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Ion channel formation by N-terminally truncated A β (4–42): Relevance for the pathogenesis of Alzheimer's disease

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Abstract

A β deposition is a pathological hallmark of Alzheimer's disease (AD). Besides the full-length amyloid forming peptides (A β _{1–40} and A β _{1–42}), biochemical analyses of brain deposits have identified a variety of N- and C-terminally truncated A β variants in sporadic and familial AD patients. However, their relevance for AD pathogenesis remains largely understudied. We demonstrate that A β _{4–42} exhibits a high tendency to form β -sheet structures leading to fast self-aggregation and formation of oligomeric assemblies. Atomic force microscopy and electrophysiological studies reveal that A β _{4–42} forms highly stable ion channels in lipid membranes. These channels that are blocked by monoclonal antibodies specifically recognizing the N-terminus of A β _{4–42}. An A β variant with a double truncation at phenylalanine-4 and leucine 34, (A β _{4–34}), exhibits unstable channel formation capability. Taken together the results presented

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Author Contributions

JG, BK and RL conceived the idea. AFM work was performed by AGK and DKB. RA performed electrophysiology experiments. CD, ThT, TEM, and MS analysis was performed by AR and JG. AGK, AR, RA analyzed and wrote a detailed explanation for all the results with JG, BK, and RL. AGK, AR, JG, BK, and RL wrote the paper.

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herein highlight the potential benefit of C-terminal proteolytic cleavage and further support an important pathogenic role for N-truncated A β species in AD pathophysiology.

Graphical abstract text

N- and C-terminal truncated A β species play an important role in the pathophysiology of Alzheimer's disease (AD). In this study, structural, morphological and electrical activity of one of the main N-truncated species A β_{4-42} was analyzed. Through EM and AFM analysis we report that A β_{4-42} forms oligomeric structures and in the membrane environment these structures have a pore-like morphology. These pores are electrically active as evident from the transmembrane current recorded through electrophysiological studies. These results have broad implications in understanding the pathophysiology of AD and help advance the design of therapeutic agents.

Keywords

truncated A β peptides; amyloid; oligomers; electrophysiology

Background

Alzheimer's disease (AD), the most common form of dementia in the world, is a progressive neurodegenerative disorder neuropathologically characterized by the presence of extracellular parenchymal and vascular A β amyloid deposits as well as intracellular neurofibrillary tangles composed of hyperphosphorylated versions of tau, a microtubule accessory protein. Parenchymal amyloid lesions adopt different morphological characteristics presenting as dense core neuritic plaques or diffuse deposits[1]. Irrespective of their dissimilar morphology, both lesions are populated by relatively short A β peptides cleaved from the much larger membrane spanning amyloid precursor protein (APP)[2]. Whereas compact plaques and vascular deposits are rich in β -sheet structures responsible for their characteristic green birefringence under polarized light after Congo red staining, and exhibit fibrillar appearance under electron microscopy (EM), diffuse plaques are non-fibrillar and Congo red negative, likely representing early phases in the development of compact plaques[3].

Multiple lines of investigation implicate amyloid peptides in the pathogenesis of Alzheimer's disease. Individuals with Down's syndrome – trisomy 21 harbor similar lesions and develop early AD-like pathology, typically by middle age[3]. Furthermore, familial mutations in the APP gene in the long arm of chromosome 21 are associated with early onset hereditary forms of AD. In addition, animal models overexpressing human APP or transgenic for familial APP mutations exhibit progressive AD-like A β amyloid pathology associated with cognitive deficits[2]. Amyloid peptides are known to be produced by the sequential processing of APP through the action of β - and γ -secretases which result in the generation of A β peptides starting at the aspartate residue at position 1 and ending at amino acids 38/ 40 / 42 [4]. Although, A β_{1-40} and A β_{1-42} have been the most frequently studied species, a wide variety of post-translational modifications – including isomerization and racemization of aspartate residues, cyclization of N-terminal glutamate, and oxidation of methionine, among others – have also been documented.

