ROLE OF THE STRUCTURAL CHARACTERISTICS OF DENDRIMERS IN THE MANIFESTATION OF THE ANTIAMYLOID PROPERTIES

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Abstract

Among the compounds able to efficiently inhibit the amyloid aggregation of proteins and decompose the amyloid aggregates that cause neurodegenerative diseases, of particular interest are dendrimers, which represent individual macromolecules with the hypercrosslinked architectures and given molecular parameters. This short review outlines the peculiarities of the antiamyloid activity of dendrimers and discusses the effect of dendrimer structures and external factors on their antiamyloid properties. The potential of application of dendrimers in further investigations on the aggregation processes of amyloid proteins as the compounds that exhibit the remarkable antiamyloid activity is evaluated.

Introduction

Dendrimers are branched macromolecular compounds with regular structures that can be obtained in a directed and controlled manner owing to the stepwise synthesis [1]. On the one hand, dendrimers possess all the useful properties of conventional polymers: high molar masses and a large number of functional groups; on the other hand, they are individual compounds with certain molar masses and sizes [2]. These features make them promising objects for biological applications, in particular, for investigation of their interaction with proteins [3].

Proteins accomplish the most important functions in the organism, including enzymatic, transport, constructional, and regulatory; they are involved in the composition of blood, *etc*. The structural conversion of proteins and associated formation of amyloid fibrils in the human body lead to the development of neurodegenerative diseases, most of which have no appropriate treatment nowadays [4]. They include Alzheimer's, Parkinson's, Huntington's, and prion diseases (Creutzfeldt–Jakob disease, Gerstmann–Sträussler–Scheinker syndrome, fatal familial insomnia, and kuru) [5, 6]. These diseases are accompanied by the appearance of amyloid plaques in the matrix of neural tissue that consist of amyloid fibrils. The fibrils are formed from certain proteins normally present in cells (beta-amyloid, alpha-synuclein, prion protein, *etc*.), which lose their normal structure and undergo unfolding and aggregation [7]. This process occurs sequentially; therefore, there are several means to affect it. Thus, the ligands can bind with a protein and block its aggregation or, in contrast, decompose the formed aggregates [8].
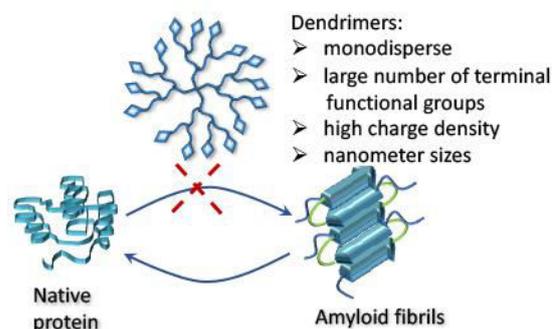
Certain advances in this field were achieved using low-molecular chemical compounds [9], such as phenol [10, 11] and cinnamic acid [12, 13] derivatives, and some polymers, for example, polystyrene sulfonate [14, 15]. However, the use of low-molecular compounds is low effective due to weak binding with the protein. The polydispersity of synthetic polymers complicates the investigation of the resulting protein–polymer complexes due to their nonuniformity. Furthermore, long and short polymer chains often produce different and sometimes even opposite effects: the long chains can inactivate the protein, while the short chains, in contrast, can promote aggregation. Therefore, the use of polymers does not afford unambiguous and reproducible results.

The promising candidates for the compounds that can affect the amyloid aggregation of proteins are dendrimers, which combine high molar masses with the monodispersity of molecular characteristics. The dendritic form of a molecule provides a large number of functional groups and, consequently, offers an opportunity to form a system with a great charge and high charge density in each macromolecule. This property enables the formation of strong complexes with proteins owing to the multivalent interaction and can be an additional advantage of dendrimers in the case when the stabilization of the protein molecule is important [16].

Peculiarities of the interaction of dendrimers with amyloid proteins

The first investigations on the antiamyloid activity of dendrimers were carried out using four generations of

poly(amidoamine) (PAMAM) and poly(propylene imine) (PPI) dendrimers of the second and fourth generations [17, 18]. The experiments were performed on scrapie-infected neuroblastoma cells. Scrapie is a disease affecting the nervous system of sheep and goats that is caused by damages in the secondary structure of the prion protein, which converts from the properly folded form (PrP^C) to the misfolded one (PrP^{Sc}) saturated by beta-sheet. It was found that dendrimers can remove PrP^{Sc} from neuroblastoma cells, and their efficiency grows with an increase in the generation number. This means that the antiamyloid activity of dendrimers is apparently connected with their great charge and high density of the charge. This was confirmed by the absence of the effect upon application of neutral PAMAM-OH dendrimers as well as linear polymer analogs. The result depended also on the dendrimer concentration and test duration. It should be noted that the appearance of PrP^{Sc} was not detected for further three weeks after the removal of the dendrimer from the medium [17].

