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Inhibition of cytotoxicity and amyloid fibril formation by a D-amino acid peptide that specifically binds to Alzheimer's disease amyloid peptide

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder. The 'amyloid cascade hypothesis' assigns the amyloid-beta-peptide (Aβ) a central role in the pathogenesis of AD. Although it is not yet established, whether the resulting Aβ aggregates are the causative agent or just a result of the disease progression, polymerization of Aβ has been identified as a major feature during AD pathogenesis. Inhibition of the Aβ polymer formation, thus, has emerged as a potential therapeutic approach. In this context, we identified peptides consisting of D-enantiomeric amino acid peptides (D-peptides) that bind to Aβ. D-peptides are known to be more protease resistant and less immunogenic than the respective L-enantiomers. Previously, we have shown that a 12mer D-peptide specifically binds to Aβ amyloid plaques in brain tissue sections from former AD patients. *In vitro* obtained binding affinities to synthetic Aβ revealed a K_d value in the submicromolar range. The aim of the present study was to investigate the influence of this D-peptide to Aβ polymerization and toxicity. Using cell toxicity assays, thioflavin fluorescence, fluorescence correlation spectroscopy and electron microscopy, we found a significant effect of the D-peptide on both. Presence of D-peptides (Dpep) reduces the average size of Aβ aggregates, but increases their number. In addition, Aβ cytotoxicity on PC12 cells is reduced in the presence of Dpep.

Keywords: Aβ/aggregation/Alzheimer's disease/cytotoxicity/D-amino acid peptide

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, which is characterized by memory loss, confusion and a variety of cognitive disabilities (Selkoe, 1999). AD, the most common form of dementia, is a devastating disease and affects five million people in Europe alone (Ferri *et al*., 2005). Today, the only definite way to diagnose AD is the *post mortem* identification of amyloid plaques and neurofibrillary tangles in the brain tissue of the patient. Recent studies indicate that imaging methods like computed tomography, positron emission tomography (PET) and magnetic resonance tomography (MRT) could be useful in distinguishing AD from other forms of dementia disorders, such as vascular dementia, Parkinson's disease and Huntington's disease. Especially, new radiotracers like fluorine-18-labelled-FDDNP (2-(1-{6-[(2-[F-18]fluoroethyl)(methyl)amino]-2-naphthyl}ethylidene) malononitrile) and carbon-11-labelled-PIB (Pittsburgh Compound B) in combination with improved PET scanning methods are promising tools for the improvement of the early diagnosis of AD and the monitoring of disease progression. The major component of the amyloid plaques is the amyloid β-peptide (Aβ) consisting of 39 to 43 amino acid residues. It is still not established, whether these plaques are a result of AD or, vice versa, AD is the result of Aβ plaques. Although final proof or disproof has not yet been provided, there is an increasing amount of evidence in favor of the latter hypothesis, and the corresponding 'amyloid model' has become more and more consistent during the last years (Selkoe, 1999; Kimura *et al*., 2003). Aβ is produced from the amyloid precursor protein (APP) by two distinct proteolytic activities, called β- and γ-secretases (Kang *et al*., 1987; Weidemann *et al*., 1989; Haass and Selkoe, 1993). These formerly proposed proteolytic activities have been assigned to certain proteins, called beta-site APP-cleaving enzyme (BACE) (Sinha *et al*., 1999; Vassar *et al*., 1999) and Presenilin-1 (Haass, 1996), the latter in combination with other factors (Edbauer *et al*, 2003).

Therapeutical approaches currently available for AD are restricted to rather unspecific interventions in processes being located far downstream in the causal chain leading ultimately to AD clinical symptoms. Substances used for these interventions include inflammation inhibitors, acetylcholinesterase inhibitors, glutamic acid receptor activators, glutamate antagonists, secretase-inhibitors, NGF-agonists and antioxidants. Acetylcholine inhibitors like donepezil, galantamine and the NMDA receptor antagonist memantine are approved for clinical use for treatment of cognitive symptoms (Blennow *et al*., 2006; Roberson and Mucke, 2006). Atypical antipsychotic drugs like risperidone are reported to show an effect in reducing disease-related behavioral symptoms like aggression, agitation and psychosis (Brodaty *et al*., 2003).

