p3 peptide, a truncated form of Aβ devoid of synaptotoxic effect, does not assemble into soluble oligomers

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Abstract In previously proposed models of Aβ soluble oligomers, the N-terminal domain $A\beta_{1-16}$, which is missing in p3 peptides, protects the hydrophobic core of the oligomers from the solvent. Without this N-terminal part, oligomers of p3 peptides would likely expose hydrophobic residues to water and would consequently be less stable. We thus suggest, based on theoretical and experimental results, that p3 peptides would have a low propensity to assemble into stable oligomers, evolving then directly to fibrillar aggregates. These properties may explain why p3 would be devoid of any impact on synaptic function and moreover, strengthen the hypothesis that Aβ oligomers are the principal synaptotoxic forms of Aβ peptides in Alzheimer disease.
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1. Introduction

Senile plaques, containing amyloid β peptides 1–40 and 1–42 (noted $A\beta_{1-40}$ and $A\beta_{1-42}$), characterize Alzheimer's disease (AD) pathology. In addition to Aβ peptides, plaques also contain amino-terminal truncated Aβ peptides, including 3-kDa fragments termed p3 peptide (corresponding to residues $A\beta_{17-40}$ and $A\beta_{17-42}$). p3 peptides are derived from the so-called "non-amyloidogenic" pathway of the amyloid precursor proteins (APP) processing, by proteolytic activities of α- and γ-secretases [1,2].

Experiments have shown that fibrillar Aβ is composed of cross-β structures [3–6]. Aβ fibril formation is preceded by various intermediates, water-soluble oligomeric states, and protofibrils peptide forms [7–11], which are suggested by experimental and clinical findings to be particularly important for the pathogenesis of AD [8,12]. Potential forms of these stable oligomeric soluble intermediates of Aβ have been recently modelled by homology with proteins sharing sequence similarities with the Aβ peptide [13,14].

Very few data on the structures of p3 are available in the literature. Dickson and colleagues [15] reported that p3 may represent a benign form of amyloid, since it lacks domains associated with activation and recruitment of glia to senile plaques. Higgins and colleagues [16] showed that p3 presents all of the structural determinants required for fibril assembly. Furthermore, Pike and colleagues [17] observed in vitro that the p3 fragment can adopt a β-sheet conformation and that amino-terminal deletions of Aβ peptide 1–16 enhance aggregation of amyloid-β peptides. However, the importance of p3 peptide accumulation in Alzheimer disease and its toxic properties are still not clear. p3 peptide can assemble into fibrils (for which no specific models or structure are available) but, to our knowledge, no oligomeric forms of p3 peptide have been identified.

In this study, we evaluate the effect of a N-terminal truncation, leading to the p3 peptide, on the structure and stability of models of Aβ monomers and oligomeric soluble Aβ assemblies, proposed in a previous work [13]. The propensity of p3 to form oligomeric intermediates was otherwise tested under the same experimental conditions where Aβ peptides form soluble oligomers.

2. Materials and methods

2.1. Molecular modelling

The coordinates of the three Aβ models were retrieved from a previous work [13]. The construction of the p3-derived models was performed using Swiss-PDB Viewer [18]. Models have been minimized to energetic convergence using GROMOS96 through the Swiss-PDB Viewer software [18]. This software has also been used to evaluate the internal energy of each model. The solvent-exposed surface has been calculated through the ASA program [19].

2.2. Experimental materials

Human recombinant $A\beta_{1-42}$ was bought from Bachem (Weil am Rhein, Germany) and p3 peptide (corresponding to $A\beta_{17-42}$) was bought from Anachem (Luton, United Kingdom). For the Western blot analysis of Aβ configuration, we used two mouse monoclonal antibodies: 6E10, an antibody raised against residues 1–16 or 4G8 raised against residues 17–24 of Aβ. 6E10 and 4G8 and all the other chemicals were obtained from Sigma (L'Isle d'Abeau, France).

2.3. $A\beta_{1-42}$ and p3 oligomerization and fibrillogenesis

We used protocols of solubilisation of Aβ and p3 described by Stine et al. [20] that selectively induce oligomers or fibrils formation of Aβ.

