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Influence of phosphorus dendrimers on the aggregation of the prion peptide PrP 185–208

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Abstract

Inhibition of fibril assembly is a potential therapeutic strategy in prion diseases. The effect of cationic phosphorous dendrimers on the aggregation process of the prion peptide PrP 185–208 was studied using a spectrofluorometric assay with thioflavin T (ThT) and Fourier transformed infrared spectroscopy in order to monitor the kinetics of the process and the changes in the peptide secondary structure. The results show that phosphorous dendrimers are able to clearly interfere with PrP 185–208 aggregation process by both slowing down the formation of aggregates (by causing a decrease of the nucleation rate) and by lowering the final amount of amyloid fibrils, a common hallmark of conformational diseases. The dendrimers effect on the aggregation process would imply their interaction with peptide monomers and oligomers during the nucleation phase.

Keywords: Amyloid; Fibril; Dendrimer; Polymer; Prion disease; Aggregation

Prion diseases are fatal neurodegenerative disorders that occur in hereditary and sporadic forms or can be transmitted from one organism to another [1]. Prion diseases result from conformational transformation from the normal cellular form of prion proteins (PrP^{C}) to the infectious scrapie isoform (PrP^{Sc}). The infectious form has a changed secondary structure from α-helical into β-sheets. PrP^{Sc} spontaneously form fibrils—amyloid-like structures. The accumulation of amyloids in the central nervous system precedes neurological dysfunction accompanied by neuronal vacuolation and astrocytic gliosis, leading eventually to death. Currently existing antiprion agents such as polyene antibiotics, anionic dyes, sulfated dextrans, anthracyclines, porphyrins, phthalocyanines, dapsone, and synthetic β-breaker peptides demonstrate only weak ability to stop prion propagation and none of them shows the ability to remove the pre-existing prions from an infected organism, so they would need to be administered prophylactically [2–10]. The first publication, which described that dendrimers have their own potentially therapeutic activity against prion diseases were published in 1999 [11]. Dendrimers are synthetic polymers characterized by a specific structure. These macromolecules consist of a core to which branched monomers are radially attached. When a layer of monomers is attached, the so-called ''generation'' of dendrimers increases. Such a synthesis results in a globular shape and many end groups on the surface. It was proved that polyamidoamine (PAMAM) and polypropylenimine (PPI) dendrimers possessing cationic amino groups on the surface were able to eliminate PrP^{Sc} from scrapie-infected neuroblastoma cells (ScN2a) [12]. The potency of both PAMAM and PPI dendrimers in purging PrP^{Sc} from ScN2a cells enhanced as the generation increased. It suggested that the presence of amino surface groups that increases with generation was crucial for antiprion activity.

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These promising results encouraged to study other types of dendrimers. Solassol et al. tested three generations of phosphorus—containing cationic dendrimers (3rd, 4th, and 5th) for anti-prion activity [13]. These dendrimers are characterized by the presence in their backbone of aminothiophosphates and they possess protonated tertiary amine groups on the surface [14]. Therefore, they have a hydrophilic surface and a hydrophobic backbone which allows very efficient membrane penetration. They are nontoxic in a broad concentration range and the presence of phosphorus atoms provides that dendrimers are stable against both nucleophilic attack and acid-catalyzed hydrolysis. It was shown that phosphorus dendrimers were able to inhibit the formation of PrP^{Sc} in ScN2a cells and remove the pre-existing PrP^{Sc} from infected cells as it was detected by Western blot after protease K digestion. Moreover, ScN2a cells lost their infectivity after the treatment with dendrimers. Even after up to eight passages PrP^{Sc} could not be detected in the cells inoculated with dendrimer-treated cells, while cells inoculated with control ScN2a accumulated a significant level of PrP^{Sc} after only three passages. The ability of dendrimers to purge pre-existing PrP^{Sc} was confirmed by incubating them with brain homogenates infected with different prion strains. Phosphorus dendrimers were the first ones that were tested *in vivo*. They inhibited PrP^{Sc} replication in the spleen of mice that had been intraperitoneally inoculated with scrapie. This is an important feature because it is known that a spleen accumulates PrP^{Sc} long before the agent reaches a central nervous system [15].

