ water solutions into the ESI source always produces the mass spectrum showed in Fig. 1c. However, it is already reported that Aβ water solutions are not very stable as they undergo aggregation and spontaneous cleavage [20] and we confirmed the two latter processes. In fact, in Fig. 4 the ESI mass spectrum of 3 days aged Aβ 1–42 solution is reported and the following considerations can be drawn: (i) the signal/noise ratio is decreased, as expected for an aged solution of an aggregation-prone peptide and (ii) one Aβ fragment is detected as a peak at *m/z* 915.5. The latter was assigned by MS/MS experiment, confirming the sequence GLMVGGVVIA and excluding the other two possibilities (VGSNKGAIIG, NKGAIIGLM), see Fig. 2S of the supporting information. Interestingly, applying a source fragmentation does not produce appreciable Aβ fragments [42] as it only changes the ratio between the multiple charged molecular ions detected, favouring the doubly charged species (see Fig. 5).

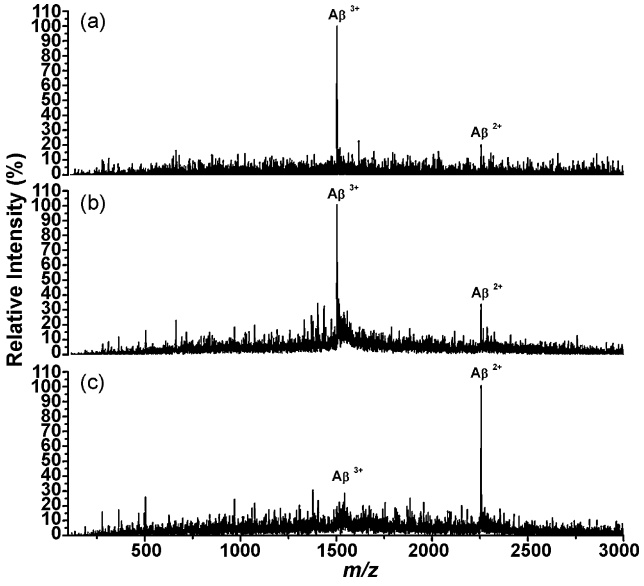


**Fig. 5.** ESI mass spectra obtained from Aβ 1–42 (10 μM) in water by applying source fragmentation energy of 50 (a), 75 (b) and 100 V (c). As for Fig. 3, the low signal/noise ratio observed for aged Aβ solutions causes the detection of other peaks originating from solution impurities that have been neglected.

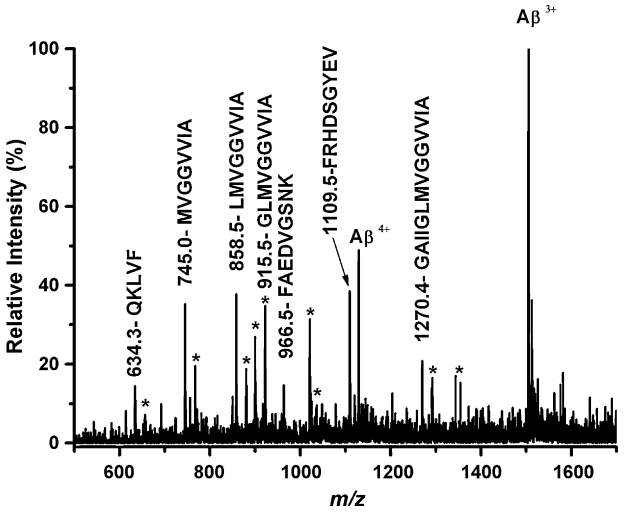


**Fig. 6.** ESI mass spectrum recorded for a Aβ 1–42 (10 μM) buffered solution containing [IDE] = 36 nM after 1 h at 37 °C. Aβ fragments assigned to IDE cleaving action are indicated. MS/MS experiment confirms the assignment for peak at *m/z* 745.0 (see Fig. 3S of the supporting information). The peaks labelled with an asterisk are due to sodiated species and/or to other peptide fragments generated by auto-hydrolysis of Aβ (see text).