Results from Aβ vaccination studies by active or passive immunization of humans (Hock *et al*., 2003) and transgenic mice (Schenk *et al*., 1999; Bard *et al*., 2000; Dodart *et al*., 2002; Seabrook *et al*., 2007) are promising. However, data on potential long-term side effects of vaccination against endogenous host proteins are not available yet. In one study, phase 2 clinical trails were halted when 6% of the patients treated with an Aβ-antibody (AN1792; Elan Pharmaceuticals Inc.) developed meningoencephalitis (Orgogozo *et al*., 2003). Other approaches targeting Aβ are the reduction of Aβ production by γ- or β-secretase-modulators (Citron, 2004) or the disruption of Aβ aggregation by small molecules or peptides. Thus, substances being able to inhibit Aβ aggregation and reduce its toxic effects are still highly

desirable. A variety of such substances were described, e.g. Congo red, haloperidol, nicotine, hexadecyl-*N*-methylpiperidiniumbromid, laminin and rifampicin (Soto, 1999). Oligomeric acylated aminopyrazoles, which prevent Aβ aggregation, were constructed by rational design (Rzepecki *et al*., 2004). Small peptides that inhibit Aβ aggregation and reduce its toxic effects are also described. Soto *et al*. designed a pentapeptid based on the central hydrophobic region in the N-terminal domain of Aβ that acts as a β-sheet breaker (Soto *et al*., 1998; Soto, 1999). Tjernberg *et al*. identified a short Aβ fragment that binds to full-length Aβ, thus preventing its assembly into amyloid fibrils (Tjernberg *et al*., 1996). The corresponding all-D-amino acyl analogue peptide of the Aβ fragment LVFFA was proven to be a Aβ fibrillogenesis inhibitor (Findeis *et al*., 1999). All these aforementioned peptides were derived by variations of the Aβ peptide itself.

Earlier, we described a mirror image phage display approach with the D-amino acid enantiomer of Aβ(1−42) as target. We identified an Aβ binding D-peptide (Dpep). We were able to show that Dpep specifically binds only to Aβ deposits in human brain tissue sections (Wiesehan *et al*., 2003). In the present study, we explore the influence of Dpep on polymerization and cytotoxicity of Aβ.

Materials and methods

Peptides

L-Aβ(1−42), DAEFRHDSGYEVHHQKLVFFAEDVGSNKG AIIGLMVGGVVIA, Dpep, QSHYRHISPAQV, all amino acids such as L-enantiomers and D-enantiomers were purchased as reversed phase high performance liquid chromatography purified products (Jerini Biotools, Berlin, Germany). Identity was confirmed by matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS) (Karas and Hillenkamp, 1988).

Preparation of Aβ samples

L-Aβ(1-42) was dissolved in sterile filtered H_2O at a concentration of 10 μM. The solution was aliquoted and lyophilized. These aliquots were dissolved in an adequate volume of PBS (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4), which did contain zero, 100 μM or 1 mM Dpep, to obtain 5-fold end concentrations of L-Aβ(1−42) in the respective assay.

Cell toxicity assays

For the investigation of Dpep influence on the cellular toxicity of L-Aβ(1−42), the MTT assay (Shearman *et al*., 1994) was accomplished in this way: 20 μl of each Aβ-solution were directly (0 h) or after one day (24 h, 37°C), respectively, added to a collagen-IV-coated microtiter plate (BD Biosciences) well containing 80 μl DMEM ('Dulbecco's modified Eagle medium' with acetyl-alanine-glutamine, 10% fetal bovine serum, 5% horse serum and 10 mg/ml gentamycin) and 2×10^4 PC12 cells grown for 24 h at 37°C in a 7.5% (v/v) CO_2 atmosphere.

