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Research paper

Identification of amyloid beta mid-domain fragments in human cerebrospinal fluid

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ABSTRACT

Amyloid beta (Aβ) is a peptide derived from processing of the membrane bound amyloid precursor protein and is a main constituent in amyloid plaques in Alzheimer's disease (AD). The excess Aβ in AD brain may be caused by altered Aβ metabolism, including reduced enzymatic degradation. Our previous enzymatic study of Aβ degradation revealed that intracellular enzymes produced several truncated Aβ mid-domain fragments. We therefore generated an antibody to enable identification of these anticipated Aβ species in cerebrospinal fluid (CSF). The produced antibody displayed affinity for the Aβ mid-domain region and 36 N-terminally truncated Aβ fragments were precipitated from human CSF and identified by liquid chromatography – mass spectrometry. 31 peptides were truncated from residue 18 up to 23, N-terminal truncation that have not previously been identified in CSF. The results show that the complexity of amyloid beta peptides circulating in the CSF is greater than previously suggested and we also demonstrate that the mid-domain antibody used can serve as an additional tool for mapping a more complete Aβ degradation profile.

1. Introduction

Amyloid beta deposits in the brain are one of the known characteristics of Alzheimer's disease [1]. The plaque forming amyloid beta peptides originates from proteolytic cleavage of the membrane bound amyloid precursor protein (APP) by β-secretase and by γ-secretase, creating Aβ peptides that are prone to aggregate [2]. The cleavage of APP by β-secretase in the luminal domain leaves a free N-terminal end, often referred to as position 1 in the Aβ sequence. The other enzymatic cleavage required to release Aβ is performed by γ-secretase and it occurs in the transmembrane domain leaving a hydrophobic C-terminus [3]. In an alternative route known as the non-amyloidogenic pathway, α-secretase cleaves between residue 16/17 resulting in APP fragments called p3 starting from position 17 when followed with a proteolytic release from the membrane by γ-secretase [4]. One of the most used biomarker in Alzheimer's disease research is measurement of Aβ1-42 and Aβ1-40 in CSF [5]. Although Aβ1-40 is present at about ten to twenty times higher concentration in CSF, Aβ1-42 is more prone to aggregate and shown to correlate better with AD neuropathology [6–9].

While the commonly used immunoassays allow monitoring of the Aβ1-42/Aβ1-40 peptides, the method only measure the intact peptide concentration, leaving their exact biochemical faith unknown. Although an increased Aβ1-42 production in body fluid of patients with inherited early-onset familiar AD is pathogenic, reduced proteolytic clearance may explain increased Aβ load in late-onset sporadic AD [10–12]. Several enzymes have been shown to degrade Aβ1-40 and Aβ1-42 [13] and many of these have been directly linked to changes in Aβ concentration in mice models [14,15]. While the exact processes behind the reduced Aβ-clearance in sporadic AD is still unknown, the truncated Aβ species in CSF reflect the processing by such degrading proteases. A more complete mapping of the catabolic Aβ-fragments may increase possibilities to detect changes of enzymatic activity from the Aβ profile.

Abbreviations: CSF, cerebrospinal fluid; Aβ, amyloid beta; AD, alzheimer's disease; LC, liquid chromatography; MS, mass spectrometry; MS/MS, tandem mass spectrometry; APP, amyloid precursor protein; IDE, insulin degrading enzyme.

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Knowledge about the Aβ species circulating in CSF can also be used for in-vitro studies to investigate the neurotoxicity and aggregation properties of the various Aβ-peptides.

An effort has therefore been put into identification of Aβ peptides in CSF [16–20]. Nano liquid chromatography – tandem mass spectrometry (nano LC-MS/MS) is a method for identifying peptides at low concentrations in limited samples. Due to the very high number of endogenous peptides and intact proteins present in CSF, immunoprecipitation has been used to pull down Aβ peptides to reduce sample complexity prior to analysis [19,21,22]. Previous investigations allowed a large number of C-terminal truncations and N-terminal truncations up to position 11 to be identified in human CSF [18]. Compared to the Aβ-peptides previously identified in CSF, our enzyme study performed in-vitro showed that several mid-domain peptides were produced from known Aβ-degrading proteases [13]. A high number of peptide products starting from amino acid in position 19 up to 21 and ending at position 30 up to 34 were found. These peptides have to our knowledge not previously been identified in CSF. The detection of such peptides is dependent upon using correct antibodies in the precipitation step. Antibodies which bind close to the N-terminus of Aβ are typically used in immunoprecipitation of CSF-Aβ, e.g. commercially available antibodies like 6E10 antibody (epitope at 4–9) which would be restricted to N-terminal truncation up to position 4 of Aβ. The 4G8 antibody (epitope at 18–22), which can capture N-terminal fragments with truncation up to residue 17 is also frequently used (Fig. 1).