The post translationally modified peptides also include different N- and C-terminally truncated A β species that have been identified in animal models and AD patients [5,6] and are likely generated by a number of A β -degrading enzymes among them neprilysin, insulin degrading- and endothelin converting-enzymes, plasmin and matrix metalloproteases[7,8]. In this sense, biochemical and mass spectrometry analyses have revealed the presence of a variety of C-terminally truncated A β species in human brain interstitial fluid (ISF) and cerebrospinal fluid (CSF), among them A β_{1-16} , A β_{1-30} , A β_{1-34} , and A β_{1-37} [9–12] likely representing the proteolytic action of local resident enzymes. Similarly, an assortment of A β peptides truncated at the N-terminus have been reported, among them A β_{2-42} , A β_{3-42} , A β_{3pE-42} , A β_{4-42} , A β_{9-42} , and A β_{17-42} [13]. A β truncations severely alter the biophysical properties of the molecule. While C-terminal cleavage of A β generates peptides that are soluble and normal components of body fluids, N-terminal truncated forms, due to their propensity for adopting β -sheet conformations, are prone to self-aggregation, are poorly soluble, and typically exhibit exacerbated cytotoxicity[1,13,14].

One of the most relevant N-terminal truncated forms, A β_{4-42} was first reported over three decades ago[3]. This peptide, preferentially found in dense core plaques and cerebrovascular deposits in AD cases and transgenic animal models [1,3,15] is also highly prevalent in patients with trisomy-21, and vascular dementia[16]. Recent studies indicate that the N-terminal truncation at Phe4 of A β results in greater hydrophobicity and higher β -sheet content of the molecule compared to A β_{1-42} leading to an augmented ability to oligomerize[1]. These biophysical properties of A β_{4-x} forms contrast with those of C-terminally cleaved A β species, generally found in aqueous-based biological fluids, which typically exhibit higher solubility and less aggregation propensity than the parent full-length peptides. Mounting evidence indicate a critical role of the lipid microenvironment in the molecular pathways affecting amyloid aggregation. In this sense, the composition of the different cellular membranes including the type of lipid molecules and cholesterol content [17,18] are known to play important roles in the oligomerization and aggregation fates of amyloid peptides. Particularly, the presence of GM1 gangliosides has been shown to increase amyloid aggregation by forming ganglioside-peptide complexes with capacity to act as amyloid seeds [19,20]. Recent NMR based studies have also explored geometric factors such as bilayer thickness in modulating binding affinity of A β_{1-40} with the membrane and propensity of oligomerization by folding into an intermediate, partially folded helical filament, which maybe a crucial step in the aggregation cascade [21,22]. Notably, *in-vitro* NMR studies of A β_{1-40} with lipid nanodiscs have reported an inhibition of fibrillation as a consequence of the trapping of aggregation intermediates, indicative of a potential use for future therapeutic avenues [23,24].

In spite of extensive studies focusing on A β , the relevance of the N- and C-terminal truncated species for AD pathogenesis as well as their contribution to the complex cellular mechanisms affected during the disease remain largely understudied. Among the most relevant pathogenic pathways under investigation, the insertion of channel structures in cellular and intracellular membranes, a two-step insertion mechanism, is certain to cause alterations in membrane potential and leakage of critical cellular ions such as calcium and potassium, ultimately damaging cellular homeostasis and survival[25–28]. The present work examines the ability of A β_{4-42} to form ion channels in lipid membranes using both

electrophysiology and AFM approaches, further demonstrating that additional C-terminal truncation of Phe4 starting peptides results in highly unstable pore structures. Overall, the current studies provide insight into the detrimental pathogenic mechanisms of N-terminally truncated A β proteoforms, contribute to a more comprehensive understanding of the three dimensional structure of A β aggregates – both in solution and in the membrane environment– and expand existing knowledge on the significance of A β heterogeneity for disease pathophysiology. All these features provide new and unique ways to design and develop therapeutic molecules targeting the aggregation and oligomerization pathways and/or blocking channel activity[29,30].