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In brief, recombinant $A\beta_{1-42}$ or p3 ($A\beta_{17-42}$) peptides were resuspended in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) to disrupt pre-existent peptide aggregates [20]. The clear solution containing dissolved peptide was then aliquoted in microcentrifuge tubes. The HFIP was then allowed to evaporate in a fume hood and resulted peptide films were stored at −80 °C. Immediately prior to use, HFIP-treated aliquots were carefully and completely resuspended to 5 mM in anhydrous dimethyl sulfoxide (DMSO) by pipette mixing and vortexing [21]. Oligomers were prepared by diluting peptides to 100 µM in ice-cold HEPES- and bicarbonates-buffered saline solution (HBBSS) with immediate vortexing and sonication. Peptides were then incubated at 4 °C for 24 h on a stirring machine. Fibrils were prepared by diluting peptides to 100 µM in 10 mM HCl prepared in ultrapure water with immediate vortexing and incubation at 37 °C for 72 h on a stirring machine.

2.4. Western blot analysis of SDS–PAGE

Gel electrophoresis and Western blot analysis were performed according to the manufacturer's protocols (Invitrogen). Samples were diluted in the NuPAGE® LDS sample buffer and applied on a 4–12% NuPAGE® bis-Tris SDS–PAGE gels. Electrophoresis was performed using MES running buffer and transferred to polyvinylidene difluoride membranes (Polyscreen® membrane, Perkin–Elmer, Paris, France). Membranes were blocked in Tween 20, 0.1%-Tris 200 mM buffered solution (TTBS) complemented with 5% bovine serum albumin (BSA) during 1 h and blots were incubated at 4 °C overnight under gentle agitation with the indicated antibody (1:1000). Incubation with the secondary peroxidase-conjugated anti-rabbit (1:50 000) antibodies was performed during 1 h at room temperature. Finally, blots were detected with an enhanced chemiluminescence Western blotting

detection system (Western Lightning™ Chemiluminescence Reagent Plus, Perkin–Elmer). Molecular weight values were estimated using the Seeblue® Plus2 pre-stained standard molecular weight marker (Invitrogen).

3. Results

3.1. Description of the Aβ soluble oligomeric models

In our previous work [13], we have constructed three structural models for $A\beta_{1-42}$ soluble oligomers on the basis of sequence similarity with fragments of three functionally unrelated proteins: the DraE adhesin, the serine protease module of Semliki Forest virus (SFVP) and the transthyretin (TTR). These potential structurally similar fragments have been identified by searching in the Protein Data Bank (PDB) [22] for sequence similarities that could be validated at the two-dimensional level using the "Hydrophobic Cluster Analysis" (HCA) method [23]. The three monomeric models were constructed on the basis of the alignment of each template with the sequence of the Aβ peptide and using MODELLER [24]. The adhesin-based model, extracted from an immunoglobulin-like domain, is composed of four antiparallel β-strands forming two two-stranded β-sheets (Fig. 1, 1A). The SFVP-based model, extracted from a β-barrel, displays four β-strands (Fig. 1, 2A). The TTR-based model consists in three main β-
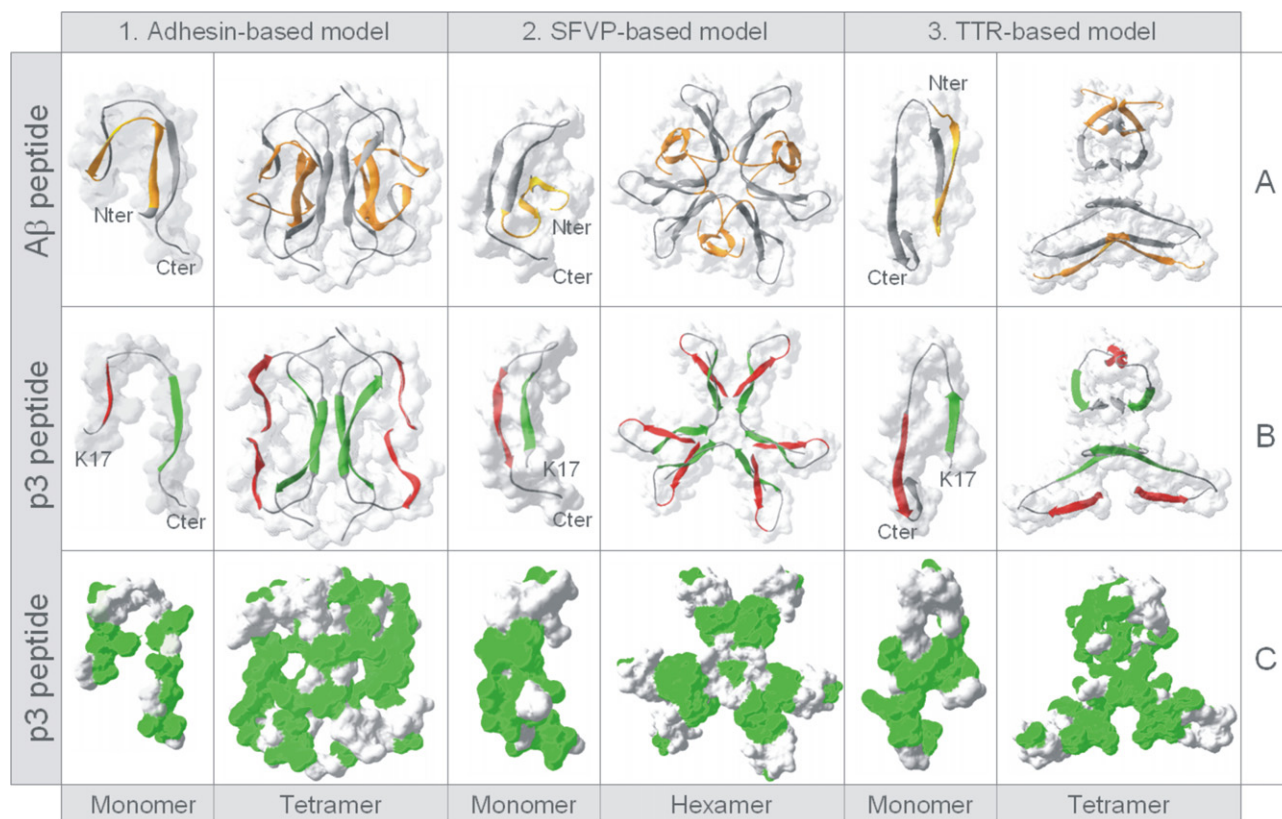