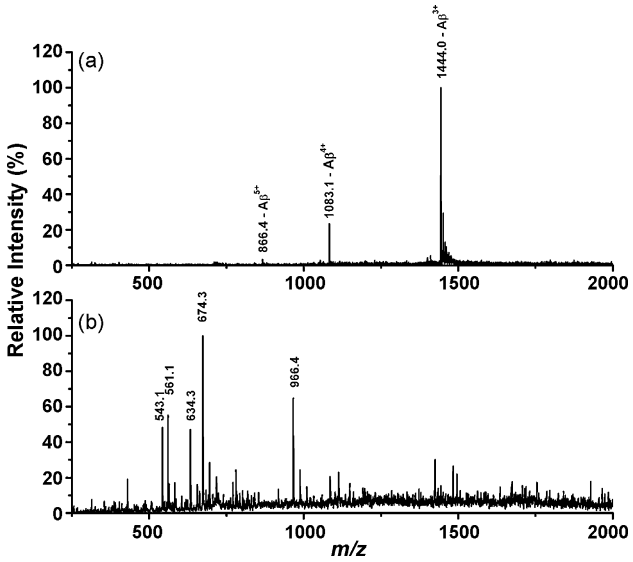


**Fig. 7.** ESI mass spectra recorded for a Aβ 1–40 (10 μM) buffered solution without (a) and with [IDE] = 36 nM (b) after 1 h at 37 °C. Aβ fragments assigned to IDE cleaving action are listed in Table 4.

It is evident from the results described above that the most suitable MS approach for detection of Aβ cleavage sites by IDE is ESI. In fact, although spontaneous cleavage of Aβ peptides in water solutions can only be minimized but not totally avoided [20], it can be normally neglected in the ESI analysis as long as Aβ freshly prepared solutions are going to be considered (see Fig. 1c).

In Fig. 6 the ESI mass spectrum recorded for a Aβ 1–42 (10 μM) buffered solution containing [IDE] = 36 nM after 1 h at 37 °C is reported. Aβ fragments assigned to IDE cleaving action are indicated (see also Table 3) and from two of them (MVGGVVIA and LMVGGVVIA) it was possible to identify the IDE cleavage sites 33–34 and 34–35 of the Aβ aminoacidic sequence. Other peaks indicated with an asterisk in Fig. 6 are easily attributed to species containing $NH_4^+$ and $Na^+$ ions that are generated from the buffered solution or to Aβ fragments formed because of spontaneous cleavage as expected (see the discussion above referring to Fig. 4).

Although the above reported result represents a new finding [15,16], the above mentioned cleavage sites have already been reported for Aβ solutions interacting with other proteolytic enzymes such as neprilysin [10,43]. Furthermore, it has already been reported that IDE specificity is directed toward the amino side of hydrophobic and basic residues [44] and the two newly reported cleavage sites obey to this rule. Although the assignment reported in Table 3 was confirmed by MS/MS experiments (see for example Fig. 3S of the supporting information), in order to confirm these new cleavage sites, we carried out the same experiment also for the Aβ 1–40 peptide and the results obtained are reported in Fig. 7. The presence of fragments MVGGVV and LMVGGVV in the mass spectrum of Fig. 7b (see Table 4) confirmed IDE cleaving action at sites 33–34 and 34–35 of the aminoacidic sequence for both peptides.

The study of Aβ solutions is widely reported to be very challenging due to the difficulties encountered in handling the samples and finding the appropriate experimental conditions that ensure reproducibility [45]. In this work, we have identified one experimental parameter that dramatically affects the detection of Aβ solutions by MALDI methods, that is the matrix/sample ratio. Too low values of the latter produce Aβ fragments because of photodegradation processes that can invalidate MALDI investigation on this kind of samples. As a low matrix/sample value could also be produced by local inhomogeneity and wrong spotting procedure among the others, we have identified ESI MS as a more suitable technique to study Aβ solutions. Particularly, ESI MS was proved to be very advantageous for the characterization of the peptide fragments produced by the enzymatic action of IDE on Aβ peptides. In fact, in this case the only Aβ fragments detected were those produced by IDE proteolytic action and the analysis of the fragmentation pattern allowed the unambiguous identification of all IDE cleavage sites. Therefore we were able not only to confirm the cleavage sites already reported in the literature (3–4 [16], 12–13, 14–15, 19–20 and 28–29 [15]), but also to identify two new ones at sites 33–34 and 34–35. As different Aβ fragments can have different neurotoxic properties [46], the identification of the two new