Subsequently, the investigations were expanded. The antiamyloid properties were observed for PAMAM [19], PPI [20], phosphorus [21], polylysine [22, 23], and different glycodendrimers [24, 25].

The dendrimers exhibited the antiamyloid properties against a series of amyloidogenic proteins and peptides: beta-amyloid peptide associated with the development of Alzheimer's disease [26, 27], alpha-synuclein involved in Parkinson's disease [28–30], tau protein associated with both Alzheimer's and Parkinson's diseases [31], and prions which cause the development of spongiform encephalopathies [17–19].

In vitro experiments confirmed the ability of dendrimers to affect the amyloid aggregation of proteins. They allowed one not only to study the effect of dendrimers on the already formed aggregates, as it was shown in the cell studies, but also to evaluate the ability of dendrimers to hamper the process of protein amyloid aggregation. Again it was shown that a key role in the antiamyloid activity was played by the dendrimer structure, more precisely, the great charge and its high density [19]. Thus, PPI dendrimers modified with guanidine groups inhibited the fibrillation of the prion protein 106-126 more efficiently than the unmodified analogs [32]. The dendrimers with guanidine groups were fully charged, whereas the unmodified ones had lower charges.

At the same time, the ability of the most frequently used amino-containing dendrimers to suppress the aggregation of proteins strongly depends on the medium pH. This dependence was studied in detail by Klajnert *et al.* [33]. The effect was caused by the protonation degree of both dendrimer and protein. The most favorable conditions for the formation of complexes between the dendrimer and the protein are in the range of low pH values of the medium, at which the high ionization degrees of the dendrimer amino groups and the protein histidine residues are achieved [17, 33, 34]. Thus, PEI and PPI dendrimers, which effectively remove PrP^{Sc} from neuroblastoma cells at pH = 4.0, did not exert any effect under neutral conditions [17, 18]. Hence, the applicability of these pH-dependent dendrimers *in vivo*, where the physiological level of pH is within neutral values (pH = 7.4), is significantly restricted.

Another important aspect which should be taken into account while using flexible dendrimers, such as PAMAM, PPI, and polylysine dendrimers, is the ability of their chains to incurve to the macromolecule center, which leads to changes in the amount of surface functional groups and, as a consequence, affects the interaction with the protein [34–36].

Furthermore, it was revealed that the use of cationic (positively charged) dendrimers is preferred. Thus, anionic PPI dendrimers of the fifth generation modified with sulfo groups led to a reduction in the content of PrP^{Sc} *in vitro* but were found to be inactive in investigations with cell lines [34]. The observed difference is likely to be explained by the low permeability of negatively charged cell membranes for anionic dendrimers.

In this respect, the promising compounds for the investigation of peculiarities of binding of amyloid proteins with dendrimers, evaluation of the effect of external factors on the complex formation, and elucidation of the main fundamental principles that account for the ability of dendrimers to affect the amyloid aggregation of proteins are the cationic dendrimers, which charge does not depend on the properties of a solvent and medium conditions, and the amount of terminal groups exposed to a solution remains unchanged. These compounds include cationic pyridylphenylene dendrimers (CPPDs) (Fig. 1).
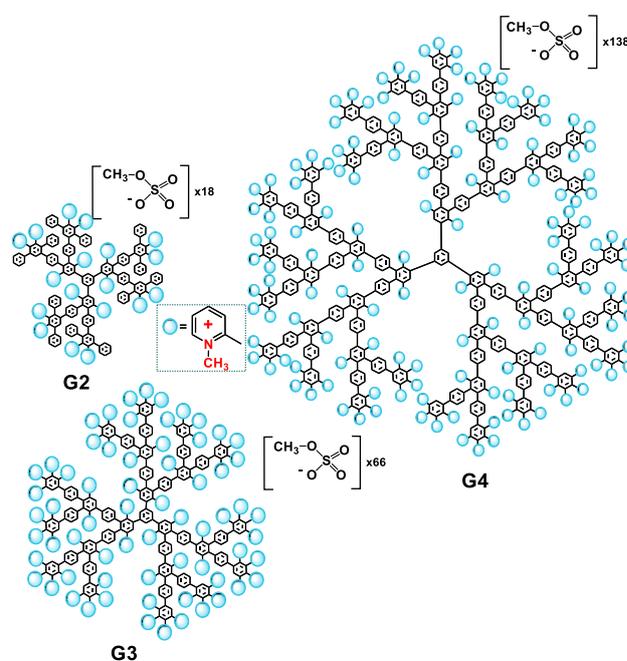

Figure 1. Cationic pyridylphenylene dendrimers of the second (G2), third (G3), and fourth (G4) generations.

