

# Detection and quantitation of cellularly derived amyloid $\beta$ peptides by immunoprecipitation-HPLC-MS

Nigel J. Clarke<sup>a</sup>, Andy J. Tomlinson<sup>a</sup>, Yasumasa Ohyagi<sup>b</sup>, Steven Younkin<sup>c,\*</sup>,  
Stephen Naylor<sup>d</sup>

<sup>a</sup>Biomedical Mass Spectrometry Facility, Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN 55905, USA

<sup>b</sup>Department of Neurology, Iizuka Hospital, 3-83 Yoshio-Machi, Iizuka City, Fukuoka 820, Japan

<sup>c</sup>Department of Pharmacology, Mayo Clinic, Jacksonville, FL 32224, USA

<sup>d</sup>Biomedical Mass Spectrometry Facility, Department of Biochemistry and Molecular Biology,  
Department of Pharmacology and Clinical Pharmacology Unit, Mayo Clinic, Rochester, MN 55905, USA

Received 2 January 1998; revised version received 1 June 1998

**Abstract** A quantitative method for detection of amyloid  $\beta$  peptides using immunoprecipitation-HPLC-mass spectrometry (IP-LC-MS) is described. Comparison of IP-LC-MS with sandwich ELISA revealed comparable results in the analysis of A $\beta$  1–40 and A $\beta$  1–42 derived from fetal guinea pig cell media and cell lysates. The use of IP-LC-MS not only allows a quantitative method for A $\beta$  1–40 and A $\beta$  1–42 peptides present in Alzheimer's disease (AD), but allows detection of other A $\beta$  peptide species that may also play a role in the onset of AD in humans.

© 1998 Federation of European Biochemical Societies.

**Key words:** Alzheimer's disease; Amyloid  $\beta$ ; HPLC; Immunoprecipitation; Mass spectrometry

## 1. Introduction

Alzheimer's disease (AD) is the major cause of senile dementia in late adult life [1]. Although this neurodegenerative disease is prevalent among the older population, definitive clinical diagnosis has proven very difficult. This, in part, is due to the lack of a simple physiological test for detection of the disease, as well as the close similarity of AD symptoms with those observed in other forms of dementia. Post-mortem examination of AD brain tissue reveals gross histopathological changes compared with 'normal' brain tissue [2]. However, the etiology of these changes, as in the deposition of the amyloid  $\beta$  peptide (A $\beta$ ) to form senile plaques [3], is not well understood. In order to understand such changes, model cell systems and transgenic animals [4,5] have been developed to mimic genetically transmitted forms of the disease. Unfortunately, investigation of such model systems has been

hampered by the lack of a sensitive, specific, and robust assay capable of detecting these changes both intra- and extracellularly.

Enzyme linked immunosorbent assays (ELISAs) [6] and sandwich ELISAs [7] (sELISAs) have been developed for the quantitative analysis of individual A $\beta$  peptides (typically A $\beta$  1–40 and A $\beta$  1–42) [8–10]. Unfortunately these assays are unable to detect A $\beta$  peptides derived from cell lysates with any great success. Moreover, subtle chemical changes to the peptides, which may have a dramatic effect on their physical and biological properties, cannot be evaluated by this approach. Identification of such modifications is vital since changes in the physical and biological properties of these peptides may play a part in the onset of the disease. Furthermore, it has been previously shown by Orlando et al. [11] that typical methods of extracting A $\beta$  from plaques and brain tissue often causes covalent modification of the peptides. These changes may affect the reliability of ELISA-based quantitation results. Finally, ELISA-based assays can only detect analytes for which antibodies have been raised. This obviously requires prior knowledge of sample composition coupled with the time-consuming effort required to raise a new antibody for each target species. All of these facts prevent ELISA-based assays from being able to identify unknown species within a sample.

In order to complement the limited information yielded by ELISA or sELISA analysis, we have developed an assay based on quantitative mass spectrometry (MS). Mass spectrometry is often the detection system of choice for structural characterization of analytes within complex biological mixtures [12–15] due to its ability to specifically detect a wide range of analyte types. This makes MS ideal for the identification of unknown species during de novo research. Furthermore, quantitation of detected species can be completed simply using either internal or external calibrants. Finally, MS analysis affords rapid and sensitive detection of a wide range of compounds at the low femtomole to high attomole range.

