Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 364 (2007) 20–25
BBRC
www.elsevier.com/locate/ybbrc

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

Barbara Klajnert [a,*], Marta Cortijo-Arellano [b], Josep Cladera [b], Jean-Pierre Majoral [c], Anne-Marie Caminade [c], Maria Bryszewska [a]

[a] Department of General Biophysics, University of Lodz, 12/16 Banacha Street, 90-237 Lodz, Poland
[b] Center of Studies in Biophysics, Universitat Autonoma de Barcelona, Catalonia, Spain
[c] Laboratoire de Chimie de Coordination, CNRS Toulouse, France

Received 19 September 2007
Available online 1 October 2007

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.

* Corresponding author. Fax: +48 42 635 44 74.
E-mail address: aklajn@biol.uni.lodz.pl (B. Klajnert).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.09.083

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

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

Changes in the fluorescence of ThT were used to monitor the formation of amyloid fibrils (Fig. 1). ThT is a highly selective dye which fluorescence depends on the formation of amyloid aggregates (fibrils). In agreement with previous results [22,23], PrP 185 formed amyloid fibrils following the characteristic sigmoid which can be interpreted as a nucleation-dependent polymerization. Adding dendrimers caused a decrease of the final concentration of fibrils. The dendrimer was most efficient at reducing the final fibrils concentration when present at a dendrimer/peptide ratio of 0.02. Both, 10 times lower, and ten times higher ratios also reduced the amount of fibrils, but not to the same extent.

Results were analysed in terms of a nucleation dependent polymerization model [27], from which the nucleation and elongation rate constants were calculated (Table 1). The process of fibrilization can be divided into a nucleation phase (known also as a lag phase) and an elongation phase. During the lag phase, peptide monomers slowly combine to form nuclei. Addition of more peptide monomers to those nuclei can, according to the model, result in the exponential elongation (fibril formation) phase, which is much faster than the lag phase. Adding dendrimers decreased both the nucleation and the elongation rate constants but the effect on the nucleation process was bigger and achieved maximum for a dendrimer/peptide ratio of 0.02 (dendrimer concentration 0.1 μmol/l) when the constant decreased five times. In the case of the elongation process the decrease was dendrimer concentration-dependent.

FTIR spectroscopy provides information on the secondary structure of proteins or peptides. Proteins known to be predominantly α-helical exhibit amide I absorptions in the spectral range 1645–1655 cm^{-1}, while proteins with β-sheet structure absorb between 1610 and 1640 cm^{-1} [29,30]. Changes in the secondary structure of peptides incubated with heparin along time are shown in Fig. 2. Bands at 1613 cm^{-1} are characteristic of aggregated β-sheet structure and bands at 1648 cm^{-1} can be assigned to a mixture of α-helices and random structures. These two bands were the main spectral characteristics of the different experimental

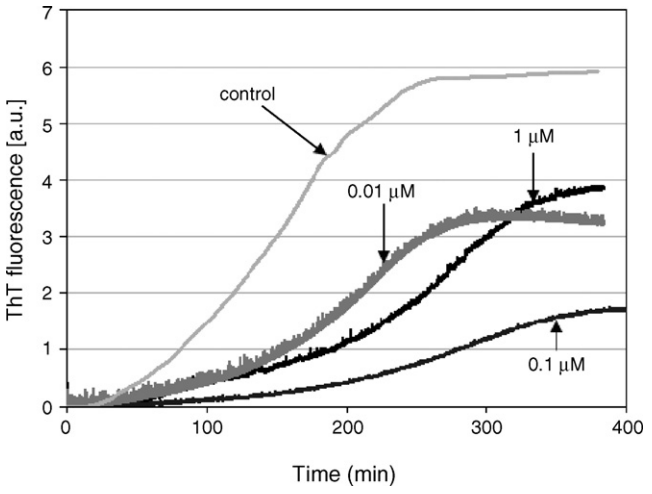

Fig. 1. Changes in ThT fluorescence during the aggregation process of PrP 185–208 in the presence of phosphorous dendrimers.