Here we aimed to develop and use an antibody with affinity between residue 21 and 34, the location of frequent cleavage sites of the previously studied catabolic enzymes, to find the anticipated mid-domain peptides in CSF samples [13]. We report new Aβ peptides from CSF samples which are heavily N- and C-terminally truncated, and show that a mid-domain Aβ-antibody can serve as a tool to help monitor a more complete degradation profile of Aβ and aid in the quest for new and better AD biomarkers.

2. Materials and methods

2.1. Production of amyloid beta mid-domain antibody and epitope mapping

The affinity-purified 12EF325 rabbit antibody was raised against human Aβ21-34 (sequence AEDVGSNKGAIIGL) and supplied from Agrisera (Vännäs, Sweden). The antigen peptide contained an N-terminal cysteine allowing it to be maleimide-conjugated to KLH. The antiserum was affinity purified on an UltraLink Iodoacetyl Resin (53155, Pierce) and eluted at pH 2.5 with 0.1 M glycine. Epitope mapping were conducted using 16 peptides each consisting of 10 amino acids spot synthesized on a cellulose membrane (JPT Technologies GmbH). Each spot contained approximately 5 nmol peptide covalently bound to the cellulose membrane. The mapping was done according to the manufacturer procedure. The membrane was soaked in methanol for 5 min, washed three times with tris buffer (50 mM tris, 137 mM NaCl, pH 8) and blocked for 2 h using 5% powdered milk in tris buffer containing 0.05% Tween 20 (tris-T). The antibody was dissolved in tris-T buffer with 0.5% dried milk to a concentration of 0.5 μg/mL and incubated for 3 h. The membrane was subsequently washed three times with tris buffer. A solution consisting of 0.4 μg/mL goat anti-rabbit IgG-HRP antibody (Southern Biotech) dissolved in tris-T buffer with 0.5% dried milk was incubated with the membrane for 2 h. The membrane was washed three times with tris buffer and detection was performed using Amersham ECL Prime Western Blotting Detection Reagent (GE Healthcare) in combination with an LAS 3000 image reader (Fujifilm).

2.2. Immunoprecipitiation of cerebrospinal fluid

About 15 mL CSF from an AD patient was collected in polypropylene tubes by lumbar puncture as previously described [23]. The CSF sample was centrifuged for 2000 g at 4 °C for 10 min to remove insoluble material and stored at −80 °C until immunoprecipitation and LC-MS/MS analysis. Immunoprecipitation was carried out from human CSF using magnetic Protein G Dynabeads (Life Technologies). A total of 100 μL bead solution was incubated with 20 μL of 1 mg/mL 6E10, 4G8 or 12EF325 antibody solution for 40 min and the solution containing excess antibody was discarded. Subsequently 2 mL of CSF was incubated with each antibody beads for 90 min at 4 °C. Beads were washed two times with 200 μL ammonium bicarbonate buffer (50 mM, pH = 7.3) followed by sample transfer into a new tube. The captured Aβ-derived peptides were eluted with 30 μL of 0.5% aqueous formic acid. The elution process was performed by vortexing for 20 min at room temperature and supernatant was transferred to new tubes and stored at 4 °C prior to analysis.

2.3. Nano liquid chromatography tandem mass spectrometry

Immunoprecipitated samples were analyzed using a nano LC-MS/MS system, consisting of a Q-Exactive mass spectrometer, and Flex Ion Source nanoelectrospray and an Easy nLC 1000 nano LC pump equipped with a 15 cm × 75 μm Accucore C4 column (all from Thermo Scientific). For sample injection, 5 μL of sample was picked up and loaded into the analytical column. Peptides were separated using a liquid gradient from 10% mobile phase B, to 45% B in 10 min and up to 95% in 2 min with a hold at 95% for another 5 min. Column flow rate was set to 800 nL/min for sample loading and 300 nL/min for gradient separation. Mobile phase A and B was