Materials and Methods

A β Peptides: synthesis and structural changes

Peptides A β _{4–42} (FRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA) and A β _{4–34} (FRHDSGYEVHHQKLVFFAEDVGSNKGAIIGL) were synthesized at ERI Amyloid Laboratory (Oxford, CT) using *N-tert*-butyloxycarbonyl chemistry. Peptides were purified by reverse phase high performance liquid chromatography using a Vydac C4 column (Western Analytical, Murrieta, CA). Molecular masses and purity of the synthetic peptides were corroborated by matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry, as previously reported [31].

Monodisperse amyloid subunit of both peptides were obtained by dissolving 1mg/ml of peptides in 1,1,1,3,3,3, hexafluoro-isopropanol (HFIP; Sigma Chemical Co., St. Louis, MO) and subsequent incubation for 4 days, which breaks down β -sheet structures and disrupts hydrophobic forces, promoting the formation of stable α -helical secondary structures [32–34]. HFIP-pretreated peptides were lyophilized and maintained frozen at –80°C, storage conditions that in our hands preserved the same unaggregated structures for up to one month, as verified by the predominance of monomeric components in Western blots and negligible fluorescence signal after thioflavin T binding.

Atomic Force Microscopy: sample preparation and imaging conditions

Proteoliposome preparation has been included in supplementary information. For AFM sample preparation, 10–20 μ l of sonicated proteoliposome solution was added to a freshly cleaved muscovite mica disk and further diluted to 50 μ l by adding HEPES/KCl/MgCl₂ buffer. The solution was incubated for 10–15 mins at room temperature on the mica disk, washed thrice, and rehydrated to 50 μ l using imaging buffer (HEPES, pH 7.4). A Bruker Multimode equipped with Nanoscope V controller, EV scanner and fluid cell was used for all AFM imaging studies (Bruker, Santa Barbara, CA). Bruker SNL-20 (silicon nitride cantilever, Al coated, k=0.08N/m, fr~8kHz in buffer or 0.24N/m, fr~32kHz in buffer) or Mikromasch XNC12 (silicon nitride tip, Au coated, k=0.08N/m, fr~7kHz in buffer) were used (Mikromasch, USA). Contact and Tapping (according to convenience and imaging stability) mode imaging was performed in 10mM HEPES at scan rates of 1–2Hz. Scanning was usually started with a larger scan size (10 μ m x 10 μ m) and then gradually reduced to smaller sizes depending upon area of interest (1 μ m x 1 μ m, 500nm x 500nm etc.). Setpoint voltage and feedback gains were optimized, and height and phase images collected. The

resulting images were processed with the Nanoscope Analysis 1.9 software provided by Bruker. The height images were first flattened (0th, 1st or 2nd order) and section analysis feature was used to extract cross sections of different regions feature heights.

Electrophysiology

POPE (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine), and POPG (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-1'-*rac*-glycerol), purchased from Avanti Polar Lipids and stored at -20°C , were dissolved in *n*-heptane to a final concentration of 1.5%. A β peptides were reconstituted in deionized water to the concentrations illustrated in Figure 4 whereas anti-A β_{4-x} monoclonal antibody 18H6 [1] was diluted to the desired concentration in deionized water and stored at -20°C .