To achieve high sensitivities for analytes derived from complex sample matrices, we have employed immunoprecipitation (IP) in conjunction with on-line HPLC with MS detection (IP-LC-MS). This facilitates minimum sample handling while maximizing the efficiency of analyte capture and concentration. IP-MS analysis of A $\beta$  peptides has been demonstrated previously by Wang et al. [16]; however, this approach was only capable of examining conditioned cell media. Our new on-line IP-LC-MS assay has allowed analysis and quantitation of cellular concentration levels of A $\beta$  peptide directly

\*Corresponding author. S. Naylor is also corresponding author.  
Fax (Naylor): +1 (507) 284-8433; (Younkin): +1 (904) 953-7370.  
E-mail: naylor.stephen@mayo.edu; younkin@mayo.edu

**Abbreviations:** AD, Alzheimer's disease; IP, immunoprecipitation; LC, liquid chromatography; HPLC, high performance liquid chromatography; MS, mass spectrometry; PATRIC, position and time resolved ion counter; APP, amyloid precursor protein; A $\beta$ , amyloid beta peptide; A $\beta$  1–40, amyloid  $\beta$  peptide consisting of APP residues 672–711; A $\beta$  1–42, amyloid  $\beta$  peptide consisting of APP residues 672–713; SIP, single ion profile; ELISA, enzyme linked immunosorbent assay; sELISA, sandwich ELISA; PBS, phosphate buffered saline; SDS, sodium dodecyl sulfate; CH<sub>3</sub>CN, acetonitrile; TFA, trifluoroacetic acid; AcOH, acetic acid; n-PrOH, *n*-propanol; RIPA buffer, 150 mM sodium chloride, 1% nonidet, 0.5% deoxycholate acid, 0.1% SDS, 50 mM Tris-base

from cell lysates. This assay is, therefore, highly relevant to the analysis of model AD systems, both *in vitro* and *in vivo*, and may help elucidate the cascade of events leading to A $\beta$  deposition and plaque formation. The assay could also find major use in determining the efficacy of drug therapies designed to lower A $\beta$  production within such model systems.

## 2. Materials and methods

### 2.1. Materials

Synthetic A $\beta$  1–40 and A $\beta$  1–42 were purchased from Bachem California Inc. (Torrance, CA, USA). BAN-50 anti-A $\beta$  1–16 antibodies were a gift from N. Suzuki (Takeda Chemical Industries, Osaka, Japan). Toyopearl beads, phosphate buffered saline (PBS), acetic acid, and formic acid were bought from Sigma Chemical Co. (St. Louis, MI, USA). All solvents were purchased from Burdick and Jackson (Muskegon, MI, USA) and were of analytical grade or better.

### 2.2. Cell lysis and immunoprecipitation of A $\beta$ peptides

Approximately  $10^7$  fetal guinea pig brain cells were raised to confluence in a 2 cm dish. They were then harvested and lysed with 2% SDS. The lysate was diluted with  $10\times$  RIPA buffer to reduce the final SDS concentration. It should be noted that cell media was removed prior to addition of SDS. Hence, cell media was not subjected to SDS contact but was subsequently prepared in an identical manner to cell lysate.

BAN-50 anti-A $\beta$  1–16 antibodies were covalently bound to Toyopearl AF Tressyl 650 M resin beads as per the manufacturer's instructions [17]. This process has been described in detail elsewhere [10]. Subsequently, 10  $\mu$ l of antibody-bound beads were added either to  $\sim 200$   $\mu$ l of cell media or  $\sim 200$   $\mu$ l cell lysate with gentle rocking at 4°C for 24 h. The beads were removed and washed  $2\times$  with 4°C PBS.

### 2.3. Elution of A $\beta$ peptides

Peptide antigens were eluted from the antibody Toyopearl beads (10  $\mu$ l) with 20  $\mu$ l of 60:40 CH<sub>3</sub>CN:H<sub>2</sub>O and stirred gently every 10 min. After 1 h of this procedure, 0.5  $\mu$ l glacial acetic acid was added and, subsequently, stirred and allowed to stand for 5 min at room temperature. In order to load the peptide antigens onto the HPLC column, the organic solution (60:40 CH<sub>3</sub>CN:H<sub>2</sub>O) was diluted with 40  $\mu$ l deionized H<sub>2</sub>O. The supernatant was drawn up into a syringe and loop-injected onto a C-8 reversed phase HPLC column.