Determination of cellular 3-(4,5-dimethyl-thiazol-2-yl) 2-5-diphenyl-tetrazolium bromide (MTT) reduction was carried out after a 24-h incubation period of the cells together with the Aβ−Dpep-DMEM mixtures described above at 37°C in a 7.5% (v/v) CO_2 atmosphere. Then, 10 μl sterile filtered solution of 5 mg/ml MTT in PBS was added and incubated for another 3 h. Medium was removed and 100 μl cell lysis buffer (99.4 ml DMSO, 0.6 ml 100% acetic acid, 10 g SDS) were added and incubated for 30 min while gently shaking. MTT reduction was determined by measuring the difference between absorbencies at 570 and 630 nm. A cell viability value of 100% was defined corresponding to MTT reduction of cells treated neither with Aβ nor with Dpep. A cell viability value of 0% was defined by treatment of the PC12 cells with 0.2% Triton-X. The percentage of MTT reduction for each measurement was calculated as the fraction of the value relative to the 100 and 0% values.

Thioflavin T assay

Thioflavin T (ThT) assays (LeVine, 1993) were carried out with minor modifications. Five microliter of each Aβ−Dpep mixture were added to 195 μl 5 μM ThT (Sigma) in 50 mM Glycin-NaOH, pH 8.5. Fluorescence was monitored immediately ($t = 0$ h) and after 24 h with a microplate reader at excitation and emission wavelengths of 440 and 490 nm, respectively (Polarstar Optima, BMG). Fluorescence of the ThT solution without addition of Aβ was subtracted from each value to correct for the fluorescence background.

Electron microscopy

Aliquots (10 μl) of the respective L−Aβ−Dpep mixtures as well as control samples of the Dpep and PBS-buffer (data not shown) were placed on 200 mesh carbon-coated formvar copper grids. After 5−10 min, excess fluid was discarded and the samples and simple PBS-buffer, as negative controls, were negatively stained with 2% (w/v) ammonium molybdate for several seconds. Finally, the specimens were viewed in a Zeiss EM-910 transmission electron microscope (TEM).

FCS measurements

Fluorescence correlation spectroscopy (FCS) measurements were performed with a Confocor I instrument (Zeiss-Evotec), equipped with an argon ion laser and filter systems for $\lambda_{EX} = 488$ nm. For fluorescence detection, Aβ(1−42)-peptide (P. Henklein, Charité Berlin) was labeled at the N-terminus with the dye OregonGreen (Molecular Probes). A 24 well micro carrier with 20 μl sample volume (MC 384/15, Evotec Technologies) was used. Adjustment of the instrument was performed before each measurement with rhodamine 6G. Pinhole was 45 μm in diameter. Focus was set 200 μm above bottom of the well. The reaction mixture contained 22 μM Aβ(1−40) unlabeled (prepared according to Fezoui *et al*., 2000), 10 nM Aβ(1−42), labeled with OregonGreen in 10 mM sodiumphosphate-buffer, pH 7.2, and different concentrations of the D-peptide. Aβ (1−42)-OG was prepared as 500 nM stock solution in 100% DMSO, stored in aliquots at −20°C and filtered through 0.45 μm nylon filters directly prior to use. Reaction mixtures contained only one OregonGreen labeled peptide per 2200 unlabeled molecules. Fluorescence fluctuations in each well were recorded forty times for 30 s per sample with 500 datapoint resolution. Corresponding autocorrelation functions were calculated by a hardware correlator card. Data evaluation was carried out with the Evotec software Multi-FCSaccess.

Results

Influence of Dpep on Aβ(1–42) toxicity

The influence of Dpep on the viability of PC12 cells was investigated by MTT reduction (Shearman *et al*., 1994) in the presence of Aβ concentrations between 10 and 250 μM. To achieve these Aβ concentrations in the cell assay, Aβ was pre-dissolved at a concentration of 10 μM in H_2O, lyophilized and re-dissolved in an adequate volume of PBS, with or without Dpep to obtain 5-fold concentrations of Aβ (10, 12.5, 25, 50, 100 and 250 μM) when compared with its end concentrations in the cell assays (2, 2.5, 5, 10, 20 and 50 μM). The toxicity of the Aβ solutions, in the presence of three different Dpep concentrations (0, 100 and 1000 μM), was determined immediately and 24 h after preparation of the Aβ solutions with or without Dpep (Fig. 1B and D).

Cell viability measured immediately after preparation of the Aβ–Dpep mixtures yielded only a small dependence on the Aβ concentrations applied in the assays (Fig. 1B). Only high Aβ concentrations (100 and 250 μM) lead to about 10% reduction of cell viability. At this time point, cell viability was not dependent on Dpep either.