Phospholipid bilayer membranes were prepared as previously described [35]. Briefly, bilayer membranes were prepared by placing a bubble of lipid dissolved in solvent onto the end of the Teflon tube of approximately 300 μm in diameter. The chamber design allows rapid introduction of solution into immediate proximity with the membrane in a volume of 50 μl . One percent agar salt bridges with 1 M KCl were used to connect the Ag/AgCl electrodes (E-207, Warner Instruments) to the solutions. Voltage clamp conditions were employed in all experiments. The side where peptides were added (*cis* side) was taken as ground. All voltages given in Figure 3 refer to the voltage of the *trans* side. Current was recorded with an "Axopatch C1" amplifier and stored by "DataTrax" system for later analysis. Membrane capacitance and resistance were monitored regularly to ensure the integrity of the membranes.

Results

Structural properties of A β_{4-42} and A β_{4-34} truncated peptides

Figure 1A illustrates the purity of the synthetic peptides used in our studies. In each case, MALDI-TOF analysis revealed the presence of a single component with a m/z value well within the range of the expected theoretical mass. The structural changes of the A β_{4-42} and A β_{4-34} synthetic homologues, pretreated, solubilized, and aggregated as described in Methods, was analyzed by CD spectroscopy, thioflavin T-binding, and electron microscopy analysis over a time-lapse of 2h. Consistent with previous findings [1], the highly fibrillogenic A β_{4-42} (Figure 1B), showed upon 2h incubation under physiological salt concentrations, a CD spectrum with a typical minimum at 218 nm, indicative of β -sheet secondary structures. In contrast, A β_{4-34} , bearing an additional C-terminal truncation display a primarily random conformation in the experimental timeframe. Thioflavin T binding, largely associated with the presence of cross β -sheet structures and typically correlating with the existence of fibrillar and/or protofibrillar components [36], also varied between both peptides. In accordance with its tendency to form β -sheet structures, A β_{4-42} exhibited much higher fluorescence values than the freshly solubilized peptide after 2h incubation (Figure 1C). Further C-terminal truncation at position 34 completely abolished the fluorescence signal, in agreement with the absence of changes in the CD profile. Figure 1 (panels D and E), illustrates the characteristics of the A β_{4-x} truncated species visualized by EM after uranyl acetate negative staining. The formation of oligomeric structures defined as

elements of < 100 nm in length with longer assemblies classified as protofibrils was evident for the 2h incubated A β ₄₋₄₂ (Figure. 1D). The C-terminally truncated A β ₄₋₃₄, displayed a lower capacity to form oligomeric species within the same time frame (Figure. 1E). The above data clearly indicates that the N-terminal truncation at position 4 induces formation of high β -sheet content elements and concomitant generation of oligomeric assemblies, all characteristics associated with pro-amyloidogenic properties. In contrast, the additional C-terminal truncation at position 34 leads to the generation of peptides less prone to oligomerization, suggestive of molecules with more accelerated clearance potential, in agreement with the reported tissue retrieval of C-terminally cleaved derivatives in water-based buffers and their presence in biological fluids[1].

A β ₄₋₄₂ and A β ₄₋₃₄ show pore-like structures in lipid bilayer

AFM images of different concentrations of freshly prepared A β ₄₋₄₂ and A β ₄₋₃₄ (outlined in methods) on mica showed densely populated clusters of different diameter distributions; ~5nm for smaller oligomeric structures, ~5nm-20nm for intermediate aggregates and >20nm for larger irregular aggregates (Figure 2A and 2B). Significantly, A β ₄₋₃₄ formed oligomers at a higher propensity (Fig 2B), and the peptides were closely packed compared to A β ₄₋₄₂ (Figure 2A). This is consistent with the difference in the number of membrane-spanning amino acids (8 amino acids for A β ₄₋₃₄ vs 16 amino acids for A β ₄₋₄₂). In general, the size of the globular A β ₄₋₄₂ (Figure 2A) was larger than the size of the globular A β ₄₋₃₄ (Figure 2B).

