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# Influence of dendrimer's structure on its activity against amyloid fibril formation

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**Abstract**

Inhibition of fibril assembly is a potential therapeutic strategy in neurodegenerative disorders such as prion and Alzheimer's diseases. Highly branched, globular polymers—dendrimers—are novel promising inhibitors of fibril formation. In this study, the effect of polyamidoamine (PAMAM) dendrimers (generations 3rd, 4th, and 5th) on amyloid aggregation of the prion peptide PrP 185–208 and the Alzheimer's peptide Aβ 1–28 was examined. Amyloid fibrils were produced in vitro and their formation was monitored using the dye thioflavin T (ThT). Fluorescence studies were complemented with electron microscopy. The results show that the higher the dendrimer generation, the larger the degree of inhibition of the amyloid aggregation process and the more effective are dendrimers in disrupting the already existing fibrils. A hypothesis on dendrimer–peptide interaction mechanism is presented based on the dendrimers' molecular structure.


*Keywords:* Dendrimer; Prion; Alzheimer; Amyloid peptide; Aggregation

Amyloid fibrils are aggregates of normally soluble peptides or proteins. There is a group of diseases that are characterized by the deposition of amyloid fibrils. Among them there are neurological disorders such as Alzheimer's and prion diseases. Thus, inhibition of fibril assembly is a potential strategy for therapeutic intervention. It has been recently shown that polyamidoamine and polypropyleneimine dendrimers are promising candidates for the treatment of prion diseases [1,2]. These relatively novel macromolecules are globular and are characterized by a densely packed surface. Due to their specific structure they are suitable for a variety of biomedical applications. Dendrimers promote the clearance of pre-existing $PrP^{Sc}$ (the abnormally folded prion protein, which forms amyloid fibrils). Dendrimers also prevent the conversion of the normal cellular $PrP^{C}$ into $PrP^{Sc}$ [1,2]. These branched polyamines are the first class of compounds that have been shown to be able to cure a prion infection in living cells. This fact received considerable interest and other types of dendrimers were tested as possible anti-prion agents [3,4].

Amyloid fibrils can be produced in vitro by exposing disease-associated peptides to destabilizing conditions. We have chosen this approach with the aim of contributing to the molecular characterization of the interactions between dendrimers and peptides.

We have used the third, fourth, and fifth generation of polyamidoamine dendrimers (PAMAM G3, PAMAM G4, and PAMAM G5) in order to study how dendrimers' structure and size determine their effect on amyloid formation. Dendrimers are built in a cyclic manner from a central core molecule that is surrounded by layers of branched monomers. The more layers are attached, the higher the so-called generation. As generation increases, the amount of surface groups increases too, so the shape of the dendrimer changes from flat and ellipsoidal to globular [5]. In case of polyamidoamine dendrimers ethylenediamine is the core molecule and branched units are constructed from

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Table 1
Characterization of used dendrimers [32]

| Name, generation | Terminal groups | Number of terminal groups | Molecular weight [Da] | Diameter [nm] |
|---|---|---|---|---|
| PAMAM, G3 | $-NH_2$ | 32 | 6,909 | 3.6 |
| PAMAM, G4 | $-NH_2$ | 64 | 14,215 | 4.5 |
| PAMAM, G5 | $-NH_2$ | 128 | 28,826 | 5.4 |

both methyl acrylate and ethylenediamine [6]. A summary of the characteristics of the dendrimers used in the present study is given in Table 1.

As a continuation of our previous studies we have chosen Alzheimer's peptide Aβ 1–28 and a segment of prion protein PrP 185–208 [7]. A structural homology has been recently described for these two sequences and a recent computational study has shown that residues 180–193 are one of the fibrilization sites in PrP [8,9].

Dendrimers were studied in a system containing heparin as a model glucosaminoglycan. It has been found that amyloid fibrils in vivo are normally associated with GAGs, and both PrP 185–208 and Aβ 1–28 have been previously shown to aggregate in the presence of heparin [10,11].

## Materials and methods

*Materials.* Synthetic peptides Aβ 1–28 [DAEFRHDSGYEVHHQ KLVFFAEDVGSNK] and PrP 185–208 [KQHTVTTTTKGENFTET DVKMMER] were purchased from JPT Peptide Technologies GmbH (Germany). Stock peptide solutions were kept in aqueous buffer at pH 7.5. Thioflavin T (T-3516) and heparin–sodium salt (H-4784) were purchased from Sigma Chemical Company. Dendrimers PAMAM G3, PAMAM G4, and PAMAM G5 were obtained from Dendritic NanoTechnologies Inc. (USA) and dissolved in aqueous buffer. All other chemicals were of analytical grade. Water used to prepare solutions was double-distilled.

