VIP Solid-State NMR spectroscopy

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Structural Basis of β-Amyloid-Dependent Synaptic Dysfunctions**

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Aggregation of β-amyloid (Aβ) peptide into oligomers and protofibrils is a hallmark of Alzheimer's disease (AD).[1] Increasing evidence shows that the primary insult in AD is caused by oligomeric species that impair the ordered function of synaptic networks.[1] Consistent with this view, oligomers were shown to affect synaptic plasticity, and they impair the long-term potentiation (LTP) in living brain tissues, a widely used model system of brain memory functions.[1,2] Using solid-state NMR spectroscopy, we here determined the residue-specific molecular conformation of a highly synaptotoxic β-amyloid oligomer structure. Our measurements reveal a stable N-terminal β strand that controls the partitioning between oligomer and protofibril formation, whereas targeting the peptide N-terminus ameliorates Aβ-dependent neuronal dysfunctions.

The presently investigated, chemically well-defined Aβ oligomers faithfully reproduce the hallmark characteristics of AD-related oligomers. Living hippocampal brain slices were exposed to different Aβ conformers (Figure 1 A), and a series of tetanic electrical stimuli were applied to evoke a long-lasting increase of the synaptic transmission, termed LTP. Oligomers, but not freshly dissolved, that is, primarily monomeric, Aβ peptide or fibrils, reduce the LTP response and therefore disturb the brain memory functions within these tissue samples (Figure 1 B). A similar oligomer-specificity is seen with cultured primary neurons, which present a significant oligomer-dependent decrease (−40 %) of their synaptic contacts (Figure 1 C and Figure S1 in the Supporting Information).

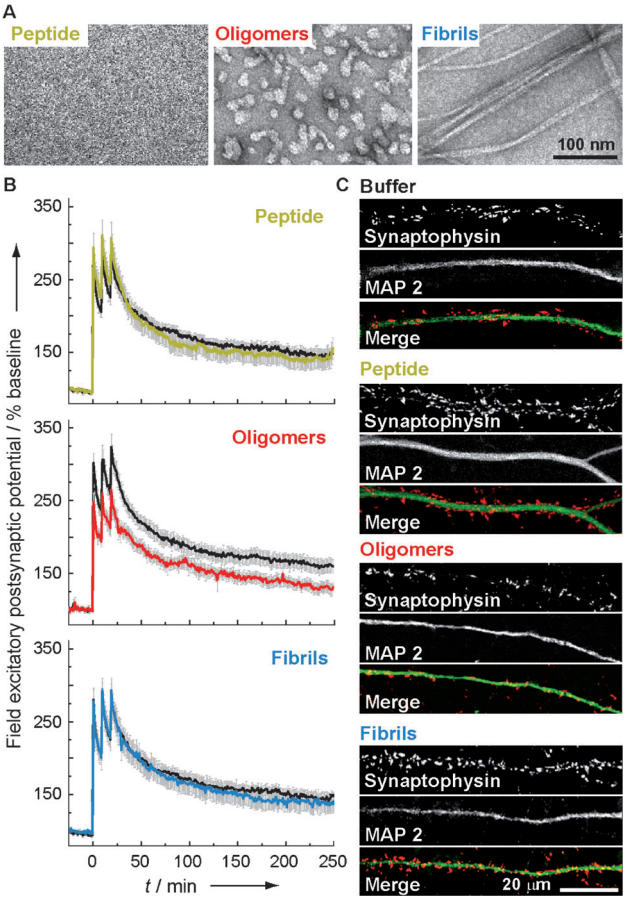

Figure 1. Neuronal dysfunctions induced by oligomers. A) TEM images of different Aβ conformers. B) Oligomers (red) significantly reduce the LTP ($p < 0.01$ analysis of variance, ANOVA, with repeated measures, $n \geq 6$), whereas fibrils (blue) and the freshly dissolved peptide (green) correspond to buffer (black). C) Synaptophysin and MAP2 double-staining of primary neurons after treatment with buffer or Aβ conformers.

The investigated oligomers show diameters from about 10 to 60 nm.[3] Amyloid-specific tracers, such as Thioflavin T or Congo red dye (see Figure S2 in the Supporting Information) and the fibril-specific B10 antibody fragment,[3] provide relatively weak binding to oligomers, whereas 8-anilinonaphthalene-1-sulfonate (ANS) shows strong interactions, indicating hydrophobic and molten globule-like oligomer properties (see Figure S3 in the Supporting Information).

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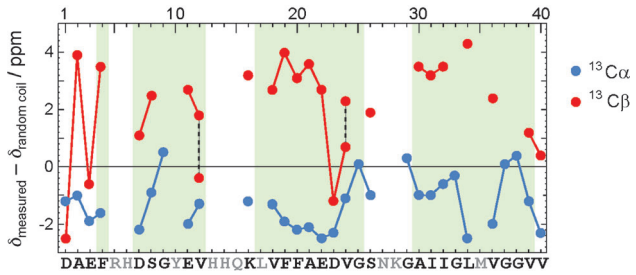

Figure 2. Secondary structure of Aβ oligomers. Measured $^{13}C\alpha$ and $^{13}C\beta$ chemical shifts δ plotted as deviations from random coil values. Green boxes: TALOS+ predictions of β-strand geometry. Gray: residue unlabeled. Dotted lines: two δ values observed.