Table 1
Nucleation (k_n) and elongation (k_e) rate constants calculated by fitting experimental data

Dendrimer concentration (μmol/l)	Dendrimer/peptide ratio	k_n (1/s)	k_e (l/mol s)
0 (control)	0	9.5×10^{-6}	8.8
0.01	0.0002	3.5×10^{-6}	8.8
0.1	0.002	1.9×10^{-6}	6.6
1	0.02	3.0×10^{-6}	6.4

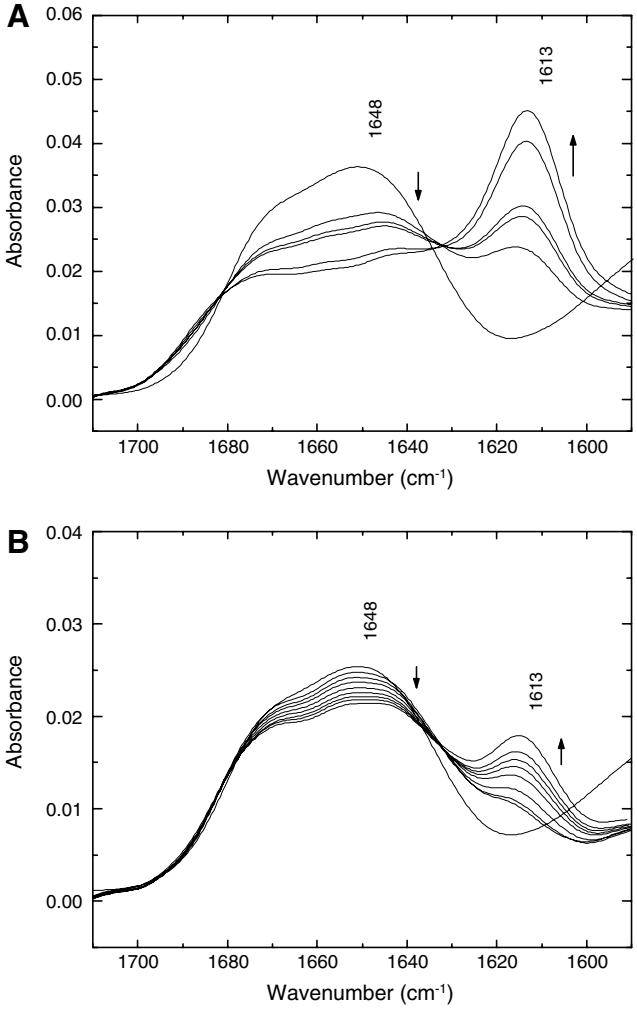

Fig. 2. Changes of Fourier-transform infrared spectra of PrP 185–208 depending on the time of incubation at pD 5.5 in the presence of heparin: (A) without dendrimer; (B) in the presence of dendrimer at a dendrimer/peptide ratio of 0.002.

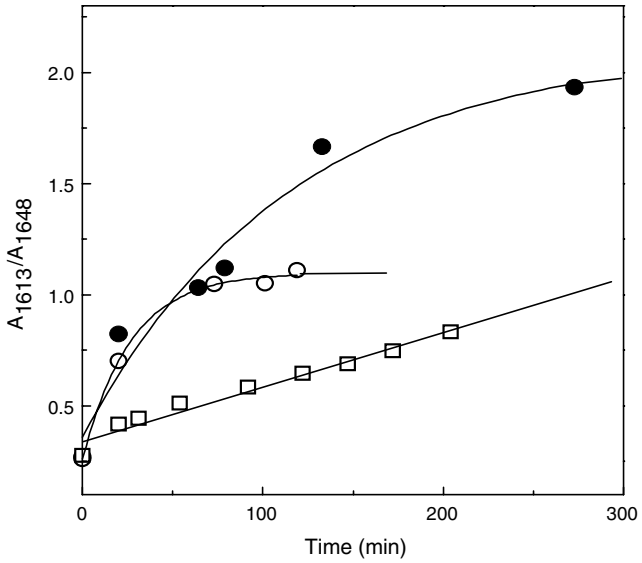

Fig. 3. Kinetics of the changes of the secondary structure. The formation of aggregated β-structure from α-helical and unordered structures has been represented as the ratio of absorbances at 1613 and 1648 cm^{-1} versus time. Full circles: control (PrP 1 mmol/l); hollow squares: PrP 1 mmol/l in the presence of dendrimer (dendrimer/peptide ratio = 0.002); hollow circles: PrP 1 mmol/l in the presence of dendrimer (dendrimer/peptide ratio = 0.02).