The presence of the quaternary nitrogen atom in the structure ensures charge stability and its independence on pH. The restricted rotation around C–C bonds which connect the aromatic rings, in turn, provides general structure rigidity [37]. Owing to this, the branches of these dendrimers do not undergo the so-called backfolding and the amount of functional groups on the surface always remains unchanged. These properties enable stable interaction of the dendrimers with biological objects, including proteins, when the medium conditions change.

The gradual exploration of the interaction of CPPDs with the complete recombinant sheep prion protein allowed for suggesting the mechanism of antiamyloid activity, the investigation of the effect of the dendrimer structure on the properties of the resulting complexes, the definition of the main

structure–activity relationships for the complex formation and their effect on the dendrimer ability to suppress the amyloid aggregation of proteins as well as decompose the formed aggregates.

Thus, it was revealed [38] that CPPD dendrimers form strong complexes with the prion protein owing to the electrostatic and hydrophobic forces. While the positively charged pyridinium units of the dendrimers interact with local negatively charged amino acid residues of the protein, the phenylene moieties are involved in the hydrophobic interactions with hydrophobic groups of the protein (Fig. 2). The complexes did not decompose upon the addition of sodium chloride up to the concentration of 1.5 mol/L and during a competitive reaction with the oppositely charged polymer, which indicates a substantial contribution of hydrophobic interactions to the complex stability.

The binding of the dendrimers with the protein did not affect significantly its secondary structure, which was confirmed by the results of circular dichroism spectroscopic and molecular dynamics studies. Moreover, it was found that the amount of molecules capable of binding with the protein reduced as the dendrimer generation increased. The contribution of hydrophobic interactions to binding also increased, which, in turn, enhanced the binding constants (1.5×10^{-5}, 1.3×10^{-6}, and 2.9×10^{-6} M^{-1} for the second, third, and fourth generations, respectively) and led to the higher stability of the complex [38].

The binding of CPPD with the prion subsequently blocked the amyloid aggregation of the prion protein. The dendrimers were found to inhibit the formation of not only amyloid fibrils but also amyloid oligomers, which currently are considered to be the most toxic amyloidogenic forms of proteins [39, 40]. The amyloid oligomers were obtained according to the procedure published by Rezaei *et al.* [41], who showed that heating of the prion protein for 2 h gives rise to oligomeric structures.

Thus, the addition of the dendrimers to the prion protein during the formation of the oligomers blocked its structural conversion and prevented aggregation. The structures obtained in the presence of the dendrimers featured essentially lower content of beta-sheet compared to the control oligomers. The additional evidence for the prevention of aggregation was a reduction in the hydrodynamic diameter of the particles that were formed in the presence of the dendrimers.

However, the most convincing experiments were carried out on neuroblastoma cell culture, which clearly indicated the efficiency of the dendrimers. The prion oligomers, being toxic, caused cell death. In contrast, the structures obtained in the presence of the dendrimers did not affect the viability of neuroblastoma cells. The effect depended on the charge of the dendrimer molecule, which increased with the generation growth. The highest activity was exhibited by the dendrimer of the fourth generation which suppressed unfolding and aggregation of the prion protein in the concentration of 0.25 µM. Hence, the dendrimers not only inhibited the aggregation but also prevented the formation of toxic structures [39, 40].

CPPDs can suppress even deeper aggregation, namely, the formation of amyloid fibrils. The efficiency of inhibition of fibrillation also increased with the growth of the dendrimer generation, which indicated an important role of a great charge in the manifestation of the antiamyloid properties. Unlike the prion amyloid fibrils, the complexes obtained in the presence of
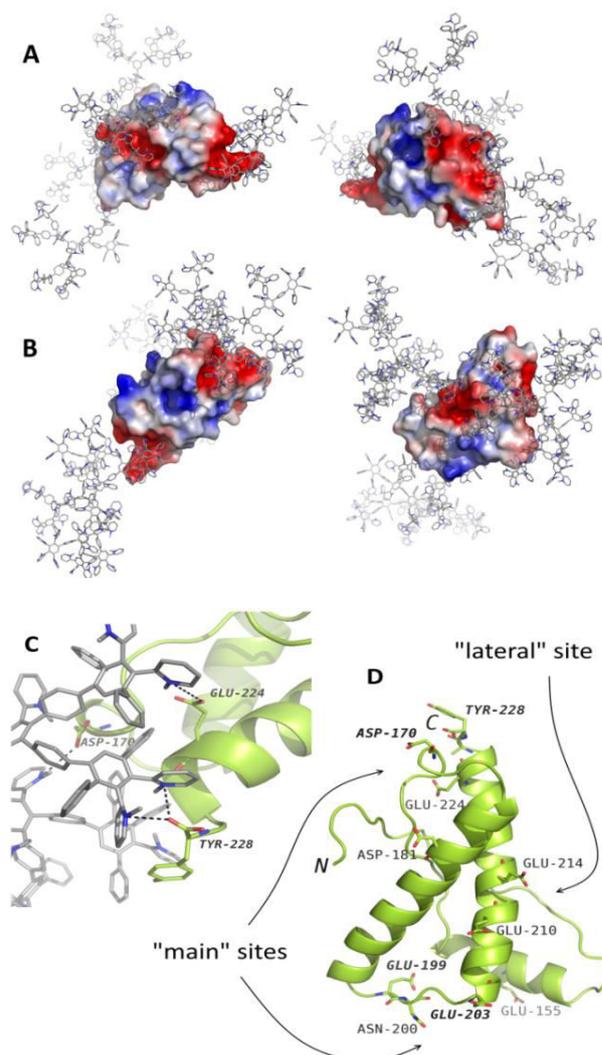