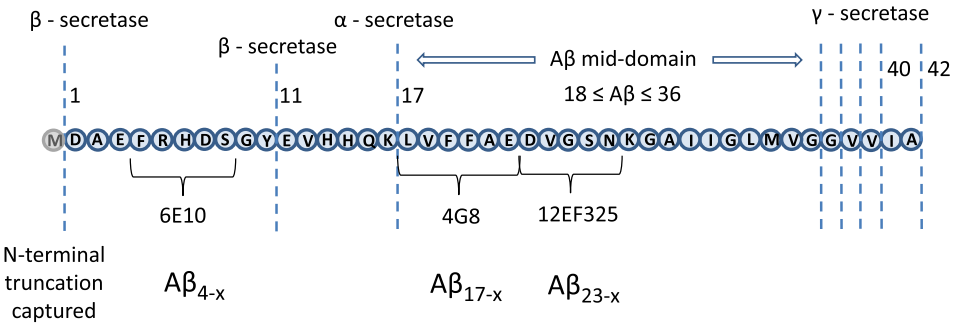

Fig. 1. Overview of the Aβ1-42 domain and epitopes of antibodies 6E10 and 4G8, and 12EF325, the latter produced for immunoprecipitation and detection of Aβ mid-domain peptides. The mid-domain area is defined here as the region between cleavages sites of α-secretase and γ-secretase.

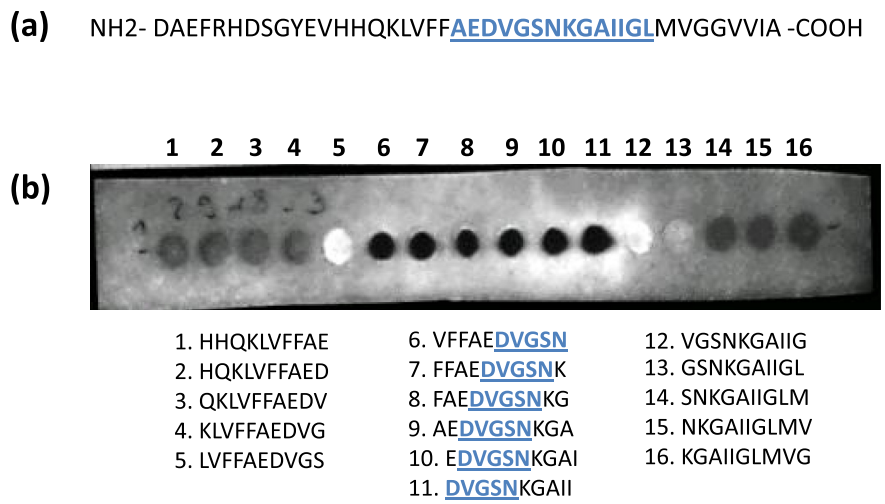

Fig. 2. (a) The Aβ1-42 sequence with the Aβ21-34 peptide used in rabbit immunization displayed as underlined letters. (b) Mapping of epitopes using 16 spot-synthesized Aβ peptides each consisting of 10 amino acids. The main binding site of the polyclonal (12EF325) antibody was determined to be from amino acid 23 to amino acid 27 (peptide 6–11). Weak binding can also be seen for peptide number 5, 12 and 13.

Table 1
Peptides identified using the 12EF325 antibody and immuneprecipitation of human CSF, followed by nano LC-MS/MS analysis.