Fig. 1. Molecular models of Aβ and p3 soluble oligomers. (A) Ribbon diagrams of the adhesin-based (1) monomeric and tetrameric, SFVP-based (2) monomeric and hexameric and TTR-based (3) monomeric and tetrameric Aβ models. The N-terminal domain with one (TTR-based model) or two β-strands (adhesin and SFVP-based models), which is truncated in p3, is colored in orange and the C-terminal domain is colored in grey. (B) Ribbon diagrams of the adhesin-based (1) monomeric and tetrameric, SFVP-based (2) monomeric and hexameric and TTR-based (3) monomeric and tetrameric p3 models. The two β-strands which compose the β-hairpin are colored in red (central β-strand) and in green (C-terminal β-strand). (C) Molecular surface of the p3 monomeric and oligomeric models with the surface corresponding to hydrophobic residues (VILFMYW) colored in green. This figure was made using Swiss-PDB-Viewer.

strands and a small C-terminal β-strand which forms a short β-sheet with its neighbour β-strand (Fig. 1, 3A). These Aβ models share a similar structure in the C-terminal region forming a β-hairpin composed of two β-strands (called the central and the C-terminal ones), which are connected by a loop. This β-hairpin is stabilized by a salt bridge between Asp23 and Lys28 which also exists in the fibrillar form of the Aβ peptide [4]. This conformation, which would form the stable core of the Aβ monomer, is relatively close to the Aβ structure core elements observed within Aβ fibrils and these two β-strands have been shown to be key determinants for fibrillogenesis [25–27]. The N-terminal part of Aβ (orange in Fig. 1, line A), which is different in the three models, is modelled by two β-strands in the adhesin and SFVP-based models and by one β-strand in the TTR-based model. These monomeric models of Aβ expose hydrophobic patches to the solvent, which make them thermodynamically unfavourable entities. In order to bury most of these hydrophobic amino acids, we constructed more stable oligomers (dimer/tetramer and trimer/hexamer for the adhesin and SFVP-based models, respectively). As the TTR template fragment used for the Aβ modelling is implicated in the formation of dimer and tetramer in TTR, we considered these native oligomers of TTR to construct dimer and tetramer of the Aβ peptide. In these three models, the β-hairpin constitutes the hydrophobic core of the oligomers which is always protected by the hydrophilic N-terminal domain of the Aβ peptide. These water-soluble oligomeric models might sample the possible conformations of the experimentally observed multimeric Aβ assemblies [13].