conditions checked (without and with dendrimers at different dendrimer/peptide ratios), although the aggregation band at 1613 cm^{-1} appeared with time at a different rate, depending on the dendrimer concentration. This fact is illustrated in Fig. 3, where the absorbance ratio A_{1613}/A_{1648} versus time is shown. It is apparent from the curves in Fig. 3 that no lag phase was measured, as one would expect since in the FTIR experiments peptide concentration is higher than in ThT experiments and moreover with this technique we detect not only the β-sheet fibrils but also other β-sheet aggregated (non fibrilar) structures present maybe in the high molecular weight oligomers typical of the nucleation phase [31]. Clearly, the presence of dendrimer at a dendrimer/peptide ratio of 0.002 slows dramatically down the aggregation process. A ten fold increase in dendrimer concentration however (dendrimer/peptide ratio of 0.02) did again accelerate the process although it reached a lower plateau, compared to the control (absence of dendrimer). Both these facts are in agreement with the observations in the ThT experiments (Fig. 1).

The effect of dendrimers on the morphology of PrP 185–208 amyloid aggregates was analyzed under electron microscope (Fig. 4). In all cases the characteristic long, interweaved fibrils were observed at the end of the sigmoids as reported in Fig. 1. This means that although decreasing in number in the presence of dendrimers, typical amyloid fibrils are present at the sigmoid plateau. The electron micrographs do not reveal the presence of any other type of aggregated species (amorphous aggregates for example) as a consequence of the presence of dendrimers.

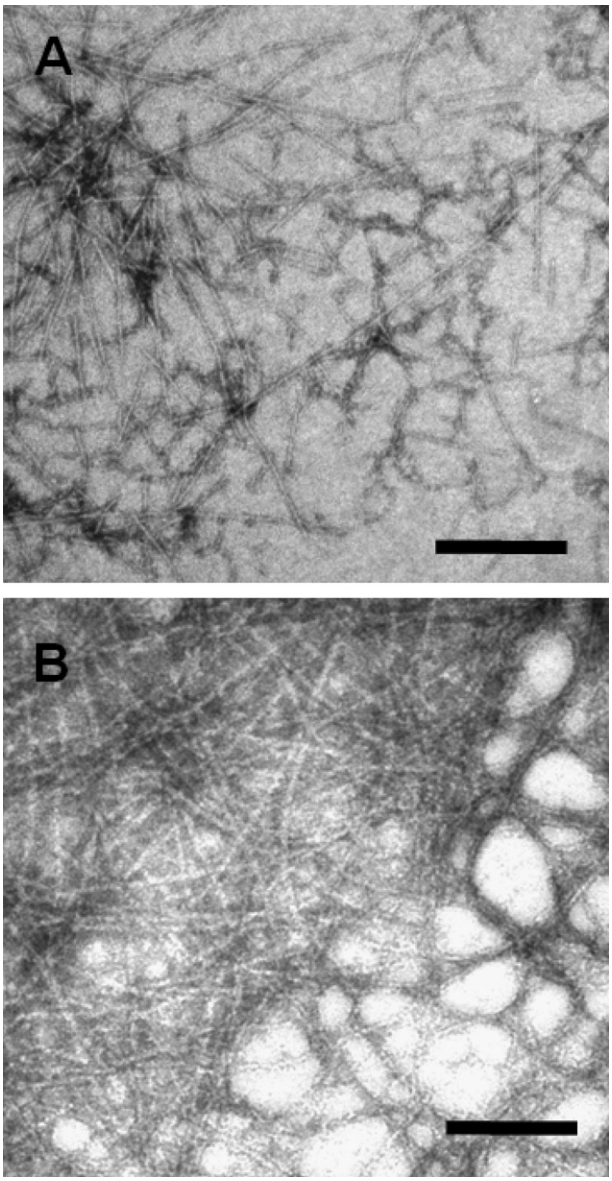

Fig. 4. Electron micrographs of PrP structures in the absence of dendrimers (A) and in the presence of phosphorous dendrimers at concentration 0.01 mmol/l (B). The length of the bar equals to 100 nm.