Oligomers are unstable in fully aqueous solutions and begin to convert into amyloid fibrils within two days (see Figure S4 in the Supporting Information). Addition of small quantities of an alcoholic co-solvent prevents this process, retaining the oligomeric morphology for more than 40 days, as shown by negative-stain transmission electron microscopy (TEM; see Figure S4 in the Supporting Information). These effects might depend on a stabilization of hydrophobic surfaces, related to naturally occurring lipid–oligomer interactions,[4] or on a strengthening of the hydrogen bond interactions caused by the organic co-solvent. They further enabled magic angle spinning solid-state NMR (MAS-NMR) spectroscopy studies with fully solvated oligomers to identify their underlying secondary structure elements.

A set of eight peptides was obtained from solid-phase chemical synthesis, each carrying selected residues that were fully ^{13}C and ^{15}N labelled (see Figure S5 in the Supporting Information). Our measurements probed a total of 75% of the residues of the peptide, covering virtually all structural regions. The recorded ^{13}C MAS-NMR correlation spectra contain well-resolved resonances (see Figure S6 in the Supporting Information), though in several cases associated with a significant line width. These properties imply the existence of multiple microscopic conformations around a preferred peptide fold, enabling structural assignments. Comparison of the observed ^{13}C chemical shifts δ with random coil values, combined with prediction of the backbone ϕ/ψ angles using the TALOS+ algorithm, identified β-strand structure in several regions of the peptide. These regions include, at least, residues 4, 7–12, 17–26, and 30–39 (Figure 2).

Our data are consistent with previous far-ultraviolet circular dichroism (far-UV CD) measurements in showing β-strand conformation.[3] Additionally, infrared spectroscopy suggested elevated levels of antiparallel β-sheet structure compared with fibrils.[3,5] While the β-strand structure at residues 17–26 and 30–39 roughly correlates with previous reports of β-sheet conformation in Aβ fibrils (see Figure S7 in the Supporting Information) and oligomers,[6–9] observation of an N-terminal β strand was highly surprising as this region is usually assumed to be unstructured.[6–9]

We thus used proline mutagenesis to probe the structural relevance of N-terminal residues. Proline residues are highly disruptive to β-sheet structure, and we inserted these residues at three N-terminal positions (2, 4, and 8, Figure 3A). At all three sites, mutagenesis does not affect the isoelectric point. Far-UV CD and ANS fluorescence detect only small, if any, structural perturbations of the oligomeric assembly with Ala2Pro (see Figure S8 in the Supporting Information), but single-particle quantification by TEM indicates a slightly enhanced formation of elongated aggregates, such as nascent protofibrils (Figure 3B). This trend is much more pronounced when mutation affected residues 4 or 8, consistent with their more central positions within the β-strand region. Both mutations significantly increase the fraction of elongated aggregates (Figures 3B and 3D), indicating that disrupting the N-terminal β strand favors protofibrils relative to oligomers.