There is an alternative method to animal- and cell-based assays to screen potential antiprion drugs. It is possible to use truncated prion peptides and in the absence of cellular factors expose them to destabilizing factors to mimic the conditions that lead to creation of fibrils. The accumulation of amyloids can be e.g. monitored by changes in the fluorescence of thioflavin T (ThT), which is sensitive to the presence of amyloid fibrils [16,17]. Additionally, it is possible to use circular dichroism (CD) or Fourier transformed infrared spectroscopy (FTIR) to follow transformation of secondary structure into β-forms characteristic for aggregates [18,19]. These structural techniques allow monitoring the kinetics of the process and give additional information about the inhibitors [20]. Such an approach has been previously applied several times to investigate the antiprion properties of PAMAM and PPI dendrimers [21–24].

In this work, we have studied the aggregation process of the prion peptide PrP 185–208 in the presence of the fourth generation of phosphorous dendrimers. This fragment of prion protein is especially interesting for several reasons. Firstly, it has been demonstrated that it easily forms fibrils [22]. Secondly, a computational study has shown that residues 180–193 are one of the fibrilization sites in the prion protein [25]. Finally, Mahfoud et al. identified a possible sphingolipid binding domain, structurally homologous in the Alzheimer's peptide 1–28 and in the fragment of prion protein 185–208 [26]. It gives hopes to translate some results when searching for efficient drugs against Alzheimer's disease, since this disorder is also associated with the formation of amyloid aggregates. The results show that phosphorous dendrimers are able to clearly interfere with PrP 185–208 aggregation process by both slowing down the formation of aggregates and by lowering the final amount of amyloid fibrils, a common hallmark of conformational diseases.

Materials and methods

Materials. Synthetic PrP 185–208 [KQHTVTTTTKGENFTETD VKMMER] was purchased from JPT Peptide Technologies GmbH (Berlin, 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. The fourth generation of phosphorous dendrimers was synthesized in the Laboratoire de Chimie de Coordination de CNRS as described previously [14]. Molecular weight of this compound ($C_{1296}H_{2256}N_{375}Cl_{96}O_{90}P_{93}S_{90}$) equals to 33702 Da. This dendrimer possesses 96 cationic end groups. The chemical structure was shown previously [13]. Dendrimers were 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 thioflavin T (ThT)—a dye, which fluorescence depends on the presence of amyloid structures. 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 was added (final concentrations of 35 μmol/l) and pH was adjusted to 5.5 with aliquots of HCl. The aggregation process was triggered by the addition of 0.041 mg/ml of heparin. Fluorescence measurements were carried out with a SLM-Aminco 8000 spectrofluorimeter. Excitation and emission wavelengths were set at 450 and 490 nm, respectively. Temperature was controlled with a thermostatic bath at 37 °C.

Kinetic analysis. Spectrofluorimetric data were analyzed in order to calculate the kinetic constants. It was assumed that the aggregation process proceeded according to a nucleation dependent polymerization mechanism [27,28]. The approach considers amyloid fibril formation as an autocatalytic process with a nucleation reaction followed by an elongation one with its respective kinetic constants, k_n and k_e. Experimental data can be fitted to such a model using the following equation:

$$f = \frac{\rho \cdot e^{(1+\rho)\cdot k\cdot t} - 1}{1 + \rho \cdot e^{(1+\rho)\cdot k\cdot t}} \quad (1)$$

where f is the fraction of the fibril form; $k = k_e \cdot a$, being a is the initial peptide concentration; and $\rho = \frac{k_n}{k}$.

In order to fit the experimental data to Eq. (1), fluorescence was converted to fraction of fibril formation, considering $f = 0$ at $t = 0$ and $f = 1$ at the plateau of each sigmoidal curve.

Changes in the secondary structure—Fourier transform infrared spectroscopy (FTIR) experiments. The peptide was lyophilized and then dissolved in Hepes/D_2O buffer (10 mmol/l) to a final concentration 1 mmol/l. pD was adjusted to 5.5. Then heparin was added (0.82 mg/ml). The sample was placed between two CaF_2 windows separated with a 50 μm spacer. FTIR spectra were recorded at 37 °C with an FTIR Mattson Polaris spectrometer, equipped with a cooled liquid nitrogen mercury–cadmium–telluride (MCT) detector, at a nominal resolution of 2 cm^{-1}. The spectrometer was continuously purged with dry air (dew point lower than −60 °C). All spectra were corrected for atmospheric water vapor contribution. Two-hundred scans were averaged per spectrum using the shuttle device. Spectra were collected as a function of time as indicated in Fig. 4. Solvent contributions were always subtracted from the spectra.

Electron microscopy. Ten microliters of sample from the fluorescence cuvette (see ThT assay) were placed on a carbon 400 mesh grid. It was