Dpep is able to inhibit cell toxicity of Aβ

When the Aβ–Dpep mixtures were applied to the cell cultures for MTT reduction assay after a 24 h incubation period at 37°C, the situation had completely changed (Fig. 1D). In the absence of Dpep, a clear reduction of cell viability by about 50% was observed for all Aβ concentrations under investigation (10–250 μM). Presence of 100 μM Dpep yielded an increase of viability to almost 70% at low Aβ

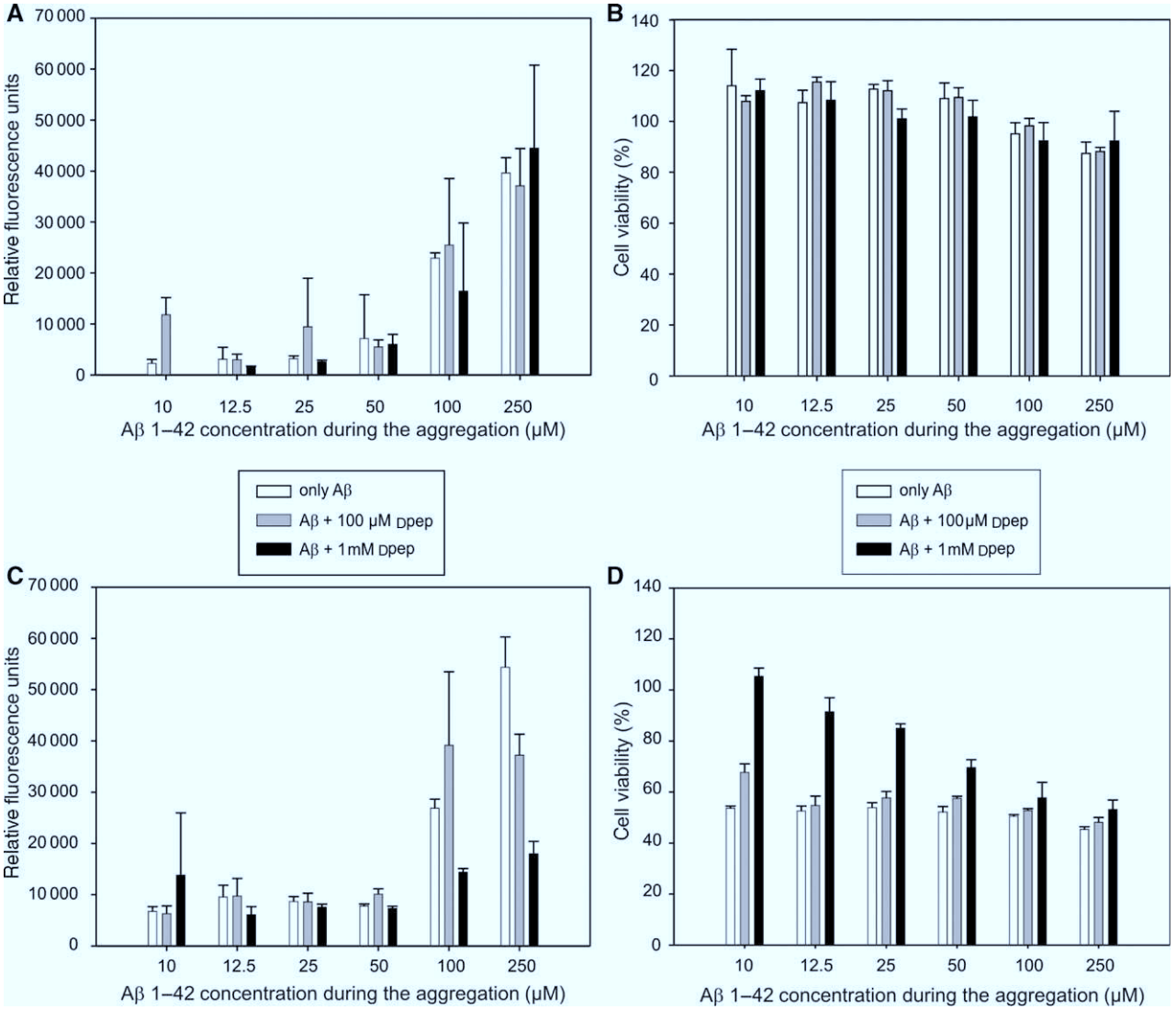

Fig. 1. Influence of Dpep on cytotoxicity and aggregate formation of Aβ. (**A** and **C**) Binding of ThT to amyloid Aβ-fibrils was determined by ThT fluorescence at Ex 440/Em 490 and shown as relative fluorescence units. All values are given as means from three measurements with the respective standard deviation. One single measurement (10 μM Aβ and 100 μM Dpep) was completely out of range for unknown reasons and was not used for further analysis. All measurements were carried out on freshly prepared Aβ(1–42) (A) and Aβ(1–42) incubated for 24 h at 37°C (C). (**B** and **D**) Cell viability for Aβ(1–42)-treated cells was measured using the MTT assay with PC12-cells. Percentages of cell viability were derived as follows: the 100% value was obtained from cells treated neither with Dpep nor with Aβ(1–42); a value of 0% was obtained by treatment of the PC12 cells with 0.2% Triton-X. All values are given as means from three measurements with the respective standard deviation. All measurements were carried out on freshly prepared Aβ(1–42) (B) and L–Aβ(1–42) incubated for 24 h at 37°C (D).

concentration (10 μM). Presence of 1 mM Dpep yielded an increase of viability up to 100% depending on the Aβ concentration. Dpep itself did not show any toxic effects, as demonstrated with cell viability values between 98 and 111% for Dpep concentrations of up to 1 mM Dpep in the absence of Aβ (data not shown).

Taken together, the data clearly demonstrate inhibitory effects of Dpep for Aβ toxicity; although, concentrations of Dpep necessary to obtain this effect are rather high and may withstand its therapeutical application.

Aβ amyloid fibril formation in the presence of Dpep as measured by ThT assay