### 2.4. HPLC conditions

A UMA HPLC system (Michrom Bioresources Inc., Auburn, CA, USA) fitted with a C-8 column (50 mm $\times$ 0.5 mm, monitor C-8 packing material, Michrom Bioresources Inc.) was utilized for all HPLC analyses. A gradient of 20% mobile phase B (90% CH<sub>3</sub>CN/7% water/3% *n*-propanol (*n*-PrOH)/0.5% acetic acid (AcOH)/0.2% trifluoroacetic acid (TFA)), 80% mobile phase A (2% CH<sub>3</sub>CN/98% water/0.5% AcOH/0.15 TFA) to 70% mobile phase B reached over 15 min at a flow rate of 30  $\mu$ l/min was utilized giving an estimated gradient delay of 4 min.

### 2.5. Mass spectrometry conditions

A Finnigan MAT 900 double sector instrument fitted with a position and time resolved ion counter (PATRIC) array detector was utilized for all MS analyses. The MS instrument was operated over a mass range of 700–1200 Da at a scan rate of 2 s/decade. The PATRIC detector was operated at a microchannel voltage of 820 V and a mass window of 8%. Eluent from the HPLC column was introduced into the MS via a fused silica capillary (180  $\mu$ m OD $\times$ 25  $\mu$ m ID, Polymicro Technologies, Phoenix, AZ, USA) which passed coaxially through the inner bore of the electrospray needle. This needle was biased at 4.8 kV with respect to the accelerating voltage of 5 kV. Ionization was by positive ion electrospray ionization with an N<sub>2</sub> sheath gas flow to help nebulization (1.5 l/min).

### 2.6. Calibration curve construction

Calibration curves for human A $\beta$  1–40 and 1–42 were constructed using known amounts of the two synthetic peptides bound to BAN-50 anti-A $\beta$  1–16 beads. The antigens were eluted from the beads and analyzed by LC-MS using the identical protocol employed with bio-

logically derived samples. Synthetic human A $\beta$  1–40 and 1–42 peptides were exposed to the beads at amounts ranging from 100 fmol, 200 fmol, 300 fmol, 500 fmol, 700 fmol, 1000 fmol and 3000 fmol for both peptides. Each calibration point was examined in triplicate and peak areas from the MH<sub>4</sub><sup>+</sup> and MH<sub>5</sub><sup>+</sup> ions combined for each peptide. Combining the peak areas allowed small variations in charge state distribution between samples to be averaged out.

## 3. Results and discussion

### 3.1. Immunoprecipitation for LC-MS and sELISA

Previous studies have demonstrated the high specificity of the BAN-50 anti-human A $\beta$  antibody for both synthetic and endogenous A $\beta$  peptides [9,10]. The epitope region has been determined to be the first 16 N-terminal amino acids and a further requirement is that there is a free amino terminus. Hence, this antibody was utilized in the IP step in the analysis of endogenous A $\beta$  peptides. Since the epitope consists of the N-terminus of the A $\beta$  peptides [10], various ragged C-termini A $\beta$  peptides would also be isolated (see later). Furthermore, this antibody had been used in a sELISA assay to quantitate A $\beta$  1–40 and A $\beta$  1–42 derived from fetal guinea pig cell media [10,18]. In order to ensure that we could directly compare the quantitation of A $\beta$  peptides by IP-LC-MS with ELISA and/or sELISA, the IP step carried out was identical for both analyses. Suzuki et al. [10] had previously demonstrated that 60:40 CH<sub>3</sub>CN:H<sub>2</sub>O afforded optimal recovery ( $\sim 85$ – $90\%$ ) of A $\beta$  peptides from BAN-50 antibodies. Furthermore, subsequently Oyagi et al. [18] has shown that, using this approach, total A $\beta$  peptide capture and recovery from spiked cell media was  $\sim 35$ – $40\%$ . In all the present analyses, samples were subjected