Peptide	Amino acid sequence	Predicted mass	Mass deviation [ppm]	Score
1–30	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGA	3388.5861	0.6	87.8
1–33	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIG	3671.7757	−0.7	145.1
1–34	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGL	3784.8598	0.5	164.6
1–37	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVG	4071.9901	0.8	90.9
1–38	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGG	4129.0116	0.4	121.6
1–39	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGV	4228.0800	0.9	40.4
1–40	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV	4327.1484	−0.2	127.5
11–30	EVHHQKLVFFAEDVGSNKGA	2211.1073	0.2	203.1
11–33	EVHHQKLVFFAEDVGSNKGAIIG	2494.2969	−0.4	155.8
11–34	EVHHQKLVFFAEDVGSNKGAIIGL	2607.3809	1.4	111.7
17–28	LVFFAEDVGSNK	1324.6663	1.8	61.1
17–33	LVFFAEDVGSNKGAIIG	1735.9145	0.4	104.3
18–28	VFFAEDVGSNK	1211.5823	−0.9	77.1
18–30	VFFAEDVGSNKGA	1339.6408	−0.3	169.0
19–28	FFAEDVGSNK	1112.5138	−0.7	99.6
19–30	FFAEDVGSNKGA	1240.5724	−0.5	158.1
19–30	FFAEDVGSNKGAIIG	1523.7620	0.2	109.1
20–29	FAEDVGSNKG	1022.4669	0.5	108.0
20–30	FAEDVGSNKGA	1093.5040	0.0	136.5
20–33	FAEDVGSNKGAIIG	1376.6936	−0.7	129.7
20–34	FAEDVGSNKGAIIGL	1489.7777	2.2	81.4
20–37	FAEDVGSNKGAIIGLMVG	1776.9080	−0.3	101.7
20–38	FAEDVGSNKGAIIGLMVGG	1833.9295	−0.3	112.8
20–40	FAEDVGSNKGAIIGLMVGGVV	2032.0663	−1.0	138.5
21–33	AEDVGSNKGAIIG	1229.6252	−0.6	65.0
21–34	AEDVGSNKGAIIGL	1342.7092	−0.8	129.7
21–37	AEDVGSNKGAIIGLMVG	1629.8396	0.5	87.4
21–38	AEDVGSNKGAIIGLMVGG	1686.8611	−0.3	106.0
21–39	AEDVGSNKGAIIGLMVGGV	1785.9295	−0.2	121.7
21–40	AEDVGSNKGAIIGLMVGGVV	1884.9979	−0.9	148.2
22–30	EDVGSNKGA	875.3985	−0.6	76.2
22–33	EDVGSNKGAIIG	1158.5881	0.0	89.2
22–34	EDVGSNKGAIIGL	1271.6721	−0.7	133.9
22–37	EDVGSNKGAIIGLMVG	1558.8025	−0.1	133.5
22–38	EDVGSNKGAIIGLMVGG	1615.8240	0.0	109.8
22–39	EDVGSNKGAIIGLMVGGV	1714.8924	−0.5	152.9
22–40	EDVGSNKGAIIGLMVGGVV	1813.9608	−1.0	57.5
23–34	DVGSNKGAIIGL	1142.6295	0.4	128.4
23–35	DVGSNKGAIIGLM	1273.6700	0.8	92.2
23–37	DVGSNKGAIIGLMVG	1429.7599	0.2	81.6
23–38	DVGSNKGAIIGLMVGG	1486.7814	0.0	81.4
23–39	DVGSNKGAIIGLMVGGV	1585.8498	0.1	71.1
23–40	DVGSNKGAIIGLMVGGVV	1684.9182	−1.2	127.3

0.1% formic acid in water and acetonitrile, respectively. An electrospray potential 2.0 kV in positive mode was used and spectra were recorded from *m/z* 350 to 2000 at a resolution of 70 000 in MS and 35 000 in MS/MS. Up to the 10 most intense signals in each MS spectra were fragmented using an isolation window of 2.0 *m/z* and a normalized collision energy of 25%. Peptides with a charge from +2 to +5 were selected for fragmentation.

2.4. Data analysis

Peptides were identified using MaxQuant v.1.5.2.8 with the built in Andromeda search engine [24] (a freeware available at www.maxquant.org). Peptide false discovery rate was set to 0.01 and a mass tolerance of 6 ppm and 20 ppm was used in MS and MS/MS, respectively. Cleavage specificity was set to unspecific and methionine oxidation and N-terminal acetylation were used as variable modifications. Peptide reports were based on a minimum Andromeda score of 40, unless otherwise stated.

2.5. Ethics statement

The research was approved by the regional ethical committee at Norwegian Southern and Eastern Regional Health Authority. The participant gave informed consent to participate in AD research.

3. Results

3.1. Amyloid beta mid-domain antibody specificity

We have previously identified several N- and C-terminally truncated Aβ peptides by allowing Aβ1-42 to be enzymatically processed by known Aβ degrading proteases [13]. Many of the identified degradation products started at residue 19, 20 and 21 and ended after amino acid residue 30, 33, 34 and could therefore not be captured by many of the commercially available antibodies (see Fig. 1). In order to identify these anticipated mid-domain peptides from CSF by immunoprecipitation – mass spectrometry, antibodies with the epitope inside the Aβ mid-domain region had to be used. The polyclonal (12EF325) antibody, which was produced by immunizing rabbits with Aβ21-34 (Fig. 2a), showed strong affinity for peptides containing the sequence DVGSN corresponding to residues 23–27 in Aβ1-42 (Fig. 2b). Weaker affinity can also be seen for peptide number 5, 12 and 13, giving an affinity for residue 22–29 in Aβ1-42.