Simultaneous to each cell viability assay, the effect of Dpep on amyloid fibril formation was determined by thioflavinT (ThT) assays (Levine *et al*., 1993). All Aβ–Dpep mixtures prepared for the cell toxicity assays were diluted 1:40 with ThT solution for determination of amyloid fibril formation (Fig. 1A and C). Dpep solutions in the absence of Aβ did not yield any detectable fluorescence signals for Dpep concentrations of up to 1 mM Dpep (data not shown).

ThT assays carried out with Aβ solutions immediately after they were prepared yielded values that roughly correlate with the concentrations of Aβ applied to the assay. ThT assay results of Aβ solutions with and without Dpep did not show significant differences from each other (Fig. 1A).

Dpep is able to reduce Aβ β-sheet formation

When the ThT assays were carried out after a 24 h incubation period of the Aβ–Dpep mixtures, a remarkably different result was obtained (Fig. 1C). Mixtures with Aβ concentrations up to 50 μM, with or without Dpep, yielded very similar fluorescence values among each other. The mixtures without Dpep containing 100 and 250 μM Aβ show a small but significant increase of β-sheet formation after 24 h. This increase is dramatically inverted for the 250 μM Aβ mixture containing 1000 μM Dpep. Thus, high concentrations of Dpep are able to reduce the amyloid fibril content of mixtures with elevated Aβ concentrations.

Cell toxicity and β-sheet formation do not correlate

Cell viability assays of Aβ samples of various concentrations with or without Dpep yielded values between 90 and 110%, regardless of the β-sheet contents as obtained from the ThT assays. After 24 h incubation, the Dpep containing Aβ samples exert highly diverse values in the cell viability assays. In contrast, the ThT assays of the same samples yielded values rather similar to each other. The most intriguing example is the 250 μM Aβ sample with three different Dpep concentrations (0, 100 and 1000 μM) after 24 h incubation. Although the total amount of Aβ in amyloid fibrils, as measured by the ThT assay, is highly dependent on the Dpep concentration, the presence of Dpep obviously did not have a significant effect on Aβ toxicity. This indicates that the ThT signal does not correlate with cytotoxicity in general. This is a very clear and interesting conclusion from the data.

