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Perspective

Amyloid-beta oligomerization is associated with the generation of a typical peptide fragment fingerprint

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Abstract Amyloid-beta (Aβ) peptide oligomerization plays a central role in the pathogenesis of Alzheimer's disease (AD), and Aβ oligomers are collectively considered an appealing therapeutic target for the treatment of AD. However, the molecular mechanisms leading to the pathologic accumulation of oligomers are unclear, and the exact structural composition of oligomers is being debated. Using targeted and quantitative mass spectrometry, we reveal site-specific Aβ autocleavage during the early phase of aggregation, producing a typical Aβ fragment signature and that truncated Aβ peptides can form stable oligomeric complexes with full-length Aβ peptide. We show that the use of novel anti-Aβ antibodies raised against these truncated Aβ isoforms allows for monitoring and targeting the accumulation of truncated Aβ fragments. Antibody-enabled screening of transgenic models of AD as well as human postmortem brain tissue and cerebrospinal fluid revealed that aggregation-associated Aβ cleavage is a highly relevant clinical feature of AD.
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1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is manifested as a gradual decline in memory and cognitive function. A number of studies indicate that soluble oligomers might account for the AD-associated decline in synaptic plasticity [1,2] and that inhibition of natural amyloid-beta (Aβ) oligomerization rescues deficits in long-term potentiation [3]. Several types of Aβ assemblies of dimeric and trimeric [1,2,4] or dodecameric (Aβ* 56) [5] nature have been observed in vitro and in vivo in transgenic mouse models, human cerebrospinal fluid (CSF) [6], and postmortem AD brain extracts [7,8], with the higher molecular weight species being considered the main neurotoxic culprit associated

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with cognitive dysfunction. Collectively, Aβ oligomers can be considered as an appealing diagnostic and therapeutic target. However, the general morphological heterogeneity and, to some extent, metastable structure renders an antibody-based targeting and detection of oligomers difficult. Therefore, the development of specific antioligomeric based therapeutics remains challenging.

CSF analyses from AD patients indicate that the presence of Aβ oligomers correlates with a concomitant decrease in Aβ42 levels. CSF levels of total and phosphorylated tau protein [9], tissue transglutaminase (tTGase) [10], ubiquitin [11], Aβ oligomers [12] as well as changes in Aβ1–42 concentration, together with the presence of particular Aβ truncations [13], have been collectively suggested as useful biomarkers in AD.

Previously, mass spectrometry (MS)–based analysis of CSF revealed a specific Aβ peptide fragment signature in sporadic AD patients [14–16], and it has been reported that truncated Aβ is known to represent more than 60% of all Aβ species found in nondemented as well as in AD individuals [17]. These findings may suggest that Aβ oligomers could consist of a heterogeneous morphological entity of full-length Aβ40 and Aβ42 as well as truncated Aβ isoforms, of which the latter may serve as an important molecular seed during peptide aggregation [18]. Similarly, a recent report showed that the aqueous phase of human AD brain extracts contained sodium dodecylsulfate (SDS)–stable Aβ species of a molecular weight range of 6–7 kDa and that these Aβ species may form part of larger Aβ aggregates [19].

In this work, we sought to identify a "molecular crosstalk" during the lag phase of Aβ peptide aggregation that typically precedes the pathologic accumulation of neurotoxic oligomers. Here, we have identified site-specific autocleavage of Aβ peptide and report a typical peptide fragment fingerprint, which may be associated with the early nucleation process of Aβ aggregation. Using targeted and quantitative MS, we reveal a highly reproducible Aβ fragment signature with a significant abundance of C-terminal peptide amidation. Moreover, we show that these truncated Aβ peptides have a particularly high propensity in forming SDS-stable low-molecular-weight oligomers of dimeric and trimeric nature. These findings have enabled us to develop novel neoepitope antibodies that selectively bind to the gradual accumulation of truncated Aβ isoforms during the early phase of peptide aggregation. Our targeted analysis of human brain tissue extracts and CSF revealed that Aβ cleavage within the peptide's β-turn region is a highly relevant feature observed in AD.

2. Methods

2.1. Aβ peptide preparation

Full-length wild type (wt) Aβ peptides Aβ1–40, arctic mutant Aβ1–40 (Arc), and Aβ1–42 Aβ (Dr. James I. Elliott, Yale University, USA) were dissolved in 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) at a concentration of 1 mg/mL, followed by a 10-minute sonication to break any preformed aggregates. HFIP solution was evaporated under a ventilated fume hood by applying a light stream of N_2 gas. The HFIP film containing the Aβ peptide was either directly resuspended in 100% dimethyl sulfoxide (DMSO; Sigma-Aldrich, Switzerland) and further diluted to 1% DMSO in a new buffer or stored dry at −20°C until use. Aβ peptide fragments comprising residues: 1–15, 1–22, 1–23, 1–24-NH_2, 1–25, 1–25-NH_2, 26–40, 24–40 (purity of ≥97%) were purchased from GenicBio Ltd. (Shanghai, China). Aβ peptide concentrations were determined by ultraviolet-visible (UV) absorbance using the peptide's molar extinction coefficient at 280 nm.

2.2. Size exclusion chromatography of Aβ42 amyloid-derived diffusible ligands and transgenic mouse brain tissue extracts

Size exclusion chromatography (SEC) fractionation was carried out using an ÄKTA Explorer FPLC (GE Healthcare) placed inside a cold (4°C) chamber. A Superdex 200 10/300GL column (GE Healthcare) was used, and samples were eluted with either 25-mM ammonium acetate (pH 8.5) or a Superdex 75 10/300GL with 20-mM Tris and 20-mM NaCl (pH, 7.5; for aggregated Aβ1–25) at a flow rate of 0.5 mL/min. Before injection, samples were centrifuged at 4°C 16,000*g* for 20 minutes, and 0.5 mL of sample supernatant was injected onto the column. Aggregated Aβ1–25 peptide was filtered using 0.22-μm filter devices before injection to prevent from injecting any large, fibrillary aggregates. Peptide elution was detected by absorbance at 280 nm, 275 nm, and 215 nm, and 0.5 ml fraction volumes were collected. Eluted fractions were either used immediately or aliquoted (50 μL) and stored at −80°C. Where indicated, samples volumes were concentrated approximately 10× in a speed vacuum.

2.3. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

Aliquots (2 μL) of samples were used for matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/TOF MS; ABI 4800 model; Applied Biosystems) measurements. Matrix solution of α-cyano-4-hydroxycinnamic acid (7 mg/mL in ACN/0.1% TFA [1:1, vol/vol]) was used for sample deposition. The sample (1 μL) was mixed with 1 μL of matrix solution, and then, 1 μL of this mixture was deposited in duplicates on the target plate and allowed to air dry. Samples were analyzed in reflectron positive mode.

2.4. Digestion of Aβ peptides

Proteolytic digestion using LysN (2 ng/uL) was performed overnight at 37°C in 50-mM ammonium

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