3.2. Immunoprecipitation followed by nano liquid chromatography – mass spectrometry

By using immunoprecipitation with the mid-domain specific antibody followed by nano LC-MS/MS we were able to identify 36 N-terminally truncated peptides in addition to 7 peptides beginning at position 1 (Table 1). All the peptides identified had a mass deviation below 2.5 ppm in addition to high scores, showing both great mass accuracy for the intact peptide and great match for the peptide fragment spectrum. In addition to a high accuracy, the observed peptide charge was in agreement with the charge expected from the peptide sequence (Table S1, supplemental material). The 36 identified peptides had N-terminal truncation at 8 different positions, from between residue 10/11 up to 22/23. Cleavage between residues 16/17 is consistent with cleaved by α-secretase, in the non-amyloidogenic pathway [25]. In addition to the well-known site between −1/1, β-secretase also has a cleavage

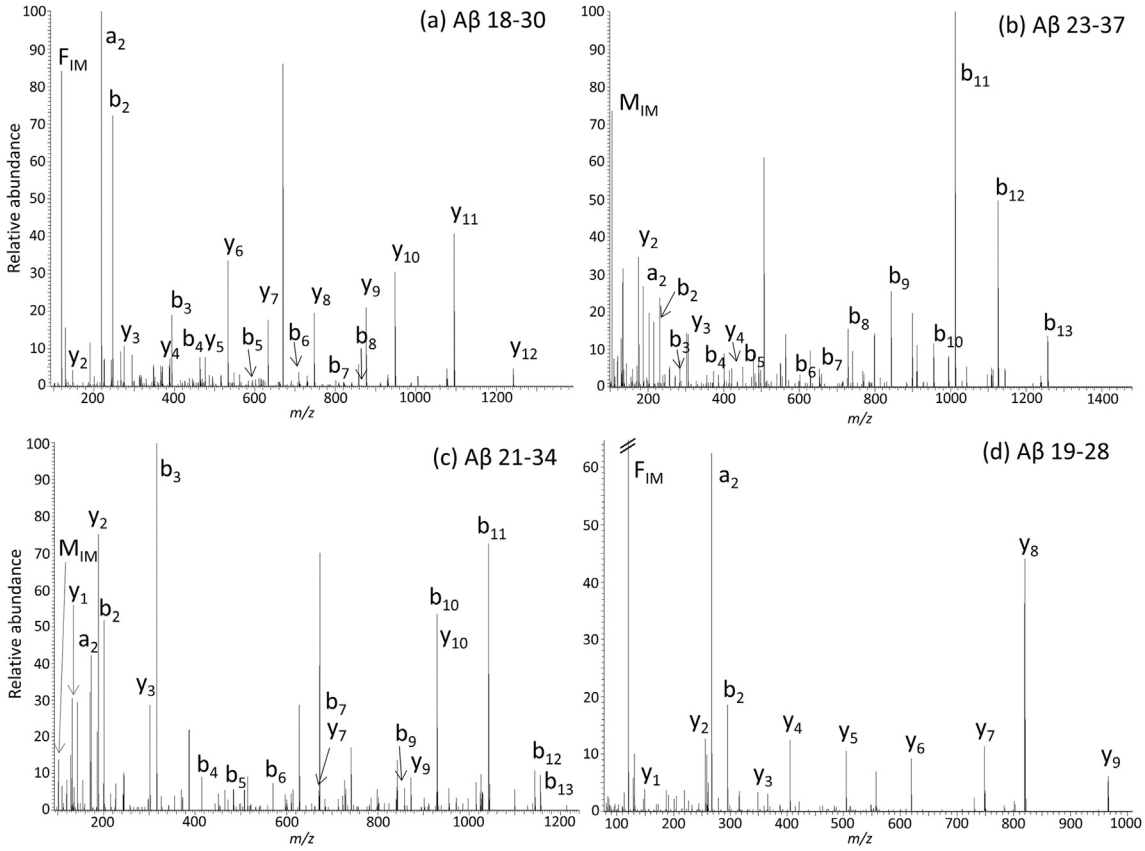

Fig. 3. Fragment mass spectra of N- and C-terminally truncated Aβ peptides. Major peaks are annotated with immonium, a-, b- and y-ions.