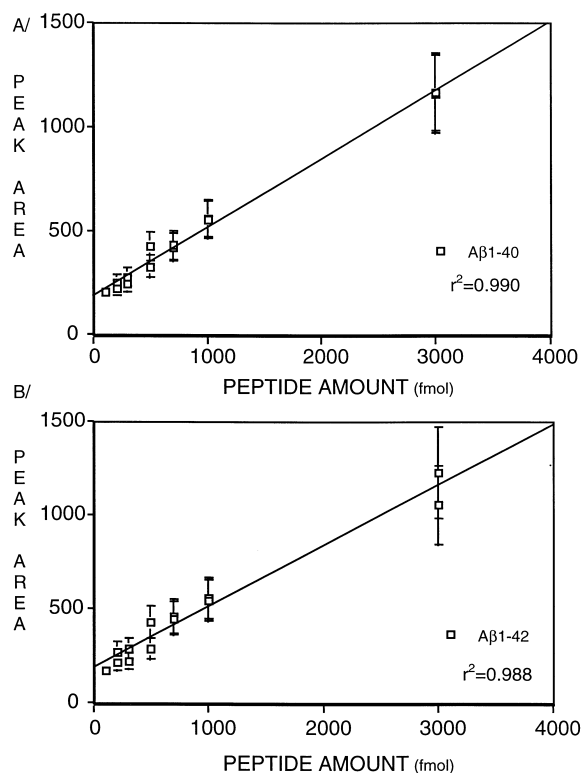


Fig. 1. Calibration curves for (A) synthetic human A $\beta$  1–40 and (B) A $\beta$  1–42 eluted from BAN-50 antibodies bound to Toyopearl resin beads.

to IP as described above and then subsequently split 50:50 for concomitant sELISA and LC-MS analysis respectively.

Calibration curves for quantitation by IP-LC-MS were constructed using synthetic A $\beta$  1–40 and A $\beta$  1–42 peptides. These peptides were bound to BAN-50 Toyopearl resin beads at varying amounts ranging from 100–3000 fmol for each peptide. All calibration points were examined in triplicate and a linear correlation was observed for both peptides over this concentration range (A $\beta$  1–40  $r^2 = 0.990$ , A $\beta$  1–42  $r^2 = 0.988$ ) (Fig. 1). Calibration curves were also validated with spiked synthetic human A $\beta$  1–40 and 1–42 samples. Various concentrations of peptide solutions were prepared and analyzed blind by IP-LC-MS. Results obtained in this way were in very good agreement with the actual concentrations bound to the beads ( $\pm 5\%$ , data not shown). These blind tests demonstrated the inter-run reproducibility of the calibration curves. It can be seen (Fig. 1) that the calibration curves for both A $\beta$  1–40 and A $\beta$  1–42 do not intercept the  $y$ -axis at zero. In part, this is due to low level chemical background. This leads to some significant electronic responses by the ultrasensitive PATRIC detection of the MAT 900. Ultimately, this results in some enhanced baseline response at sample levels below 10–50

femtomoles, and this phenomenon has been observed elsewhere [19].

### 3.2. Quantitation of endogenous A $\beta$ peptides – comparison IP-LC-MS and IP-sELISA

Cell media and lysate from fetal guinea pig brain cells were collected separately. Each sample (200  $\mu$ l) was then subjected to IP, and then each was split into two equal volumes. One half of each sample was quantified using sELISA, as described previously [8,10]. The other half of each sample was subjected to LC-MS analysis and quantitation. In the case of the IP-LC-MS analysis of 3 day old conditioned cell media abundant ion responses for both A $\beta$  1–40 and A $\beta$  1–42 peptides were observed. The data show the selected ion profile (SIP) for the  $MH_4^{4+}$  1083.5 (Fig. 2A) and  $MH_4^{4+}$  1129.6 (Fig. 2B) for A $\beta$  1–40 and A $\beta$  1–42, respectively. The inserts show the summed raw data from beneath these responses. In both cases the  $MH_4^{4+}$  and  $MH_5^{5+}$  ions were clearly observed with signal-to-noise ratios greater than 10:1 for A $\beta$  1–40 (Fig. 2A) and 5:1 for A $\beta$  1–42 (Fig. 2B). Mathematical transformation of the detected A $\beta$  1–40 and 1–42 peptide ions yielded molecular weights in excellent agreement with the expected values (A $\beta$