AFM images of A β ₄₋₄₂ and A β ₄₋₃₄ when reconstituted in a DOPC bilayer show multiple different forms of oligomeric species of different sizes present in the bilayer membrane as well as some clustered together around membrane edges and defects (Figure 3A for A β ₄₋₄₂ and 3B for A β ₄₋₃₄). At a lower molar ratio (1:1000) of A β ₄₋₄₂:DOPC, much lesser membrane disruption was observed, and oligomeric species were easier to detect and differentiate from DOPC bilayer over the mica substrate. High resolution AFM images of A β ₄₋₄₂ oligomers interacting with DOPC membrane reveal annular pore-like structures with outer diameter 12–16 nm (+/-2.3 nm, accounting for tip convolution) with a central dip, indicative of a peptide pore in the membrane. These are very similar to A β _{PE3-42} pore-like structures reported previously [37,38] (Figure 3C and inset attached with C). Similar structural clusters of diameter 14–18.5 nm with a pore-like morphology are observed for A β ₄₋₃₄ reconstituted with DOPC bilayer, although a clear dip in the pore structure was not resolvable (Figure 3D and inset attached with D). The height protrusion of these pore-like structures above the DOPC planar surface is found to be about 0.9~1.2 nm. These pore-like structures were found to be distributed throughout the sample with varying spatial and number density.

A β ₄₋₄₂ forms a stable and long-lived ion channel in lipid bilayer

Figure 4A shows the results of an experiment where A β ₄₋₄₂ was added to the aqueous phase bordering a phospholipid membrane. The horizontal traces represent current passing through the membrane as a function of time under voltage clamp conditions. The current was extremely close to zero prior to the addition of peptide. After the addition of peptide (first arrow), there is a lag phase, and then the current begins to rise in a stepwise manner

ultimately resulting in a large (negative) and stable current through the membrane representing the insertion of multiple ion channels in the membrane. The current increase is represented by a downward deflection of the current trace, indicating that the current is in the negative direction, as a negative voltage was applied to the membrane. When the voltage is reversed to a positive voltage, the current direction reverses, but is approximately equal in magnitude, indicating the channels do not turn on or off with voltage. These channels appear to have significant stability over time and relatively long lifetimes comparable to those exhibited by A β _{1–42} [39]. At the point indicated by the second arrow, zinc chloride was added to the solution. A short time later the magnitude of the negative current was reduced by the addition of zinc, similar to the previous findings of zinc blockade of A β _{1–42} [40].

A β _{4–34} forms unstable and short-lived ion channels

Figure 4B shows the results of a lipid bilayer experiment where peptide A β _{4–34} was added to the solution bathing the membrane on one side. In the initial phase of the experiment the current passing through the membrane is close to zero reflecting the very low permeability to ions of the unmodified phospholipid membrane. After the addition of A β _{4–34}, there is a lag phase followed by the appearance of spontaneous fluctuations in the current. These fluctuations represent the insertion and opening of ion channels in the membrane. These channels appear to have very poor stability and short lifetimes (< 1 sec). The channels flicker rapidly open and closed, and do not exhibit the more typical stability of full length A β peptides (1–40 and 1–42)[41].

Monoclonal 18H6, specific to the N-terminal truncation at A β Phe4, blocks activity of A β _{4–42} ion channels

Figure 4C shows an experiment where A β _{4–42} was added to the solution bounding the membrane. After a lag there is a significant increase in the membrane current represented by a downward deflection as the voltage at that time across the membrane was a negative voltage therefore resulting in a negative or downward current. Monoclonal antibody 18H6, specific for N-terminal truncated peptides, was added to the solution bounding the membrane (arrow). This resulted in a rapid reduction of the magnitude of the negative current indicating partial blockade of the channel by the antibody. This suggests not only that the current induced in the membrane was specific to A β _{4–42}, but also that the N-terminal epitope that recognized the antibody was still accessible to the side of the membrane to which the peptide was added. That is, A β _{4–42} is oriented in the membrane with its N-terminus facing the side to which it has been added.