site in position 10/11, which can explain the peptide N-terminal at residue 11 [26]. To our knowledge none of the 36 identified N-terminally truncated Aβ fragments have previously been reported in CSF and no N-terminal truncation above position 17 has been reported. None of the identified peptides had oxidation of the methionine residue, located in position 35. While oxidation of methionine can be a post-translational modification, oxidation can also occur during sample preparation and storage [27,28]. Four of the fragment spectrums from Aβ peptide identifications that required the mid-domain antibody in immunoprecipitation can be seen in Fig. 3, where Aβ18-30 (Fig. 3a) have been cleaved at the first residue above the proteolysis site of α-secretase. Interestingly the peptide Aβ23-37 (Fig. 3b) is derived from cleavage between amino acid 22/23 and to our knowledge no cleavage site between these amino acids have been identified with insulin degrading enzyme (IDE), cathepsin D, endothelin converting enzyme or neprilysin [13]. A total of six identified peptides were cleaved between residue 22/23 indicating proteolytic activity by angiotensin converting enzyme, an enzyme shown to cleave at this position [29]. Alternatively this cleavage can be explained through processing by glutamyl aminopeptidase that can cleave glutamic acid (at position 21) from the peptide N-terminus [30]. Aβ21-34, the peptide that was used to generate the 12EF325 antibody by immunization was identified in CSF (Fig. 3c). This peptide has also been identified from enzymatic digestion of Aβ1-42 with both cathepsin D and IDE [13].

From the N-terminal truncated peptides 10 different C-termini were identified (Table 1). Seen aside from the observed Aβx-37 up to Aβx-40 peptides from C-terminal γ-secretase cleavage, 21 peptides were C-terminally truncated from between residue 35/36 down to 28/29 (e.g. Aβ19-28, Fig. 3d.), where the latter is also a known site of IDE proteolysis [13]. Peptides with C-terminal ends were observed from all residues from 28 up to 40, with the exception of residue 31, 32, and 36. The lack of Aβ peptides ending at amino acid 31, 32 and 36 is also in agreement with previous findings in CSF [18,19]. No fragments ending at position 42 were identified by MS/MS search. This may be explained by the lower abundance of the Aβ1-42 peptide and therefore also a lower abundance of the Aβx-42 proteolysis products.

The intact Aβ1-40 and Aβ1-38 frequently measured in immunoassays was also identified from the tandem mass spectrometric method using the search engine, and the peptides can be seen in the mass spectrum (Fig. 4a) and the fragment spectrum (Fig. 4b). Mostly b-ions were found in the fragment mass spectra, a natural consequence when the positively charged lysine in position 28 is far from the C-terminal end of the Aβ-peptide. Brinkmalm et al. have showed that a mass spectrometer equipped with an alternative fragmentation technique can be used to improve the fragmentation of Aβ1-38 and Aβ1-40 [18].

In order to further evaluate the findings from the 12EF325 antibody CSF-immunoprecipitates, 12EF325 was exchanged with 4G8 or 6E10 antibodies. Otherwise the same procedure was performed. A total of 26 peptides were identified and as expected, all the identified APP derived peptides contained the epitope of the respective antibody (Table 2). Immunoprecipitation with 6E10 antibody, N-terminal truncation from position −5 and position 1 was identified. C-terminal truncations were identified between all residues from 14/15 up to 20/21 in addition to truncation between