3.2. Effect of a N-terminal truncation on the Aβ models, leading to p3 peptide

The effect of the lack of the N-terminal segment in p3 relative to Aβ on the structure and stability of the peptide was considered by truncating the N-terminal domain (residues 1–16) of the $A\beta_{1-42}$ monomeric models (orange in Fig. 1, line A). Following these constructions, p3 peptides would be limited to the β-hairpin (red and green in Fig. 1, line B), which is well conserved between the three proposed Aβ soluble models. This β-hairpin exposes many hydrophobic residues (in green on monomers surface in Fig. 1, line C) with only one hydrophilic patch on the D23–K28 loop.

To construct p3 oligomers, we assembled monomers in various ways and try to burry most of the hydrophobic amino acids. Whatever assemblies we built, the resulting oligomers always exposed to solvent the hydrophobic β-hairpin core, and present a high positive energy. In order to evaluate the stability of p3 oligomeric forms compared to those of Aβ, we have built the p3 models (Fig. 1, line B) based on the tetrameric and hexameric models of Aβ (Fig. 1, line A). The absence of the N-terminal segment of Aβ, which protects the hydrophobic core of

the Aβ oligomers from solvent, makes these Aβ-based oligomeric models of p3 unstable (tetramers and hexamers in Fig. 1, line C).

We calculated the final minimized energies of the oligomeric forms of Aβ and p3 (Table 1). The p3 oligomeric models are all characterized by a positive energy, whereas all Aβ models present a strong negative one. Then, we compared the proportion of hydrophobic residues accessible to solvent (colored in green in Fig. 1, line C) in p3 and Aβ models. The solvent-exposed hydrophobic surfaces increased in the p3 peptide compared to the Aβ oligomeric forms from ~20% to 60% (Table 1) and the buried surface areas within the oligomers are logically smaller in p3 compared to Aβ (from 30 to 50%; Table 1).

In conclusion, the estimated energy of the Aβ-based oligomeric models of p3 is always positive, the models expose hydrophobic patches to the solvent and bury a small proportion of their accessible surface within the oligomeric intermediates, which render their existence thermodynamically unfavourable. We thus hypothesized that the p3 peptide cannot form stable soluble oligomers in the same way as Aβ does.

3.3. Experience shows that p3 peptides do not form oligomeric intermediates but mainly fibrils

To validate this hypothesis, we performed Western blot analysis on the different human peptides $A\beta_{1-42}$ or p3 incubated in either oligomers-forming conditions during 24 h at 4 °C in a solution with physiologic pH and ionic strength or in fibrils-forming conditions during 72 h at 37 °C in 10 mM HCl [20]. Aβ oligomers appear to be relatively stable structures, as SDS-resistant species have been isolated from post-mortem human brains and cell lines [28,29]. We determined whether SDS-resistant Aβ oligomers could be identified and resolved by SDS–PAGE and Western blotting from these experimental solutions. The Immunoblots were incubated with Aβ monoclonal antibodies 6E10, recognizing Aβ 1–16 residues and 4G8, recognizing Aβ 17–24 residues.

In oligomers-forming conditions, both primary antibodies revealed the presence of monomers, dimers, trimers and tetramers of $A\beta_{1-42}$ (Fig. 2, lanes 1 and 3) and high molecular weight oligomers (especially with the primary antibody 6E10). In fibrils-forming conditions, we observed the presence of monomers and dimers (with 4G8) but no tetramers. A fainted signal revealed a moderate presence of high molecular weight oligomers, while most of $A\beta_{1-42}$ were converted in high molecular weight fibrils that remained in the well.

p3 peptide blots revealed with the primary antibody 6E10, recognizing Aβ1–16 residues, did not show any signal as expected (data not shown). With 4G8, synthetic human p3 peptide exposed to oligomers or fibrils-forming conditions exhibits a very similar electrophoretic migration profile showing only monomers without any low molecular weight

Table 1
Final minimized energies (kJ/mol), percentage of solvent-exposed hydrophobic surfaces and buried surface within oligomers (Å² per monomer) of the three oligomeric models of Aβ and the corresponding p3 oligomeric models, with the percentages of increase of the percentage of solvent-exposed hydrophobic surfaces or reduction of buried surface area between the Aβ and p3 oligomers within brackets