*Formation of amyloid fibrils—ThT assay.* The process of aggregation was monitored using the dye thioflavin T (ThT), whose fluorescence depends on the presence of amyloid structures [12,13]. A stock solution of peptide (1.2 mmol/l) in Tris buffer, pH 7.5, was diluted to a final concentration of 50 μmol/l. Then ThT and heparin were added (final concentrations of 35 μmol/l and 0.041 mg/ml, respectively) and pH was adjusted to 5.5 with aliquots of HCl. Fluorescence measurements were performed at 37 °C upon continuous shaking using a microplate reader (Wallac 1440 VICTOR$^3$ V Multilabel Counter from Perkin-Elmer). Aggregation kinetics were monitored by measuring the fluorescence intensity every 375 s using 450-nm excitation and 490-nm emission filters.

*Electron microscopy.* Ten microliters of sample from the fluorescence experiment (see previous paragraph) was placed on a carbon 400 mesh grid. It was dried and the excess of solution was removed with a filter paper. The sample was stained with 2% uranyl acetate for 2 min, dried, and then viewed using a Hitachi H-7000 electron microscope.

## Results

The fluorescence of thioflavin T is normally used to monitor the formation of amyloid fibrils. Figs. 1 and 2 show the fluorescence variation of ThT for Aβ 1–28 and PrP 185–208 in the absence and presence of increasing concentrations of dendrimers. The time-dependent increase in ThT fluorescence follows a sigmoidal curve typical of a nucleated polymerization reaction. Peptide monomers slowly combine to form non-fibrilar structures known as nuclei (lag phase). Addition of peptide monomers to these nuclei and combination of nuclei, together with a conformational transition which implies the formation of fibrilar β-sheet structures, results in the so-called elongation phase

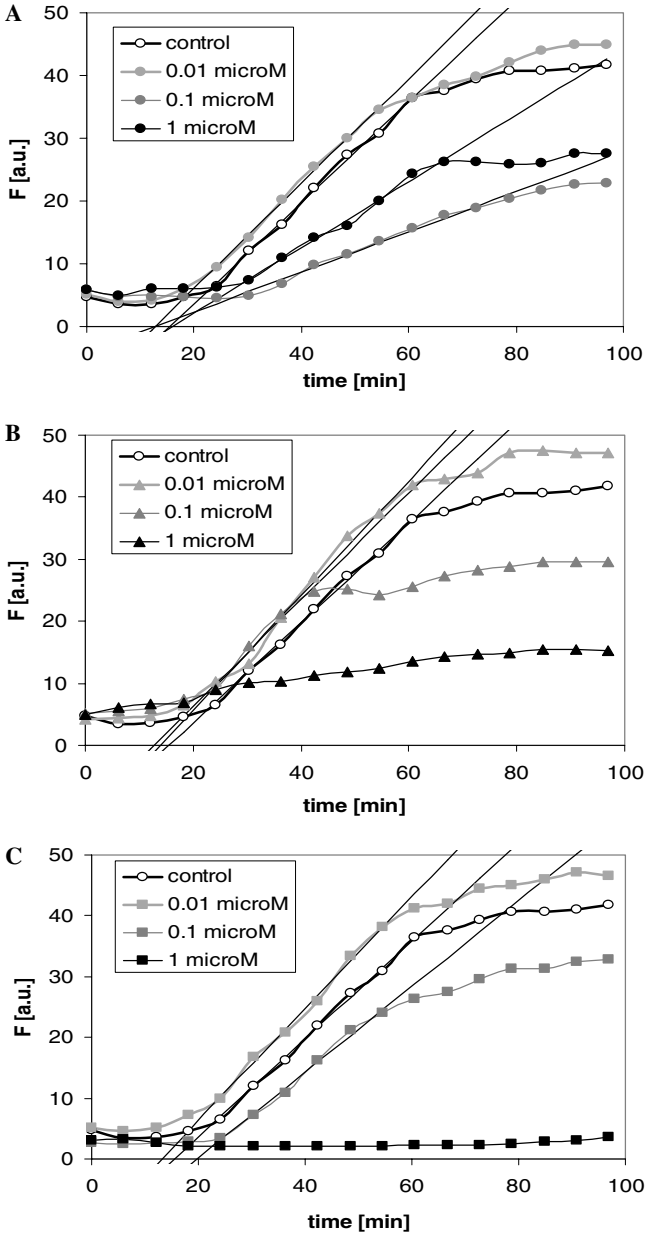


Fig. 1. Changes in fluorescence of ThT during the aggregation processes of Aβ 1–28 peptide in the presence of PAMAM G3 (A), PAMAM G4 (B), and PAMAM G5 (C).