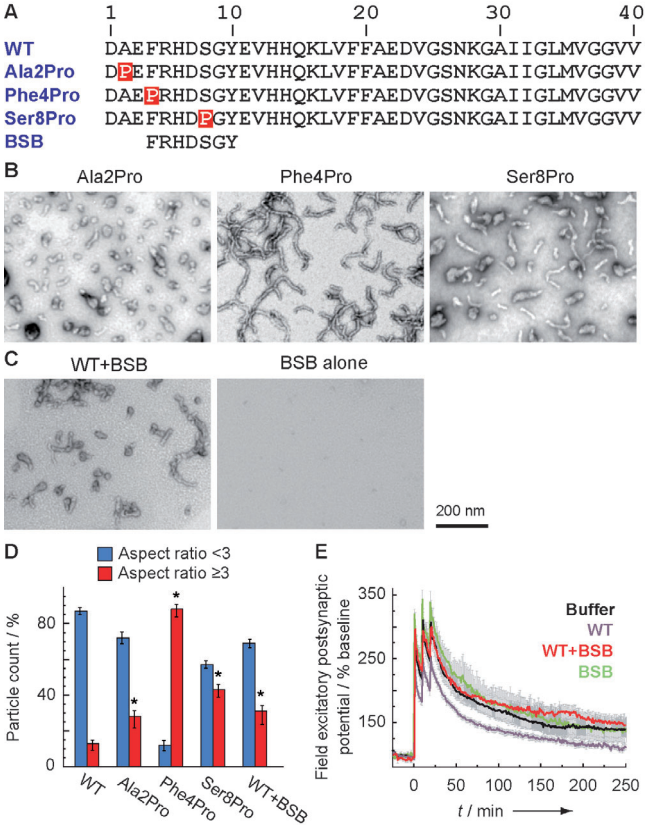

Figure 3. Targeting the peptide N-terminus by interference. A) Amino acid sequences of wild-type (WT) and mutant Aβ (Ala2Pro, Phe4Pro and Ser8Pro) as well as of the β-sheet breaker (BSB) peptide. B) TEM image of aggregates derived from Ala2Pro, Phe4Pro, and Ser8Pro. C) TEM images of Aβ + BSB and BSB alone, showing aggregates only in the Aβ-containing sample. Aggregates with BSB are more elongated than in WT only (Figure 1A). To test the BSB peptide, stock solutions of Aβ(1–40) (500 μM) and BSB peptide (5 mM) were prepared in 100% hexafluoroisopropanol (HFIP). Equal volumes (20 μL) from each stock were mixed (40 μL) and subsequently diluted ten-fold into 360 μL water. The solution was further processed as described in the standard oligomer preparation protocol. D) Quantitative TEM analysis from data in (C) shows more elongated particles (length/width = aspect ratio ≥ 3) upon mutation or BSB peptide addition to wild-type Aβ. Significance (*, $p = 0.05$, $n = 3$) established with a two-sided unpaired *t*-test. E) LTP measurements with Aβ oligomers (blue), buffer (black), BSB peptide (green), and BSB + Aβ (red).

Interestingly, significant literature evidence has also argued that Aβ protofibrils do not possess a stable N-terminus. Published $^{13}C\alpha$ and $^{13}C\beta$ chemical shifts were random coil-like for residues 1–12,[6] and N-terminal mutagenesis was negligibly destabilizing to protofibrils and favored protofibrils over mature fibrils.[10,11] To further test the structural role of the N-terminus we used the β-sheet breaker (BSB) approach. This methodology uses peptide fragments from β-strand segments to block full-length peptide assembly.[12]

We find that addition of a BSB peptide corresponding to residues 4–10 of Aβ does not entirely block the assembly of peptides; instead, it increases the yield of elongated, protofibril-like aggregates (Figure 3C,D). No such structures are seen without Aβ, showing that a full-length peptide is required to construct these aggregates (Figure 3C). Collectively, BSB and peptide mutagenesis show that the N-terminus controls the partitioning between oligomer and protofibril formation.

Finally, we used LTP measurements to test the possible consequences of interfering with the N-terminal strand for the biological properties of the investigated oligomers. We find that a mutation-dependent enhancement of protofibril formation does not counteract the LTP disturbances seen with wild-type Aβ oligomers. Phe4Pro protofibrils are equally LTP-effective as wild-type Aβ oligomers (see Figure S9 in the Supporting Information); that is, even structurally polymorphic Aβ aggregates are able to induce neuronal disturbances, which is consistent with previous data.[13,14] By sharp contrast, addition of a BSB peptide to a wild-type Aβ peptide potently neutralizes the LTP activity of the latter when present as oligomers (Figure 3E). Hence, it is possible to ameliorate Aβ-dependent neuronal dysfunctions interference strategies by targeting the peptide N-terminus and without fully blocking all peptide assembly (Figure 3D).

Our observations are highly compatible with recent neuropathological data, linking AD to high-molecular weight Aβ oligomers or protofibrils similar to the aggregated states described here.[15] However, disease manifestations are highly variable in humans in terms of clinical presentation and histopathological features.[16] In addition, a plethora of structural Aβ polymorphs has been reported to occur,[17] including much smaller, though still biologically active, structures.[18] Thus, there may be no uniquely pathogenic Aβ conformation with sole responsibility for AD etiology, and multiple structural states or the process of aggregation itself could be required to elicit the full spectrum of Aβ-dependent neuronal disturbances.[19]

The presently documented role of the peptide N-terminus in stabilizing Aβ oligomers argues that this sequence region could be instrumental for AD. Evidence for N-terminal stability in Aβ fibrils or peptide has been provided by biochemical analysis.[20,21] Indeed, LTP-dependent memory deficits can be ameliorated with BSB peptide fragments derived from the Aβ N-terminus. Considerable, though scattered, previous evidence also points towards an implication of N-terminal epitopes within AD. For example, this region has been used for AD immunotherapy,[22] whereas N-terminal pyroglutamate modifications or allelic mutations were shown to promote the onset of AD and Aβ aggregation in vitro,[17] and evidence for N-terminal stability in Aβ fibrils or peptide has been provided by biochemical analysis, cryo electron microscopy and molecular modelling.[23,24] We therefore suggest that addressing the peptide N-terminus with suitable molecules could represent an attractive strategy for targeted intervention.[25]

Experimental Section

The oligomers were prepared by dissolving Aβ(1–40) peptide at 2.5 mg mL^{-1} concentration in 100% 1,1,1,3,3,3-hexafluoroisopropanol and incubated (15 min, room temperature). The solution was then diluted ten-fold with water, and after further incubation (15 min, room temperature) large aggregates were removed by centrifugation (14000g, 15 min). Neuronal dysfunction measurements were carried out as described,[2] ^{13}C chemical shifts were assigned based on two-dimensional ^{13}C–^{13}C correlation MAS-NMR spectroscopy.

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