Consequently, Dpep's effect on the amyloid fibril content of Aβ samples is not mirrored by a similar effect on cell viability values of the respective samples. Vice versa, at Aβ concentrations lower than 100 μM, Dpep did not have a significant effect on amyloid fibril content, but increased cell viability values dramatically.

That strongly suggests that Dpep is able to modify the toxicity of Aβ amyloid fibrils either by simply covering them or by influencing their composition without changing the ThT signal.

Aggregate formation of Aβ in the presence of Dpep as detected by electron microscopy

To examine the amyloid fibril formation in the Aβ Dpep mixtures, we prepared electron micrographs (EM) from Aβ Dpep mixtures with Aβ concentrations of 10 and 250 μM Aβ in absence or presence of 1 mM Dpep with an incubation time of 24 h (Fig. 2).

The micrographs of 10 μM Aβ without Dpep yielded mainly short amyloid fibrils with small amounts of amorphous material and a small amount of longer amyloid fibrils (Fig. 2A). Ten micromolar samples in the presence of Dpep showed after 24 h of co-incubation almost no EM-detectable amyloid fibrils (Fig. 2B). This is not in perfect accordance with the respective ThT assays (Fig. 1C). Possibly, the ThT assay is not sensitive enough to discriminate between different samples with Aβ aggregates below a certain threshold. Another explanation could be that ThT values do not exactly correlate with amyloid fibril content, but rather with β-sheet content of amyloid fibrils and amorphous material in sum. The EM pictures of 250 μM Aβ in absence of Dpep (Fig. 2C) show more amyloid fibrils and aggregates than those of 250 μM Aβ in presence (Fig. 2D) of 1 mM Dpep. This is much more in accordance with the respective ThT values (Fig. 1C).

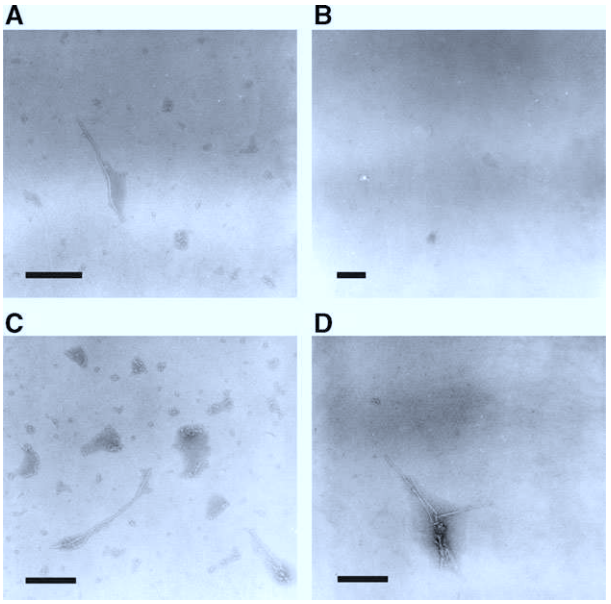

Fig. 2. Electron microscopic analysis of negatively stained samples showing the effect of Dpep on Aβ amyloid fibril formation. (**A** and **B**) Ten micromolar Aβ(1–42) with (B) and without (A) 1 mM Dpep were incubated for 24 h. (**C** and **D**) Two hundred and fifty micromolar Aβ(1–42) with (D) and without (C) 1 mM Dpep were incubated for 24 h. The samples were subsequently placed on 200 mesh carbon-coated formvar copper grids and negatively stained with 2% (w/v) ammonium molybdate. The black bars indicate 200 nm.

Control samples with PBS and Dpep solved in PBS did not yield any detectable structures. The EM results suggest that Dpep strongly influences fibrillogenesis of Aβ.

Aggregate formation of Aβ in the presence of Dpep as measured by FCS

Fluorescence correlation spectroscopy (FCS) allows the observation of single fluorescently labeled molecules diffusing free in solution. By measuring the fluorescence fluctuations caused by single molecules excited within the confocal volume element, the diffusion coefficient can be determined, which together with an assumption regarding the shape of the molecule allows for an estimation of the corresponding molecular weight of the diffusing species. In the case of aggregating Aβ, the system is expected to be highly polydispers. Aggregates of many different sizes and different shapes will be present. In addition, some of the material might already be insoluble and therefore inaccessible to the method. The obtained autocorrelation curves were fitted with a two component (fluorescent probe free and bound to aggregated species) system. The diffusion time of the first component was determined separately by measuring the fluorescent probe alone. This was used to calculate the diffusion time of the second component as well as the relative amounts of both from the measurements of the fluorecent probe in Aβ Dpep mixtures. The diffusion time of the second component represents a weight averaged diffusion time for all aggregated species in solution.