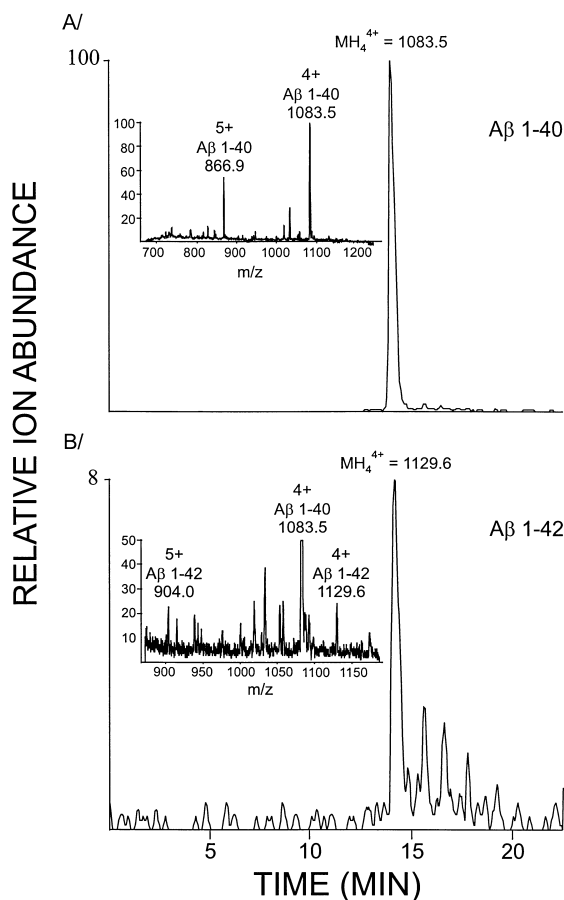


Fig. 2. Results from IP-LC-MS of conditioned cell medium. A: Data for A $\beta$  1–40 from conditioned cell medium. The foreground figure shows the single ion profile for the  $4^+$  ion from this peptide while the insert shows the summed raw data from beneath the peak. B: Data for A $\beta$  1–42 from conditioned cell medium. The foreground figure shows the single ion profile for the  $4^+$  ion from this peptide while the insert shows the summed raw data from beneath the peak.

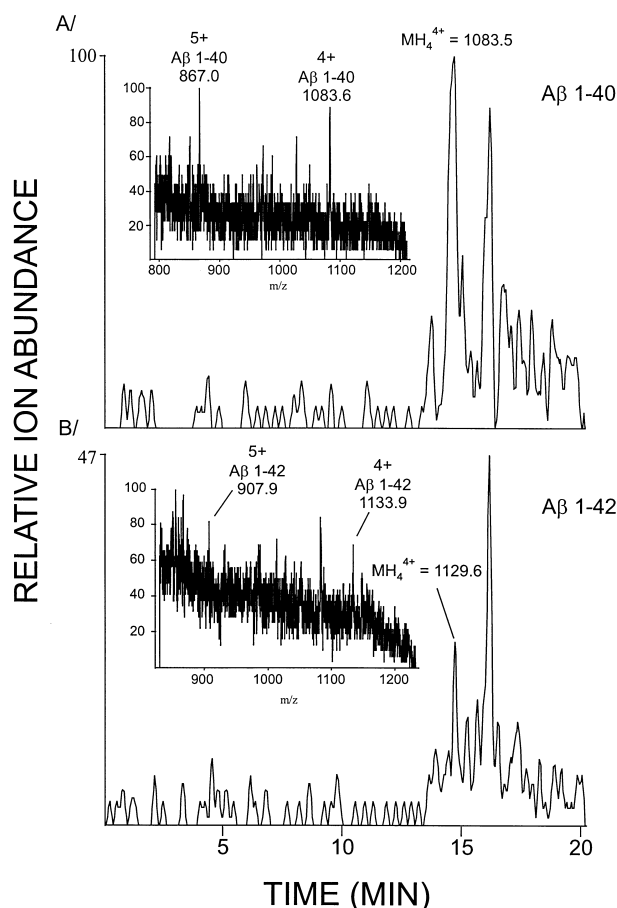


Fig. 3. Results from IP-LC-MS of cell lysate. A: Data for A $\beta$  1–40 from the cell lysate. The foreground figure shows the single ion profile for the  $4^+$  ion from this peptide while the insert shows the summed raw data from beneath the peak. B: Data for A $\beta$  1–42 from the cell lysate. The foreground figure shows the single ion profile for the  $4^+$  ion from this peptide while the insert shows the summed raw data from beneath the peak.