Discussion

N-terminally truncated peptide A β _{4–42}, a major fibrillar component of parenchymal plaques and vascular deposits, has a high tendency to adopt β -sheet conformations and lead to the rapid formation of oligomeric assemblies, structures that are considered today the most pathogenic amyloid species associated with the development and progression of AD. Indeed, previous work indicates that this truncated form exhibits even higher aggregation/oligomerization tendency than the parent full-length A β _{1–42} molecule itself and is an invariable component of the plaque cores [1,3]. Also consistent with previous reports

demonstrating the protective effect of C-terminal cleavages for A β oligomerization and toxicity, A β_{4-34} lacking the C-terminal portion of A β_{4-42} exhibits a random-coil conformation and very little oligomerization propensity in the current experimental timeframe.

The results presented herein clearly indicate that both A β_{4-42} and A β_{4-34} can differentially form ion permeable channels in phospholipid bilayer membranes. The results are not totally surprising given previous reports of channel formation by peptides containing the full length A β_{1-42} sequence [42,43] or its N-terminal truncated species A β_{3pE-42} [38] and A β_{17-42} [44,45] as well as smaller fragments such as A β_{25-35} [46,47] all of which are capable of forming ion permeable channels, albeit with varying electrophysiological characteristics. What is striking about the results presented here is the marked difference in physiologic properties between the two ion channels tested. A β_{4-42} exhibits channels that appear quite stable in the timeframe of our experiments with lifetimes lasting many seconds to minutes. The conductance induced by these channels is quite large and stable over the time course of our experiments. The conductance also appears to be independent of voltage which is a typical characteristic of full length A β_{1-42} and A β_{1-40} [48,49]. Moreover, A β_{4-42} is blocked by zinc added from the same side as the peptide, sharing this property with A β_{1-42} [48]. In contrast A β_{4-34} forms channels that appear to be unstable during the time course of our experiments and rapidly flicker open and shut. This instability is consistent with the less hydrophobic nature of the C-terminal truncated peptide A β_{4-34} . It seems to be less comfortable in the hydrophobic environment of the membrane and less stable, therefore. Because the insertion of small numbers of ion channels in a planar lipid bilayer membrane is an inherently variable process, we cannot make firm conclusions about the relative channel forming activity of both peptides from the present data. Many factors including lipid and ionic composition, temperature, and molecular crowding, among others, can affect channel activity. Still, the contrast between the two A β variants examined here is striking.

This physiologic evidence is consistent with the AFM images presented here in which A β_{4-42} clearly shows multimeric aggregates in the membrane, clustered around a central pore region. This would be compatible with the large, stable, long lasting conductance (current fluctuations) that we see in the electrophysiological experiments and also consistent with AFM images of similar A β peptides including A β_{1-42} , A β_{pE3-42} , A β_{9-42} and A β_{17-42} [37,44,45,50,51]. In contrast, the AFM images of A β_{4-34} do not show a clearly defined pore structure, suggesting that under the AFM imaging conditions, pore structures are highly unstable as is the case in the electrophysiological experiments. It should also be noted that A β_{4-42} is blocked by zinc in a manner quite similar to that of A β_{1-42} and A β_{1-40} [40,48] implying that the receptor site in the pore for zinc is similar in both peptides.

Notably, the A β_{4-42} channel activity can be specifically blocked by the antibody 18H6 which is specific for A β peptides N-truncated at position 4. This demonstrates that the N-terminus of this peptide is clearly accessible to the aqueous solution surrounding the membrane on the side that the peptide was added to, providing valuable information about the orientation of the A β N-terminus in the functional channel structure. This finding will be extremely useful for further molecular modeling of potential ion channel structures in the lipid membrane.