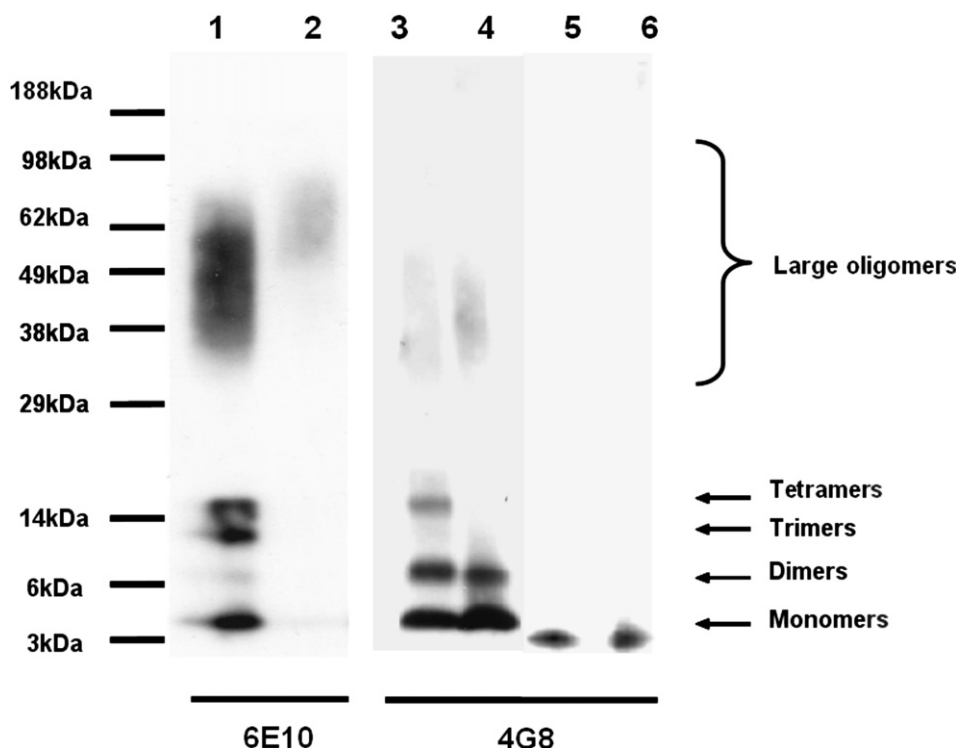|	Energy (kJ/mol)		% Hydrophobic surface		Buried surface (Å²)	
	Aβ models	p3 models	Aβ models	p3 models	Aβ models	p3 models
Adhesin-based	−4553	858	43	53 (+23)	1570	748 (−52%)
SFVP-based	−3583	837	33	54 (+64)	796	538 (−32%)
TTR-based	−3348	512	44	58 (+32)	511	266 (−48%)

Fig. 2. Western blot analysis of human Aβ$_{1-42}$and p3 under oligomers or fibrils-forming conditions. Western blot analysis of SDS–PAGE were performed on blots incubated in the primary antibody 6E10 (mouse monoclonal against Aβ residues 1–16) or 4G8 (mouse monoclonal against Aβ residues 17–24). Blots revealed Aβ$_{1-42}$ monomers, dimers, trimers, tetramers and large oligomeric assemblies ranging from 30 to 70 kDa for samples incubated under oligomers-forming conditions (lanes 1 and 3). Smearing between tetramer and monomer, may indicate interconversion between these assemblies during electrophoresis (lane 1). Aβ$_{1-42}$ samples incubated under fibrils-forming conditions contained high molecular weight immunoreactive Aβ that remained in the well, less abundant large oligomers (mostly detected with 6E10, lane 2), dimer and monomer (with 4G8, lane 4). In oligomers-forming conditions, 4G8 revealed an abundant tetramer band which was not detected under fibrils-forming conditions, although dimers and monomers were present in both conditions. p3 samples incubated under oligomers or fibrils-forming conditions only contained monomers (lanes 5 and 6), while high molecular weight fibrils remain in the well.

oligomers (Fig. 2, lanes 5 and 6). As for Aβ, high molecular weight fibrils remain in the well.

In conclusion, Western blot analysis confirms that truncation of 1–16 prevents the formation of stable oligomers, suggesting that the N-terminal 1–16 residues of Aβ$_{1-42}$ are essential for the formation of stable, water-soluble oligomeric intermediates.

4. Discussion–conclusion

The hydrophilic N-terminal amino acids 1–16 of Aβ$_{1-42}$, does not belong to the β-sheet network of the Aβ fibril, since this portion of the peptide in the fibril is flexible [30], insensitive to replacement by proline [31], and sufficiently exposed in the amyloid fibrils to account for rapid protease digestion in protease digestion experiments [32]. These data suggest that this N-terminal region is too disordered to be part of the tightly packed β-sheet in the fibrils. Heparan sulfate proteoglycans [33], metals like Al(III), Fe(III), Zn[5] and Cu[5] [34–36] or proteins like CLAC ("Collagen-like Alzheimer amyloid plaque component") [37] bind to residues which are located in this region. Within the models of Aβ soluble oligomers we have recently proposed [13] that the N-terminal domain, modelled by one or two β-strands, is always accessible to solvent, which is consistent with available experimental data.