Discussion

The characterization of the molecular mechanisms by which different peptides and proteins related to conformational diseases form amyloid aggregates and the possibility of interfering with such a process are part of the strategies directed to find ways of preventing and healing these pathologies. Many proteins related or not to different pathologies, with no sequencial homology and fragments of these proteins have been shown to be able to form amyloid fibrils in vitro. PrP 185–208, a fragment of the human prion protein, is known to form amyloid aggregates in vitro, in the presence of heparin at pH 5.5, following the typical nucleation-dependent polymerization kinetics [22–24]. We have previously reported that the aggregation

of PrP 185–208 can be affected by PAMAM dendrimers. In the present work, we have used this peptide as a model system in order to check the capacity of phosphorus-containing dendrimers to interfere with the aggregation process. The kinetic analysis of the ThT fluorescence experiments clearly show that: (1) the presence of phosphorous dendrimers decreases the final amount of amyloid fibrils (inhibitory effect) and (2) dendrimers decrease the nucleation rate, that is, they have an effect on the nucleation phase of the polymerization process. The ThT results, seem therefore to indicate that, when present during the peptide aggregation process, phosphorus dendrimers do interfere with the molecular interactions between peptide monomers and/or oligomers, characteristic of the nucleation phase, that give eventually fibrils. This is in agreement with previous experiments. Solassol et al. demonstrated the retention of prion protein on phosphorus dendrimer-coated amberlite beads, therefore they postulated that these dendrimers can bind to prion protein molecules [13].

Until now no experimental structural data existed on PrP 185–208. Infrared spectra show that at pH 7.5 the peptide has a spectrum which denotes a mixture of helical and unordered structure, comparable to the infrared spectra of other amyloid peptides in non-aggregating conditions [32]. Upon aggregation at pH 5.5, the appearance of characteristic band at 1613 cm^{-1} reveals an effect of dendrimers on the aggregation kinetics, compatible with what it is observed in the ThT experiments, at the same dendrimer/peptide ratios. In both cases the existence of an optimum dendrimer concentration, corresponding to a maximal dendrimer effect on the rate of the nucleation phase, becomes apparent: the maximal effect is observed at a dendrimer/peptide ratio of 0.002. A tenfold lower or tenfold higher ratio gives a faster nucleation phase, although in all cases the amount of final fibrils formed is lower than for the control.

When we consider the interactions between peptides and dendrimers, the fact that dendrimer activity is dependent on cationic surface groups leads us to the conclusion that electrostatic interactions are probably predominant. Our experiments were conducted at pH 5.5 that mimicked conditions that occur in acidic cell compartments like lysosomes, which are presumably the place where dendrimers interact with PrP^{Sc} [12]. At pH 5.5 approximately 70% of terminal tertiary amines on the surface of dendrimers are protonated. It is possible that individual cationic groups on the surface of dendrimers might bind to monomers or oligomers during the lag phase. Moreover the dendrimer might pull away bound peptide molecules from the aggregated structures and/or prevent such molecules from reaggregating. The large number of surface groups and a high level of their protonation explain why relatively low concentrations of dendrimers were active against bigger concentration of the peptide.

Time-course experiments demonstrated that phosphorus dendrimers are able to remove quickly existing PrP^{Sc} molecules and the clearance occurred within 4 h of treatment [13]. Because phosphorus dendrimers are able to clear preexisting PrP^{Sc}, their mechanism of action cannot simply involve binding to monomers and inhibiting de novo synthesis. Similarly as in the case of e.g. PAMAM dendrimers [23], the breakage of oligomers or whole fibrils caused by dendrimers cannot be excluded.

If dendrimers are going to be considered as therapeutic agents for prion diseases, some difficulties must be overcome first, e.g. the problem of crossing the blood–brain barrier. In the case of phosphorus dendrimers, there is no data reporting their entry into the central nervous system through the blood–brain barrier and for such big molecules it may turn out to be impossible. It seems likely that dendrimers would need to be administered directly to cerebrospinal fluid or perhaps synthesized as prodrugs capable of crossing blood–brain barrier [12]. However this does not exclude the possibility of using them in the treatment as post-exposure prion prophylaxis in the way proposed for other molecules [33].

Acknowledgments

Study sponsored by Grant No. 2 P05F 008 30 from the Polish Ministry of Science and Higher Education and by NATO Collaborative Linkage grant no EAP. C.L.G. 9811751.