Presence of Dpep reduces the diffusion time of the Aβ aggregates (Fig. 3A) indicating that on average, the size of Aβ aggregates is significantly smaller than in absence of Dpep. Interestingly, the number of Aβ aggregates is increased in the presence of Dpep when compared with samples without Dpep (Fig. 3B).

The mean diffusion time of 1 ms matches to a mean diffusion coefficient of 1.8×10^{-7} cm^2/s and corresponds to aggregates of five million Da assuming a spherical shape (Equation: $D = \omega_1^2/4\tau\text{diff}$; ω_1 is the radius of the confocal volume element). In the presence of Dpep, the mean diffusion coefficient of measured aggregates is 5.5×10^{-7} cm^2/s and corresponds to a molecular weight of only 200 000 Da. Molecular weights will appear smaller the more the shape deviates from a sphere. The reduction in aggregate size caused by Dpep seems to be at least in parts be balanced by an increase of the relative amount of aggregates as shown in Fig. 3B.

Thus, Dpep leads to a decrease of the average Aβ particle size, but increases the number of Aβ particles.

Therefore, the results from the FCS measurements are well suited to bring the ThT assay results (Fig. 1A and C) in accordance with the EM pictures (Fig. 2). In the presence of Dpep, the average particle size of Aβ aggregates drops to only 200 000 Da. This suggests that most of the aggregates were not EM-visible but contributed to the ThT signal.

Discussion

Substances that preferentially bind Aβ oligomers or amyloid fibrils can be expected to promote their formation, as was indeed observed (Kuner *et al*., 2000; Lowe *et al*., 2001). Other substances act like beta-sheet breakers and affect the amyloid fibril formation and cytotoxicty of Aβ (Soto, 1999). Furthermore, an increasing number of investigations report that besides amyloid fibrils, protofilaments (Walsh *et al*., 1999), oligomers or even smaller Aβ units may be toxic to cells (Lambert *et al*., 1998).

Thus, it was of great interest and of importance for potential diagnostic applications of Dpep, to investigate any effect of Dpep on the cytotoxicity of Aβ. Although extrapolation of the results obtained from *in vitro* systems to *in vivo*