We suggest here that the p3 oligomers could not durably exist by themselves, and quickly evolved to fibrillar forms, where the exposed hydrophobic patches are buried. This hypothesis is strengthened by the experimental finding that solution of p3 exposed to oligomers-forming conditions does not assemble into stable oligomers but only into fibrils, whereas Aβ$_{1-42}$ in the same concentration does. Our results are in agreement with those of Pike and colleagues [17] who observed that amino-terminal deletions of Aβ peptide 1–16 enhance aggregation of amyloid-β peptides. Similarly to the Aβ$_{1-42}$ peptide, p3 peptide inserted in the membrane, is likely to be in helical conformation. We thus propose that, after cleavage by the α- and γ-secretases and extraction from the membrane, the α helical p3 peptide would quickly adopt a β-hairpin conformation. This p3 monomer, highly hydrophobic, would rapidly evolve to fibrils with no soluble intermediates forms.

Aβ peptides are believed to have a causative role in the pathogenesis of Alzheimer disease. Because Aβ assembles into a variety of higher-order structures that include low molecular weight oligomers, protofibrils and fibrils, questions have emerged on the neurotoxic potential of these structures. If fibrillar forms of Aβ that are present in the amyloid plaques are neurotoxic in primary neuronal culture, the relatively weak correlations between fibrillar plaque density and severity of dementia led to a re-evaluation of the role of soluble Aβ oligomers in Alzheimer disease pathophysiology. Several studies

plead for the implication of soluble Aβ in cellular disturbance observed in Alzheimer disease. Indeed strong correlations between soluble Aβ levels and the extent of synaptic loss and/or cognitive impairment have been demonstrated [28]. Further, more studies have revealed that soluble oligomeric forms of Aβ are involved in the disruption of synaptic plasticity and toxicity in vitro and in vivo [8,38]. Because p3 is generated through the so-called non-amyloidogenic pathway, only few studies have been specifically designed to evaluate its impact on neuronal function. However, Wash et al. [29] have used p3 peptide solution to rule out the possible role of this truncated Aβ on synaptic plasticity. They revealed that p3 solution are devoid of any impact on synaptic function [29]. In the present study, we demonstrate that human p3 peptide in solution cannot form stable oligomeric intermediates, as human Aβ does. The impossibility to form p3 stable soluble oligomers combined to the absence of impact on synaptic function of p3 solution revealed by Walsh and colleagues [29] may be interpreted as a new set of data that highlighted the role of soluble oligomers in the amyloid-β mediated synaptotoxicity.

In conclusion, by using a combine theoretical and experimental approach, we demonstrated that the intuitive evidence of the absence of implication of the non-amyloidogenic processing of APP in the physiopathology of Alzheimer disease may rely on the impossibility to form stable soluble oligomers from the truncated form of $A\beta_{1-42}$: the p3 peptide.

References

[1] Annaert, W. and De Strooper, B. (2002) A cell biological perspective on Alzheimer's disease. Annu. Rev. Cell Dev. Biol. 18, 25–51.

[2] Selkoe, D.J. (2004) Cell biology of protein misfolding: the examples of Alzheimer's and Parkinson's diseases. Nat. Cell. Biol. 6, 1054–1061.

[3] Balbach, J.J., Petkova, A.T., Oyler, N.A., Antzutkin, O.N., Gordon, D.J., Meredith, S.C. and Tycko, R. (2002) Supramolecular structure in full-length Alzheimer's β-amyloid fibrils: evidence for a parallel β-sheet organization from solid-state nuclear magnetic resonance. Biophys. J. 83, 1205–1216.

[4] Lührs, T., Ritter, C., Adrian, M., Riek-Loher, D., Bohrmann, B., Döbeli, H., Schubert, D. and Riek, R. (2005) 3D structure of Alzheimer's amyloid-β(1–42) fibrils. Proc. Natl. Acad. Sci. USA 102, 17342–17347.

[5] Petkova, A.T., Ishii, Y., Balbach, J.J., Antzutkin, O.N., Leapman, R.D., Delaglio, F. and Tycko, R. (2002) A structural model for Alzheimer's β-amyloid fibrils based on experimental constraints from solid state NMR. Proc. Natl. Acad. Sci. USA 99, 16742–16747.