References

[1] S.B. Prusiner, Scrapie prions, Annu. Rev. Microbiol. 43 (1989) 345–374.
[2] C.F. Farquhar, A.G. Dickinson, Prolongation of scrapie incubation period by an injection of dextran sulphate 500 within the month before or after infection, J. Gen. Virol. 67 (1986) 463–473.
[3] D. McKenzie, J. Kaczkowski, R. Marsh, J. Aiken, Amphotericin B delays both scrapie agent replication and PrP-res accumulation early in infection, J. Virol. 68 (1994) 7534–7536.
[4] L. Ingrosso, A. Ladogana, M. Pocchiari, Congo red prolongs the incubation period in scrapie-infected hamsters, J. Virol. 69 (1995) 506–508.
[5] F. Tagliavini, R.A. McArthur, B. Canciani, G. Giaccone, M. Porro, M. Bugiani, P.M.-J. Lievens, O. Bugiani, E. Peri, P. Dall'Ara, M. Rocchi, G. Poli, G. Forloni, T. Bandiera, M. Varasi, A. Suarato, P. Cassutti, M.A. Cervini, J. Lansen, M. Salmona, C. Post, Effectiveness of anthracycline against experimental prion disease in Syrian hamsters, Science 276 (1997) 1119–1122.
[6] L. Manuelidis, W. Fritch, I. Zaitsev, Dapsone to delay symptoms in Creutzfeldt-Jakob disease, Lancet 352 (1998) 456.
[7] K.T. Adjou, R. Demaimay, J.-P. Deslys, C.L. Lasmézas, V. Beringue, S. Demart, F. Lamoury, M. Seman, D. Dormont, MS-8209, a watersoluble amphotericin B derivative, affects both scrapie agent replication and PrP accumulation in Syrian hamster scrapie, J. Gen. Virol. 80 (1999) 1079–1085.
[8] C. Farquhar, A. Dickinson, M. Bruce, Prophylactic potential of pentosan polysulphate in transmissible spongiform encephalopathies, Lancet 353 (1999) 117.
[9] S.A. Priola, A. Raines, W.S. Caughey, Porphyrin and phthalocyanine antiscrapie compounds, Science 287 (2000) 1503–1506.
[10] C. Soto, R.J. Kascsak, G.P. Saborío, P. Aucouturier, T. Wisniewski, F. Prelli, R. Kascsak, E. Mendez, D.A. Harris, J. Ironside, F. Tagliavini, R.I. Carp, B. Frangione, Reversion of prion protein conformational changes by synthetic beta-sheet breaker peptides, Lancet 355 (2000) 192–197.