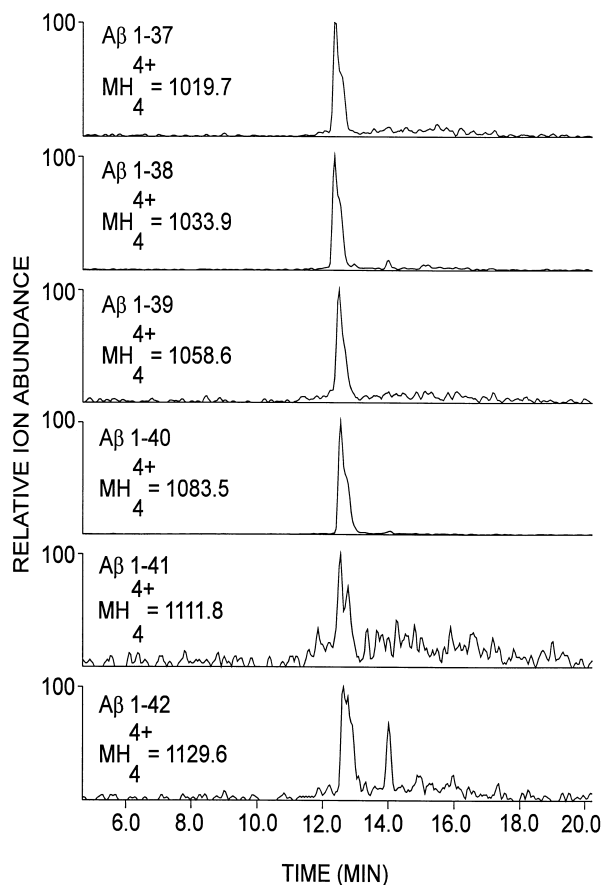


Fig. 4. Detected Aβ peptide population from conditioned cell medium. These single ion profiles show a representative example of the various endogenous Aβ peptides detected within the sample.

1–40  $M_r$ , measured 4330.0 Da, expected 4329.9 Da; Aβ 1–42  $M_r$ , measured 4514.2 Da, expected 4514.1 Da).

A similar analysis of cell lysate (Fig. 3A and B) yielded Aβ 1–40 and 1–42 responses at significantly reduced signal ion abundance. However, it is interesting to note that the relative intensities of Aβ 1–42 versus Aβ 1–40 in the cell lysate (23:100) were greater than that observed in the conditioned medium (8:100). This may suggest Aβ 1–42 is not secreted from the cell with the same efficiency as Aβ 1–40. This observation could also be explained by secreted Aβ 1–42 forming fibrils more rapidly than Aβ 1–40 in cultured medium, thereby reducing the concentration of free Aβ 1–42 available for capture by the antibody. Several studies have suggested this to be the case in vitro [20,21] and an examination of this property of Aβ 1–42 using the new IP-LC-MS assay is ongoing.

It should also be noted that in the LC-MS analysis of the cell lysates a number of significant responses eluting after both Aβ 1–40 (Fig. 3A) and Aβ 1–42 (Fig. 3B) can be observed.

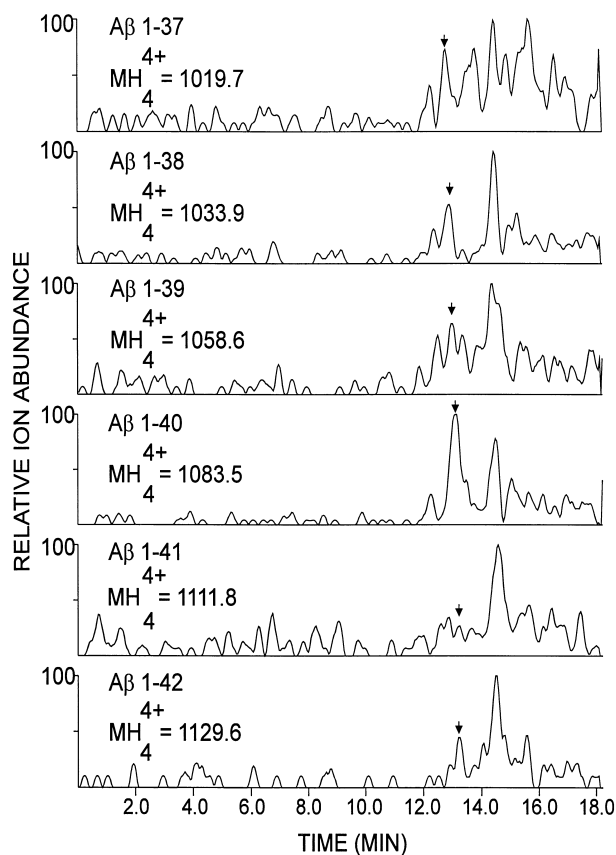


Fig. 5. Detected Aβ peptide population from cell lysate. These single ion profiles show a representative example of the various endogenous Aβ peptides detected within the sample.