[6] Thompson, L.K. (2003) Unraveling the secrets of Alzheimer's β-amyloid fibrils. Proc. Natl. Acad. Sci. USA 100, 383–385.

[7] Hartley, D.M., Walsh, D.M., Ye, C.P., Diehl, T., Vasquez, S., Vassilev, P.M., Teplow, D.B. and Selkoe, D.J. (1999) Protofibrillar intermediates of amyloid β-protein induce acute electrophysiological changes and progressive neurotoxicity in cortical neurons. J. Neurosci. 19, 8876–8884.

[8] Kayed, R., Head, E., Thompson, J.L., McIntire, T.M., Milton, S.C., Cotman, C.W. and Glabe, C.G. (2003) Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. Science 300, 486–489.

[9] Kirkitadze, M.D., Condron, M.M. and Teplow, D.B. (2001) Identification and characterization of key kinetic intermediates in amyloid β-protein fibrillogenesis. J. Mol. Biol. 312, 1103–1119.

[10] Walsh, D.M., Hartley, D.M., Kusumoto, Y., Fezoui, Y., Condron, M.M., Lomakin, A., Benedek, G.B., Selkoe, D.J. and Teplow, D.B. (1999) Amyloid β-protein fibrillogenesis. Structure and biological activity of protofibrillar intermediates. J. Biol. Chem. 274, 25945–25952.

[11] Yong, W., Lomakin, A., Kirkitadze, M.D., Teplow, D.B., Chen, S.H. and Benedek, G.B. (2002) Structure determination of micelle-like intermediates in amyloid β-protein fibril assembly by using small angle neutron scattering. Proc. Natl. Acad. Sci. USA 99, 150–154.

[12] Walsh, D.M. and Selkoe, D.J. (2007) Abeta oligomers – a decade of discovery. J. Neurochem. 101, 1172–1184.

[13] Dulin, F., Callebaut, I., Colloc'h, N. and Mornon, J.P. (2007) Sequence-based modeling of Abeta42 soluble oligomers. Biopolymers 85, 422–437.

[14] Mathura, V.S., Paris, D., Ait-Ghezala, G., Quadros, A., Patel, N.S., Kolippakkam, D.N., Volmar, C.H. and Mullan, M.J. (2005) Model of Alzheimer's disease amyloid-beta peptide based on a RNA binding protein. Biochem. Biophys. Res. Commun. 332, 585–592.

[15] Dickson, D.W. (1997) The pathogenesis of senile plaques. J. Neuropathol. Exp. Neurol. 56, 321–339.

[16] Higgins, L.S., Murphy Jr., G.M., Forno, L.S., Catalano, R. and Cordell, B. (1996) P3 beta-amyloid peptide has a unique and potentially pathogenic immunohistochemical profile in Alzheimer's disease brain. Am. J. Pathol. 149, 585–596.

[17] Pike, C.J., Overman, M.J. and Cotman, C.W. (1995) Amino-terminal deletions enhance aggregation of beta-amyloid peptides in vitro. J. Biol. Chem. 270, 23895–23898.

[18] Guex, N. and Peitsch, M.C. (1997) SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. Electrophoresis 18, 2714–2723.

[19] Richmond, T.J. (1984) Solvent accessible surface area and excluded volume in proteins. Analytical equations for overlapping spheres and implications for the hydrophobic effect. J. Mol. Biol. 178, 63–89.

[20] Stine Jr., W.B., Dahlgren, K.N., Krafft, G.A. and LaDu, M.J. (2003) In vitro characterization of conditions for amyloid-beta peptide oligomerization and fibrillogenesis. J. Biol. Chem. 278, 11612–11622.

[21] Klein, W.L. (2002) Abeta toxicity in Alzheimer's disease: globular oligomers (ADDLs) as new vaccine and drug targets. Neurochem. Int. 41, 345–352.

[22] Berman, H.M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T.N., Weissig, H., Shindyalov, I.N. and Bourne, P.E. (2000) The Protein Data Bank. Nucleic Acids Res. 28, 235–242.

[23] Callebaut, I., Labesse, G., Durand, P., Poupon, A., Canard, L., Chomilier, J., Henrissat, B. and Mornon, J.P. (1997) Deciphering protein sequence information through hydrophobic cluster analysis (HCA): current status and perspectives. Cell. Mol. Life Sci. 53, 621–645.