**Table 3**
Assignment of the Aβ peptide fragments detected by ESI MS for a Aβ 1–42 (10 μM) buffered solution containing [IDE] = 36 nM after 1 h at 37 °C.

| Aβ 1–42 fragments | Residues number | Calculated mass (*m/z*) | Experimental mass (*m/z*) |
|---|---|---|---|
| QKLVF | 15–19 | 633.8 | 634.3 |
| MVGGVVIA | 35–42 | 744.9 | 745.0 |
| LMVGGVVIA | 34–42 | 858.1 | 858.5 |
| GLMVGGVVIA | 33–42 | 915.1 | 915.5 |
| FAEDVGSNK | 20–28 | 966.0 | 966.5 |
| FRHDSGYEV | 4–12 | 1109.1 | 1109.5 |
| GAIIGLMVGGVVIA | 29–42 | 1269.6 | 1270.4 |

The detected fragments are produced by the enzyme proteolytic action and allow the identification of two unreported cleavage sites by IDE at position 33-34 and 34-35 of the aminoacidic sequence. MS/MS experiments confirmed the reported assignment (see for an example Fig. 3S of the supporting information).

**Table 4**
Assignment of the Aβ peptide fragments detected by ESI MS for a Aβ 1–40 (10 μM) buffered solution containing [IDE] = 36 nM after 1 h at 37 °C.

| Aβ 1–40 fragments | Residues number | Calculated mass (*m/z*) | Experimental mass (*m/z*) |
|---|---|---|---|
| GAIIGL | 29–34 | 542.7 | 543.1 |
| MVGGVV | 35–40 | 560.7 | 561.1 |
| QKLVF | 15–19 | 633.8 | 634.3 |
| LMVGGVV | 34–40 | 673.9 | 674.3 |
| FAEDVGSNK | 20–28 | 966.0 | 966.4 |

As for Aβ 1–42, also in this case the detected fragments are produced by IDE proteolytic action, confirming the two cleavage sites at position 33-34 and 34-35 of the aminoacidic sequence. MS/MS experiments confirmed the reported assignment.

cleavage sites above reported contributes to the elucidation of the IDE role in regulating the accumulation and toxicity of Aβ peptides in the brain.

Finally, it should be noted that the proposed ESI MS approach is not very suitable for the quantitative analysis of the Aβ fragments produced by the action of IDE, due to the different ionization efficiencies that peptides with different p*K* and solubility might have in ESI. However, it is still possible to carry out enzyme activity measurements of Aβ solutions by monitoring the intensity of one specific fragment produced by IDE as long as a calibration curve has been previously obtained by ESI MS analysis of standard peptide fragment solutions. Examples of the described approach have been already successfully reported by our group for different enzymes [47].

## 4. Conclusions

AP MALDI, ESI and MALDI-TOF MS were applied for the analysis of Aβ solutions and a detailed investigation on the changes that different experimental procedures induce on the mass spectra obtained was carried out. Data showed that low matrix/sample ratios can be responsible for Aβ fragments detection by AP MALDI and MALDI-TOF MS, possibly leading to wrong IDE cleavage sites identification. On the contrary, ESI MS does not involve the use of matrix and Aβ fragments are not detected in freshly prepared peptide solutions. For this reason the latter method was chosen as the most suitable MS technique for the investigation of IDE cleaving action onto Aβ peptides. Particularly, two new IDE cleavage sites at position 33–34 and 34–35 of the aminoacidic sequence for both Aβ 1–40 and Aβ 1–42 peptides were identified by ESI MS.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijms.2009.02.008.

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