The results presented here give further weight to the idea that N-terminal truncated peptides that are more prone to aggregation and more toxic to neurons than comparable full length A β species may play a key role in the pathogenesis of Alzheimer's disease. This idea may be critical in the formulation of therapeutic approaches to inhibiting or reducing amyloid deposition in the brain using antibodies or small molecule inhibitors targeted toward N-terminal truncated peptides. We have previously reported on the channels formed by A β_{9-42} and A β_{17-42} [44,45]. These N-terminally truncated peptides do not form amyloid, but are toxic to neurons, allow calcium ions to enter cells, and do aggregate in the presence of membranes, forming ion channels. These features are all inhibited by millimolar zinc ion, indicating that calcium entry and neurotoxicity directly result from ion channel formation, leading us to propose this pathway as an alternative mechanism for AD.

Similarly, we have also studied the structure and biological activity of A β_{pE3-42} , an N-terminal truncated amyloid peptide where after the loss of the first 2 amino acids, the third position glutamate is converted to pyroglutamate via intramolecular dehydration by the enzyme glutamyl cyclase, creating a lactam ring. While glutamate is a charged polar group and typically seeks an aqueous environment, the pyroglutamate is more nonpolar and seeks the hydrophobic environment of the membrane. Pyroglutamate-modified A β represents a dominant fraction of A β oligomers found in AD brains, while control brains show much lower concentrations of pE A β [52]. Plaques formed with pE A β appear earlier in the course of the AD-like illness due to trisomy 21 (Down's syndrome) and transgenic A β_{pE3-42} mice develop an earlier and more severe illness than those with A β_{1-42} [52]. Lipid bilayer experiments showed that A β_{pE3-42} formed channels more rapidly and exhibited a larger fraction of high conductance (100–200 pSiemens) channels compared to A β_{1-42} . [38].

The precise mechanisms leading to the development and progression of AD are complex and multifactorial involving crosstalk among different cellular pathways. Over the years, different lines of investigation have provided support for a central role of A β in the disease pathogenesis [53]. It is now clear that the conformational transition from soluble to fibrillar material is a highly complex process modulated by a variety of factors, among them the presence of post translational modifications in the A β molecule including N- and C-terminal truncations as studied in the current work. The interlinked cellular pathways compromised by A β – many of them shared by those elicited by non-A β oligomeric conformations associated with other forms of cerebral amyloidosis [36,54] – are currently under active investigation. Among the most studied pathways, induction of apoptotic/cell death mechanisms, oxidative stress, metabolic/mitochondrial dysfunction, and the ability of amyloid subunits to assemble into functional ion channel-like structures in lipidic environments are the most relevant [55,56].

Insertion of channel structures in cellular and intracellular membranes has the potential to alter homeostasis and metabolism since cells depend on intact membrane structures for a wide variety of functions. Beta sheet conformers are well-suited to forming pore-like structures in a hydrophobic environment which may account for their widespread presence in three-dimensional amyloid and non-amyloid pores. Cells depend on intact membrane structures for a wider variety of cellular functions. Non-specific ion channels, such as those described by us and other laboratories, insert leakage pathways into membranes that

interfere with their biological function causing dissipation of membrane potential and leakage of critical calcium and potassium ions [27]. Ultimately cells are forced to work harder and use up their energy stores. Ion channel formation and its potential modulation carry vast potential for the development of novel therapeutic strategies. Indeed, in spite of extensive research efforts, disease-modifying therapies that may prevent or slow the rate of AD progression are still lacking. While biochemical, genetic, and longitudinal imaging analyses provide strong support to the role of A β aggregation in initiating disease pathogenesis, the clinical trials as conducted so far have not panned out. Current consensus in the research community indicates that A β -modifying therapies are not beneficial for symptomatic patients and those in the late stage of disease when A β fibrillogenesis and brain deposition is extensive [57]. Targeting ion channel formation and its deleterious effects on cellular function either independently or as part of future combinatorial therapies is certainly a promising alternative to address the cross-talk mechanistic paths involved in the disease. A better understanding of the relevance of A β_{4-42} and other N-terminal truncated fragments will certainly provide insight into the role of A β heterogeneity in the complex mechanisms of Alzheimer's pathophysiology and open new avenues for translational opportunities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1: Biophysical and structural analysis of A β 4-42 and A β 4-34 truncated peptides. The purity of the peptides was assessed via MALDI-TOF (A) performed in a Bruker Daltonics Autoflex MALDI-TOF mass spectrometer (Bremen, Germany) in linear mode using standard instrument settings at the New York University Mass Spectrometry Core for Neuroscience. External calibration was performed using human adrenocorticotrophic hormone peptide 18–39 (average mass = 2465.68 Da) and insulin (average mass = 5733.49 Da). In all cases MS spectra were processed and analyzed by FlexAnalysis (Bruker Daltonics). The different A β homologues, pre-treated in HFIP and reconstituted in