[11] S. Supattapone, H.-O.B. Nguyen, F.E. Cohen, S.B. Prusiner, M.R. Scott, Elimination of prions by branched polyamines and implications for therapeutics, Proc. Natl. Acad. Sci. USA 96 (1999) 14529–14534.
[12] S. Supattapone, H. Wille, L. Uyechi, J. Safar, P. Tremblay, F.C. Szoka, F.E. Cohen, S.B. Prusiner, M.R. Scott, Branched polyamines cure prion-infected neuroblastoma cells, J. Virol. 75 (2001) 3453–3461.
[13] J. Solassol, C. Crozet, V. Perrier, J. Leclaire, F. Béranger, A.-M. Caminade, B. Meunier, D. Dormont, J.-P. Majoral, S. Lehmann, Cationic phosphorus-containing dendrimers reduce prion replication both in cell culture and in mice infected with scrapie, J. Gen. Virol. 85 (2004) 1791–1799.
[14] C. Loup, M.A. Zanta, A.M. Caminade, J.P. Majoral, B. Meunier, Preparation of water-soluble cationic phosphorus-containing dendrimers as DNA transfecting agents, Chem. Eur. J. 5 (1999) 3644–3650.
[15] C.I. Lasmezas, J.Y. Cesbron, J.P. Deslys, R. Demaimay, K.T. Adjou, R. Rioux, C. Lemaire, C. Locht, D. Dormont, Immune system-dependent and -independent replication of the scrapie agent, J. Virol. 70 (1996) 1292–1295.
[16] H. LeVine 3rd, Thioflavin T interaction with synthetic Alzheimer's disease beta-amyloid peptides: detection of amyloid aggregation in solution, Protein Sci. 2 (1993) 404–410.
[17] M.R.H. Krebs, E.H.C. Bromley, A.M. Donald, The binding of thioflavin T to amyloid fibrils: localisation and implications, J. Struct. Biol. 149 (2005) 30–37.
[18] C. Selvaggini, L. De Goia, L. Cantu, E. Ghibaudi, L. Diomede, F. Passerini, G. Forloni, O. Bugiani, F. Tagliavini, M. Salmona, Molecular characteristics of a protease-resistant, amyloidogenic and neurotoxic peptide homologous to residues 106–126 of the prion protein, Biochem. Biophys. Res. Commun. 194 (1993) 1380–1386.
[19] M. Salmona, P. Malesani, L. De Goia, S. Gorla, M. Bruschi, A. Molinari, F. della Vedova, B. Pedrotti, M.A. Marrari, T. Awan, O. Bugiani, G. Forloni, F. Tagliavini, Molecular determinants of the physicochemical properties of a critical prion protein region comprising residues 106–126, Biochem. J. 342 (1999) 207–214.
[20] J. Masel, V.A.A. Jansen, Designing drugs to stop formation of prion aggregates and other amyloids, Biophys. Chem. 88 (2000) 47–59.
[21] P.M.H. Heegaard, H.G. Pedersen, J. Flink, U. Boas, Amyloid aggregates of the prion peptide PrP106–126 are destabilised by oxidation and by the action of dendrimers, FEBS Lett. 577 (2004) 127–133.
[22] B. Klajnert, M. Cortijo-Arellano, M. Bryszewska, J. Cladera, Influence of heparin and dendrimers on the aggregation of two amyloid peptides related to Alzheimer's and prion diseases, Biochem. Biophys. Res. Commun. 339 (2006) 577–582.
[23] B. Klajnert, M. Cortijo-Arellano, J. Cladera, M. Bryszewska, Influence of dendrimer's structure on its activity against amyloid fibril formation, Biochem. Biophys. Res. Commun. 345 (2006) 21–28.
[24] B. Klajnert, J. Cladera, M. Bryszewska, Molecular interactions of dendrimers with amyloid peptides: pH dependence, Biomacromolecules 7 (2006) 2186–2191.
[25] I.B. Kuznetsov, S. Rackovsky, Comparative computational analysis of prion proteins reveals two fragments with unusual structural properties and a pattern of increase in hydrophobicity associated with disease-promoting mutations, Protein Sci. 13 (2004) 3230–3244.
[26] R. Mahfoud, N. Garmy, M. Maresca, N. Yahi, A. Puigserver, J. Fantini, Identification of a common sphingolipid-binding domain in Alzheimer, prion, and HIV-1 proteins, J. Biol. Chem. 277 (2002) 11292–11296.
[27] R. Sabate, M. Gallardo, J. Estelrich, An autocatalytic reaction as a model for the kinetics of the aggregation of beta-amyloid, Biopolymers 71 (2003) 190–195.
[28] M. Kamihira, A. Naito, S. Tuzi, A.Y. Nosaka, H. Saito, Conformational transitions and fibrillation mechanism of human calcitonin as studied by high-resolution solid-state ^{13}C NMR, Protein Sci. 9 (2000) 867–877.
[29] A. Elliot, E.J. Ambrose, Structure of synthetic polypeptides, Nature 165 (1950) 921–922.
[30] E.J. Ambrose, A. Elliot, Infrared spectroscopic studies of globular protein structure, Proc. R. Soc. Lond. Ser. A 208 (1951) 75–90.
[31] M.D. Kirkitadze, M.M. Condron, D.B. Teplow, Identification and characterization of key kinetic intermediates in amyloid beta-protein fibrillogenesis, J. Mol. Biol. 312 (2001) 1103–1119.
[32] P.E. Fraser, D.R. McLachlan, W.K. Surewicz, C.A. Mizzen, A.D. Snow, J.T. Nguyen, D.A. Kirschner, Conformation and fibrillogenesis of Alzheimer Abeta peptides with selected substitution of charged residues, J. Mol. Biol. 244 (1994) 64–73.
[33] S. Sethi, G. Lipford, H. Wagner, H. Kretzschmar, Postexposure prophylaxis against prion disease with a stimulator of innate immunity, Lancet 360 (2002) 229–230.