[24] Marti-Renom, M.A., Stuart, A.C., Fiser, A., Sanchez, R., Melo, F. and Sali, A. (2000) Comparative protein structure modeling of genes and genomes. Annu. Rev. Biophys. Biomol. Struct. 29, 291–325.

[25] Hilbich, C., Kisters-Woike, B., Reed, J., Masters, C.L. and Beyreuther, K. (1992) Substitutions of hydrophobic amino acids reduce the amyloidogenicity of Alzheimer's disease βA4 peptides. J. Mol. Biol. 228, 460–473.

[26] Pike, C.J., Walencewicz-Wasserman, A.J., Kosmoski, J., Cribbs, D.H., Glabe, C.G. and Cotman, C.W. (1995) Structure–activity analyses of β-amyloid peptides: contributions of the β25–35 region to aggregation and neurotoxicity. J. Neurochem. 64, 253–265.

[27] Xu, Y., Shen, J., Luo, X., Zhu, W., Chen, K., Ma, J. and Jiang, H. (2005) Conformational transition of amyloid β-peptide. Proc. Natl. Acad. Sci. USA 102, 5403–5407.

[28] McLean, C.A., Cherny, R.A., Fraser, F.W., Fuller, S.J., Smith, M.J., Beyreuther, K., Bush, A.I. and Masters, C.L. (1999) Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. Annu. Neurol. 46, 860–866.

[29] Walsh, D.M., Klyubin, I., Fadeeva, J.V., Cullen, W.K., Anwyl, R., Wolfe, M.S., Rowan, M.J. and Selkoe, D.J. (2002) Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature 416, 535–539.

[30] Török, M., Milton, S., Kayed, R., Wu, P., McIntire, T., Glabe, C.G. and Langen, R. (2002) Structural and dynamic features of Alzheimer's Aβ peptide in amyloid fibrils studied by site-directed spin labeling. J. Biol. Chem. 277, 40810–40815.

[31] Williams, A.D., Portelius, E., Kheterpal, I., Guo, J.T., Cook, K.D., Xu, Y. and Wetzel, R. (2004) Mapping Aβ amyloid fibril secondary structure using scanning proline mutagenesis. J. Mol. Biol. 335, 833–842.

[32] Kheterpal, I., Williams, A., Murphy, C., Bledsoe, B. and Wetzel, R. (2001) Structural features of the Aβ amyloid fibril elucidated by limited proteolysis. Biochemistry 40, 11757–11767.

[33] Bame, K.J., Danda, J., Hassall, A. and Tumova, S. (1997) Aβ(1–40) prevents heparanase-catalyzed degradation of heparan sulfate glycosaminoglycans and proteoglycans *in vitro*. A role for heparan sulfate proteoglycan turnover in Alzheimer's disease. J. Biol. Chem. 272, 17005–17011.

[34] Atwood, C.S., Moir, R.D., Huang, X., Scarpa, R.C., Bacarra, N.M., Romano, D.M., Hartshorn, M.A., Tanzi, R.E. and Bush, A.I. (1998) Dramatic aggregation of Alzheimer Aβ by Cu(II) is induced by conditions representing physiological acidosis. J. Biol. Chem. 273, 12817–12826.

[35] Kawahara, M., Muramoto, K., Kobayashi, K., Mori, H. and Kuroda, Y. (1994) Aluminum promotes the aggregation of Alzheimer's amyloid β-protein *in vitro*. Biochem. Biophys. Res. Commun. 198, 531–535.

[36] Mantyh, P.W., Ghilardi, J.R., Rogers, S., DeMaster, E., Allen, C.J., Stimson, E.R. and Maggio, J.E. (1993) Aluminum, iron, and zinc ions promote aggregation of physiological concentrations of β-amyloid peptide. J. Neurochem. 61, 1171–1174.

[37] Hashimoto, T., Wakabayashi, T., Watanabe, A., Kowa, H., Hosoda, R., Nakamura, A., Kanazawa, I., Arai, T., Takio, K., Mann, D.M. and Iwatsubo, T. (2002) CLAC: a novel Alzheimer amyloid plaque component derived from a transmembrane precursor, CLAC-P/collagen type XXV. EMBO J. 21, 1524–1534.

[38] Shankar, G.M., Bloodgood, B.L., Townsend, M., Walsh, D.M., Selkoe, D.J. and Sabatini, B.L. (2007) Natural oligomers of the Alzheimer amyloid-beta protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor-dependent signaling pathway. J. Neurosci. 27, 2866–2875.