physiologic salt concentration containing buffer, were incubated at 37°C for up to 2 hours. (B) CD spectra of A β ₄₋₄₂ (red line) and A β ₄₋₃₄ (black line) incubated 2h under physiological salt concentrations. Graph is representative of three independent experiments; (C) Fluorescence evaluation of Thioflavin T binding to the respective synthetic homologues (50 μ M) either freshly reconstituted (open bars) or after 2h incubation (patterned bars). Results are expressed in arbitrary units (A.U.) and represent the mean $\pm$ SEM of three independent experiments after subtraction of blank levels. (D) Structural assessment of A β ₄₋₄₂ 2h oligomerization visualized by EM after negative staining. (E) EM analysis of A β ₄₋₃₄ oligomerization after 2h incubation. In both (D) and (E), bar represents 100 nm

Figure 2: AFM images (height and amplitude error) of Aβ₄₋₄₂ and Aβ₄₋₃₄ interaction with DOPC bilayer.

A) Aβ₄₋₄₂ and **B)** Aβ₄₋₃₄ incubated on plane mica surface show distribution of monomers and oligomers. The white horizontal line depicts a section through the scanned area whose corresponding profile is shown below the image (A: scan size = 1.4μm, height scale = 1.9nm; B: scan size = 1.4μm, height scale = 3.2nm).

Figure 3: AFM images of amyloid beta ion channels when reconstituted in lipid bilayer.

A) $A\beta_{4-42}$ reconstituted DOPC bilayer showing clusters of protein distributed (scan size = 3.5 μ m, height scale = 9.5nm). **B)** $A\beta_{4-34}$ reconstituted DOPC bilayer showing clusters of protein distributed (scan size = 1.2 μ m, height scale = 5.6nm). **C.)** $A\beta_{4-42}$ reconstituted DOPC bilayer at low peptide concentration (1:1000) shows intact bilayer with oligomeric peptides (blue dotted circles) in pore-like morphology. The inset in **C)** shows the pore-like structure in isometric view (scan sizes = 1 μ m, inset scan size 50nm, height scale 2.3nm). **D)** Amplitude error (which is proportional to the feedback signal and very sensitive to height changes on the surface) image of $A\beta_{4-34}$ reconstituted DOPC bilayer at low peptide concentration (1:1000) shows intact bilayer with oligomeric peptides (black dotted circles) in pore-like morphology. The inset in **D)** shows the pore-like structure in isometric view. (scan sizes = 650nm, inset scan size = 60nm; height scale = 4.6mV)

Figure 4. Lipid bilayer membrane conductivity initiated by truncated A β peptides.

A) Conductivity initiated by $A\beta_{4-42}$ peptide. **B)** Conductivity initiated by $A\beta_{4-34}$ peptide. **C)** $A\beta_{4-42}$ conductivity inhibition by monoclonal antibody 18H6. In all cases: chamber solution contains 100 mM NaCl, 20 mM Tris-Citrate buffer pH 7.4. Membrane formed from POPE/POPG 2:1 w/w, final concentration of lipids 1.5% in n-heptane. Top lines represent transmembrane currents, bottom lines - holding potential.