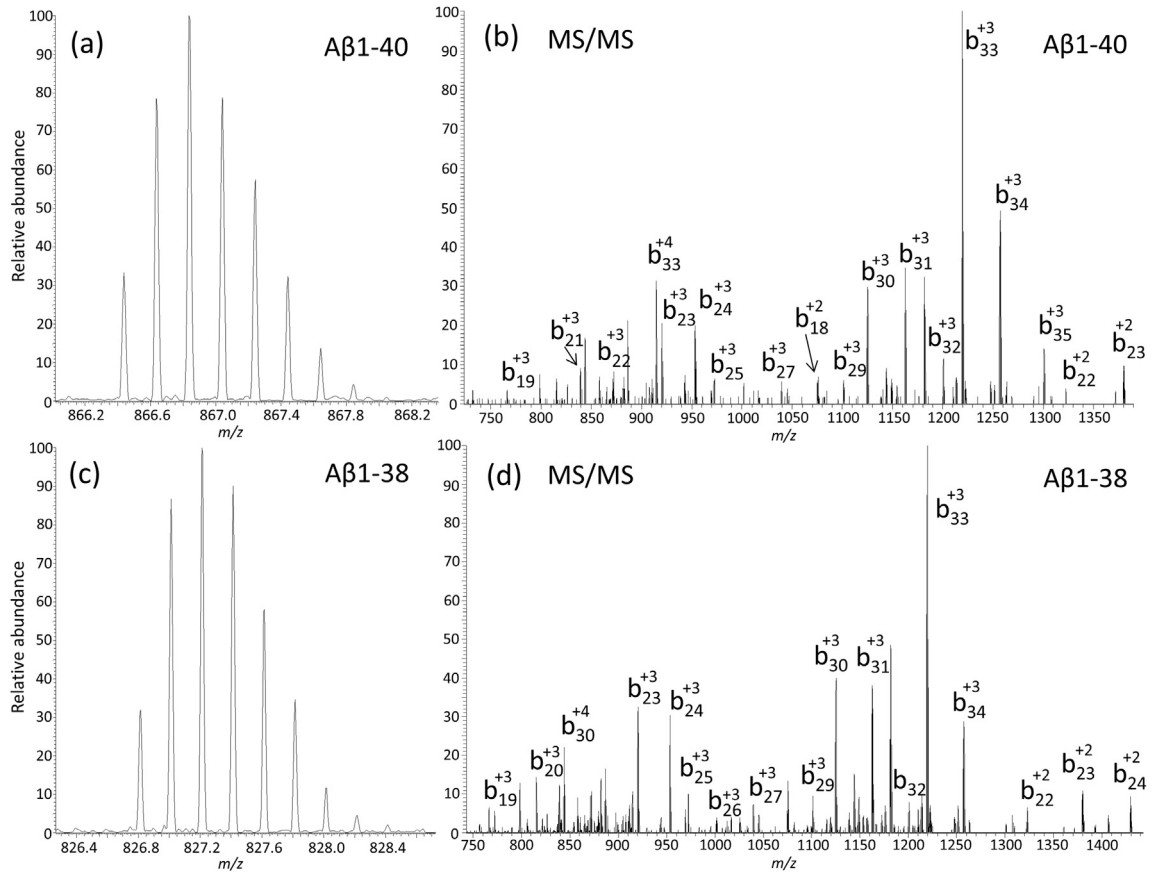

Fig. 4. The intact Aβ1-40 and Aβ1-38 frequently measured in immunoassays was also identified from immunoprecipitates of CSF using the 12EF325 Aβ mid-domain specific antibody. The mass and fragment spectrum for Aβ1-40 can be seen in Fig. 4a and b, while Fig. 4c and 4d displays the mass and fragment spectrum for Aβ1-38.

Table 2
Peptides identified from immunoprecipitation of human CSF sample using 4G8 and 6E10 antibodies, followed by nano LC-MS/MS analysis. The same CSF sample and precipitation procedure as in Table 1 was used.

Antibody	Peptide	Amino acid sequence	Predicted mass	Mass deviation [ppm]	Score
6E10	−5–15	SEVKMDAEFRHDSGYEVHHQ	2400.0553	−1.3	53.6
	1–14	DAEFRHDSGYEVHH	1697.7182	0.0	177.9
	1–15	DAEFRHDSGYEVHHQ	1825.7768	−0.3	175.6
	1–16	DAEFRHDSGYEVHHQK	1953.8718	−0.9	148.4
	1–17	DAEFRHDSGYEVHHQKL	2066.9559	−0.3	237.8
	1–18	DAEFRHDSGYEVHHQKLV	2166.0243	0.4	117.3
	1–19	DAEFRHDSGYEVHHQKLVF	2313.0927	0.0	179.8
	1–20	DAEFRHDSGYEVHHQKLVFF	2460.1611	0.1	103.9
	1–33	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIG	3671.7757	0.0	54.9
	1–38	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGG	4129.0116	2.6	4.0[a]
	1–40	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV	4327.1484	0.9	7.4[a]
4G8	1–30	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGA	3388.5861	−0.7	109.8
	1–33	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIG	3671.7757	−0.3	150.1
	1–34	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGL	3784.8598	0.7	160.8
	1–37	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVG	4071.9901	0.6	156.0
	1–38	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGG	4129.0116	0.4	179.6
	1–39	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGV	4228.0800	0.4	151.2
	1–40	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV	4327.1484	−0.3	181.0
	11–30	EVHHQKLVFFAEDVGSNKGA	2211.1073	−0.4	167.9
	11–33	EVHHQKLVFFAEDVGSNKGAIIG	2494.2969	−0.3	185.6
	11–34	EVHHQKLVFFAEDVGSNKGAIIGL	2607.3809	0.6	142.9
	11–38	EVHHQKLVFFAEDVGSNKGAIIGLMVGG	2951.5327	0.0	51.5
	11–40	EVHHQKLVFFAEDVGSNKGAIIGLMVGGVV	3149.6696	−0.6	152.9
	17–28	LVFFAEDVGSNK	1324.6663	0.8	110.1
	17–30	LVFFAEDVGSNKGA	1452.7249	0.7	172.3
	17–33	LVFFAEDVGSNKGAIIG	1735.9145	0.9	96.8

[a] Peptides are reported with a score below 40. The mass spectra of these two peptides are shown in Fig. S2, Supplemental material.