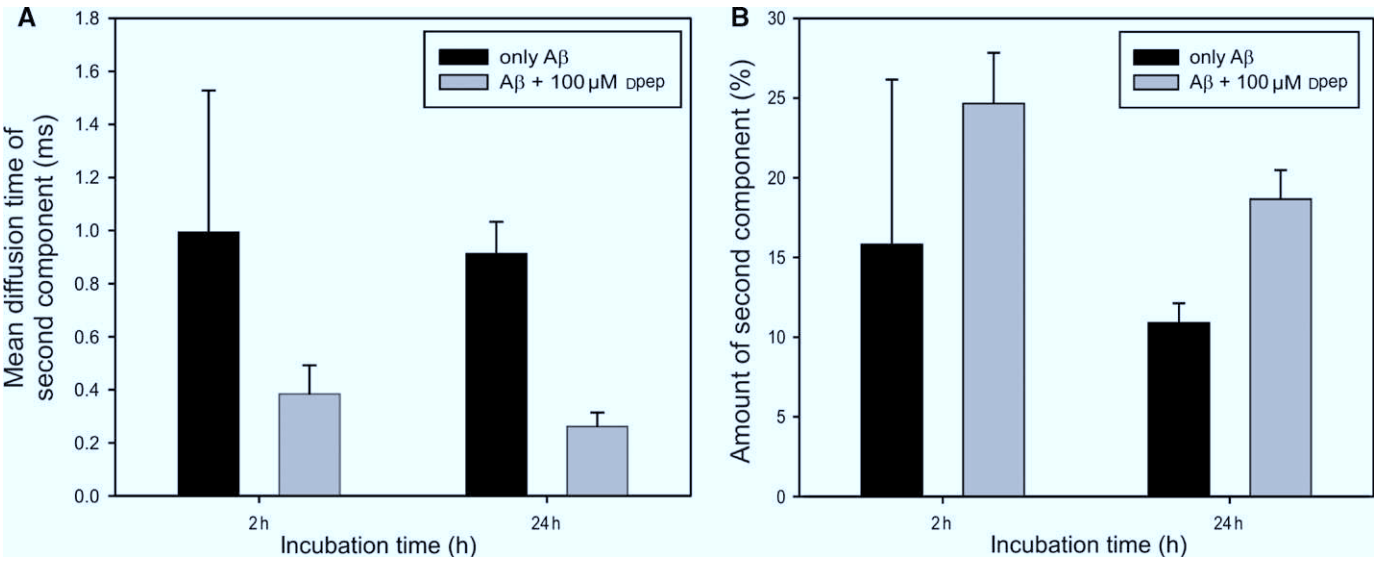

Fig. 3. FCS measurement of Aβ-aggregation. Reaction mixtures containing 22 µM Aβ(1–40), 10 nM Aβ(1–42)-OG in 1 × PBS were incubated with and without 100 µM Dpep for 2 or 24 h at room temperature. From a two-component fit of the autocorrelation functions, a weight-averaged diffusion time (**A**) and the relative amount of aggregated species (**B**) were determined. Standard deviations result from averaging 40 measurements of 30 s each and from averaging two independent experiments. The obtained fluorescence correlation data were analyzed applying a model of two independent components as implemented in the Evotec Access 2.0 software. The diffusion time of the first component comprising the monomeric Aβ was fixed to the values determined for a solution containing fluorescently labeled Aβ alone measured within the same experiment. The diffusion time of the second component comprises a weight average for all associates of Aβ present in the solution and is used as a measure of weight averaged size of aggregates.

mechanisms of the disease requires all necessary caution, we want to discuss potential mechanisms of Dpep for either inhibition of toxicity or amyloid fibril formation.

Taken together all FCS, ThT and EM data, Dpep dramatically reduces Aβ particle size, but increases the overall number of aggregates. Further, the higher the Dpep–Aβ ratio is, the more significant is the positive effect of Dpep on cell viability.

If we assume that Dpep binds to Aβ amyloid fibrils, protofilaments and other oligomers, it can be expected to reduce their toxic effects by simply covering them. Such an effect should depend roughly on the ratio of Dpep and Aβ. That is exactly what could be observed in the experiments. The highest cell viability was observed for the highest Dpep–Aβ ratio (Fig. 1C).

Formation and growth of amyloid fibrils requires existence of free binding sites on aggregates for further adsorption of Aβ molecules. If Dpep binds to all kinds of Aβ aggregates, it should hinder formation of amyloid fibrils from smaller intermediates as well as growth of existing amyloid fibrils or protofibrils. Thus, in a simple competition reaction, Dpep can be expected to suppress ongoing attachment of Aβ molecules during amyloid fibril growth. The same competition hinders fusion of Aβ aggregates to larger structures. The FCS data reported here show an increase in the number of particles and a decrease in average size (Fig. 2). This is in accordance with this model.

Most interestingly, after a 24 h incubation period, a lower amyloid fibril content is observed than before, indicating that Dpep in fact can actively reduce β-sheet content or destroy amyloid fibrils. The concentrations of Dpep necessary to observe these effects may be too high to be suited for preventive or therapeutic use in its present form. It should, however, be considered that Aβ concentrations within the brain are by far lower than those used in the described *in vitro* cytotoxicity assays, and therefore, treatment of AD patients with Dpep could possibly lead to a breakdown and clearance of existing amyloid deposits.

Taken all data together, Dpep may not only serve as a molecular marker for Aβ aggregates as shown previously (Wiesehan *et al*., 2003), but also is able to inhibit toxic effects of Aβ. Although both effects of Dpep on Aβ, reduction of amyloid fibril formation and reduction of toxicity, do not perfectly correlate to each other, they may have a common basis, which could simply be binding to and covering Aβ aggregates.

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