However, these same responses are not observed in the LC-MS analyses of cell media (Fig. 2). Transformation of the raw MS data contained in each of the peak responses seen in Fig. 3A and B revealed molecular masses corresponding to numerous oligomers of SDS. There were no MS data to indicate that these responses were possible Aβ peptide dimers, trimers, or tetramers. The detection of SDS oligomers only in the cell lysate is consistent with the fact that the detergent was only used in cell lysate but not in cell media preparations.

Comparison of the quantitation of Aβ 1–40 and Aβ 1–42 in cell media and cell lysate by IP-LC-MS versus IP-sELISA is summarized in Table 1. Overall, relatively close agreement was observed for the two techniques. However, it is noteworthy that IP-LC-MS analysis consistently indicated slightly lower amounts of Aβ 1–40 and Aβ 1–42 were present than determined by IP-sELISA. We believe this may be due to possible antibody cross reactivity with nontarget materials leading to some lack of specificity in IP-sELISA. It has previously been noted that ELISA often overestimates analyte

Table 1

A comparison of the results for samples quantitatively measured by sELISA and the new IP-LC-MS assay

	Cell medium sELISA	Cell medium IP-LC-MS	Cell lysate sELISA	Cell lysate IP-LC-MS
Aβ 1–40	3693 fmole	3492 fmole	263 fmole	212 fmole
Aβ 1–42	371 fmole	320 fmole	42 fmole	< 100 fmole

Cell lysate concentrations of Aβ 1–42 were below current quantitation levels although detectable Aβ 1–42 peptide responses were observed.

amounts compared to on-line chromatography-MS analysis due to this problem [22].

### 3.3. Detection of other A $\beta$ 1–x peptides by IP-LC-MS

An additional powerful feature of IP-LC-MS is the ability to detect other A $\beta$  peptides present in both the cell media and cell lysate. It can be seen in Figs. 4 and 5 that numerous other components aside from A $\beta$  1–40 and 1–42 are present. These responses were tentatively identified as A $\beta$  peptides based on their detected molecular weights. Fig. 4 shows a selection of the major MH $_4^{4+}$  peptide ion responses identified during the analysis of a single conditioned media sample. Intense signals were observed with a very low background noise level. This clarity of signal is due to the highly efficient capture and concentration step provided by the immunoprecipitation protocol in combination with the sample cleanup afforded by HPLC. Fig. 5 shows the identical peptide species detected within a typical cell lysate sample. As expected, the peptide concentration is much lower in this sample, and this is reflected in the reduced signal intensity observed for the detected MH $_4^{4+}$  peptide ions. Indeed, in some cases (e.g. A $\beta$  1–41) it was very difficult to observe a chromatographic response for the peptide. However the mass spectral data obtained at the expected retention time allowed tentative identification on the basis of a measured molecular weight. It can also be seen that numerous other responses are also obtainable and, as described earlier, are due to SDS oligomer ions.

Since, at present, there is no definitive proof that a particular form of A $\beta$  peptide is solely responsible for the onset and/or progress of AD, it would seem prudent to observe as broad a range of endogenous molecules as possible. In this way new information may be revealed about the cascade of events leading to formation of senile plaques and the gross histopathological changes in the AD brain. To complete such a study using sELISA technology, large numbers of different antibodies would be required to detect the myriad family of possible A $\beta$  peptides. Such an undertaking would be extremely difficult and time consuming. The use of a broad-spectrum detection system such as mass spectrometry, however, requires only a single well-characterized antibody for the initial screening and a single MS experiment to detect and identify all the peptides captured. The results presented here highlight the efficiency of the new assay in terms of resources, time, and the sample numbers and volumes required.

## 4. Conclusions

The newly developed approach of IP-LC-MS provides quantitative data in good agreement with the previously validated IP-sELISA approach. In the present analysis, IP-sELISA appears to offer a slight advantage in terms of limits of detection over IP-LC-MS. However, the latest generation of instruments that are now commercially available offer  $\sim$ 10–100-fold enhancement in sensitivity over the MAT 900 used in the present study, and hence offer sensitivity performance comparable with sELISA assays. However, the specificity of IP-LC-MS for individual A $\beta$  detection is superior to IP-ELI-

SA and IP-sELISA. Also the flexibility of MS as a detection system yields much greater information from a single sample than is possible using the same sELISA assay. The use of on-line HPLC-MS reduces sample handling to a minimum and increases the ability to automate this assay allowing examination of a wider range of compounds within a single experiment. Finally, the use of mass spectrometry should allow the detection and identification of modifications to a peptide (such as point mutations, adduction, phosphorylation or oxidation). These factors can all directly affect the behavior of a peptide and may be crucial in determining the mode of action for A $\beta$  peptides within Alzheimer's disease.