33/34. The N-terminus of Aβ -5–15 is located prior to the β-secretase cleavage site and the peptide ends at residue 15, before the suggested cleavage site of α-secretase. This observation is in agreement with the findings of Brinkmalm and Halim et al., where the Aβ peptides with N-terminus below position 1 only extended up to amino acid 15 [18,19]. These Aβ-x peptides would therefore not extend to the mid-domain region or be captured by the 12EF325 mid-domain antibody.

By using 4G8 for immunoprecipitation, N-terminal truncations up to position 11 and 17 were observed (Table 2). Cleavage between residue 10/11 and 16/17 is in agreement with proteolysis with β- and α-secretase. C-terminal truncations between residue 28/29, 29/30, 30/31, 33/34 and 34/35 also fit with the truncations identified from 12EF325 precipitation. No cleavage between residue 31/32 32/33 or 36/37 was observed, which again is in agreement with identifications with the 12EF325 antibody.

4. Discussion

While measurements of Aβ1-40/1–42 in CSF are valuable biomarkers in Alzheimer's disease research the identification and measurement of Aβ degradation products may shed light on the mechanisms behind AD. We have previously identified several N- and C-terminally truncated Aβ peptides from enzymatic degradation of Aβ1-42 [13] and proposed that these truncated Aβ peptides were also present in CSF. For the first time, we here show that antibodies against the Aβ mid-domain region allowed identification of 36 novel CSF Aβ peptides. More than half of the identified N-terminally truncated fragments were also C-terminally truncated and can therefore not be captured by the commercially available antibodies specific for e.g. the Aβ38 or Aβ40 C-terminal end.

These results show that the high complexity of Aβ peptides circulating in the cerebrospinal fluid require antibodies with epitopes covering different regions of the Aβ sequence for a complete peptide profiling when immunoprecipitation is used for sample preparation. Large number of studies in which antibodies at or adjacent to the N-terminus has been used have given a focus on N-terminal truncations of Aβ, while the findings presented here may shift focus towards any truncations across the whole sequence for a more complete mapping. Identifying a more complete degradation profile may give a further understanding of Aβ processing and clearance in vivo in humans and help to identify disease specific differences. The newly identified Aβ peptides also allow the study of aggregation and toxicity for the novel Aβ peptides both in vitro and in vivo. Cleavage between residues 22/23 has not been identified as a proteolytic site for the most commonly studied Aβ degrading enzymes suggesting that additional enzymes are involved in Aβ degradation. The identification of Aβ1-14, Aβ1-15 but no peptide products with N-terminus at residue 15 or 16 can be explained by a concerted cleavage by β- and α-secretase, as suggested by Portelius et al. [31].

No cleavage sites were identified between residues 24 to 28 with any of the antibodies used, and considering that amino acids 24-28 make up the central residues in a β-turn this suggest that the structure may be resistant to proteolysis [32]. If the structure between amino acids 24–28 is resistant to Aβ degrading proteases, these residues are a target for antibody generation as the epitope would remain intact during Aβ processing. Antibodies that cover the region from residue 21 to 29 have also been suggested as therapeutic applications [33]. The 12EF325 antibody covers this region that has been suggested to be important in Aβ aggregation and this or a similar humanized monoclonal antibody may well have therapeutic applications.

Altogether the mid-domain antibody served as a tool for identifying novel Aβ peptides which allowed identification of a more complete Aβ peptide profile. Being able to identify an extensive Aβ profile is important for future clinical studies as differences in Aβ degradation peptides between AD and non-demented controls could reveal differences in enzyme activity and give rise to specific peptide targets as new biomarkers. We are therefore currently working with method development for the

quantification of these newly identified Aβ peptides in AD and control groups.

Conflict of interest

The authors declare that there is no actual or potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biochi.2015.03.022.

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