## References

- [1] Katzman, R. and Kawas, C. (1994) in: R.D. Terry, R. Katzman and K.L. Bick (Eds.), *Alzheimer Disease*, Raven Press, New York, pp. 105–122.
- [2] Prelli, F., Castano, E., Gilkener, G.G. and Frangione, B. (1988) *J. Neurochem.* 51, 648–651.
- [3] Soto, C., Castano, E., Frangione, B. and Inestrosa, N. (1995) *J. Biol. Chem.* 17, 3063–3067.
- [4] Duff, K. (1997) *Trends Neurosci.* 20, 279–280.
- [5] Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F. and Cole, G. (1996) *Science* 274, 99–102.
- [6] Wright, P.F., Nilsson, E., Van Rooj, E.M., Leleta, M. and Jeggio, M.H. (1993) *Rev. Sci. Tech.* 12, 435–450.
- [7] Tahara, T., Usuki, K., Sato, H., Ohashi, H., Morita, H., Tsumura, H., Matsumoto, A., Miyazaki, H., Urabe, A. and Kato, T. (1996) *Br. J. Haematol.* 93, 783–788.
- [8] Gravina, S.A., Ho, L., Eckman, C.E., Long, K.E., Otvos, L., Younkin, L.H., Suzuki, N. and Younkin, S.G. (1995) *J. Biol. Chem.* 270, 7013–7016.
- [9] Scheuner, D., Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N., Bird, T.D., Hardy, J., Hutton, M., Kukull, W., Larson, E., Levy-Jahad, E., Vitanen, M., Peskind, E., Poorkaj, P., Schellenberg, G., Tanzi, R., Wasco, W., Lannfelt, L., Selkoe, D. and Younkin, S.G. (1996) *Nature Med.* 2, 864–870.
- [10] Suzuki, N., Cheung, T.T., Chai, X.-D., Odaka, A., Otvos, L., Eckman, C., Golde, T.E. and Younkin, S.G. (1994) *Science* 264, 1336–1340.
- [11] Orlando, R., Kenny, P.T.M. and Zagorski, M.G. (1992) *Biochem. Biophys. Res. Commun.* 184, 686–691.
- [12] Kumar, R., Hunziker, W., Gross, M., Naylor, N., Londowski, J.M. and Schaefer, J. (1994) *Arch. Biochem. Biophys.* 308, 311–317.
- [13] Kurian, E., Prendergast, F.G., Tomlinson, A.J., Holmes, M.W. and Naylor, S. (1997) *J. Am. Soc. Mass Spectrom.* 8, 8–14.
- [14] Miller, D.L., Papayannopoulos, I.A., Styles, J., Bobin, S.A., Lin, Y.Y., Biemann, K. and Iqbal, K. (1993) *Arch. Biochem. Biophys.* 301, 41–52.
- [15] Mori, H., Takio, K., Ogawara, M. and Selkoe, D.J. (1992) *J. Biol. Chem.* 267, 17082–17086.
- [16] Wang, R., Sweeney, D., Gandy, S.E. and Sisodia, S.S. (1996) *J. Biol. Chem.* 271, 31894–31902.
- [17] Application bulletin #28A23DS, Tosohaas.
- [18] Oyagi, Y. (1997) personal communication.
- [19] Tomlinson, A.J., Benson, L.M., Johnson, K.J. and Naylor, S. (1994) *Electrophoresis* 15, 62–71.
- [20] Hilbich, C., Monning, U., Grund, C., Masters, C. and Beyreuther, K. (1993) *J. Biol. Chem.* 268, 26571–26577.
- [21] Jarrett, J.T. and Lansbury Jr., P.T. (1993) *Cell* 73, 1055–1058.
- [22] Gleispach, H., Huber, E., Fauler, G., Kerble, R., Urban, C. and Leis, H.J. (1995) *Nutrition* 11, 604–606.