Figure 2. Results of the modeling of the interaction of the prion protein with G2 (**A**) and G3 (**B**) by molecular dynamics. The dendrimers are depicted in gray color with blue nitrogen atoms. The protein is colored according to the surface electrostatic potential: the positively charged regions are blue, the negatively charged ones are red. Extended image of the interaction of the dendrimers with the protein (**C**). Amino acid residues involved in the dendrimer binding (**D**). (Reprinted with permission from S. Sorokina *et al.*, *RSC Adv.*, **2017**, *7*, 16565–16574. DOI: 10.1039/C6RA26563D. Copyright (2017) Royal Society of Chemistry)

the dendrimers were susceptible to proteolysis. Therefore, besides the own ability of dendrimers to hamper amyloid aggregation, other cell mechanisms can be involved in their antiamyloid activity under *in vivo* conditions, thus, facilitating and supporting the dendrimer action.

It is important to note that, owing to the strong association with the dendrimers, the resulting prion protein–dendrimer complexes did not exhibit infectivity, which is characteristic of unfolded forms (unfolded monomers, oligomers, and protofibrils) of the prion protein. In other words, the prion protein loses its amyloidogenic properties in complexes with dendrimers [40].

Obviously, the arrangement of the binding sites of the ligands under consideration is highly important for the antiamyloid properties. The model for the interaction of CPPD with the prion protein demonstrates that the binding of these

dendrimers affects the region 190-200, which is involved in the amyloid transformation of the protein [38]. Owing to this, the dendrimers can block the amyloid transformation of the prion protein and prevent its aggregation. The hydrophobic interactions and multipoint contacts, which are realized owing to a great amount and high density of surface functional groups, enable strong binding and do not allow the complex to dissociate, which affords the reliable stabilization of the protein by the dendrimer. The aggregation inhibition occurs at the neutral pH values, whereas the polyamine compounds explored earlier were active only at the low values of pH [17–19].

Dendrimers can not only prevent aggregation of proteins but also decompose the already formed amyloid aggregates. The addition of CPPD to insoluble amyloid aggregates of the prion protein led to their decomposition owing to the formation of strong complexes with the dendrimer and conversion of the resulting complexes to a soluble state [42]. The activity of the dendrimers increased with the growth of a generation (charge) and the process occurred at the physiological value of pH equal to 7.4. The resulting complexes were stable and did not undergo repeated aggregation owing to the hydrophobic interactions observed earlier.

Since the treatment of nervous system diseases is complicated by the existence of the blood–brain barrier (BBB), the nanoscale sizes of the dendrimers play no small part. The permeability of BBB for the dendrimers was multiply confirmed in a range of investigations [43–45]. It is believed that the dendrimers can penetrate through BBB owing to the binding with specific receptors on the surface of endothelial cells [46]. A crucial role is played by the molecule sizes, which must not exceed 100 nm. A similar mechanism is realized during modification of the dendrimer surface with receptor-specific ligands, for example, low-density lipoprotein receptor-relative protein-1 (LRP1) or transferrin [47, 48]. In this case, the dendritic structure ensures the molecule multifunctionality. Whereas one part of the functional groups takes part in the covalent binding of a specific ligand, the other remains active for the manifestation of the antiamyloid properties. At the same time, many diseases of the nervous system are accompanied by the pathomorphological changes in BBB, which lead to loosening of the basal membrane and an increase in its permeability. This offers an opportunity for passive diffusion of nanoscale molecules. Santos *et al*. [49] showed that PAMAM dendrimers modified with poly(ethylene glycol) (PEG) were detected in the brain of mice in 24 h after the intravenous injection. It should be noted that the modification of the terminal PEG groups considerably reduced the interaction of endothelial cells with the membrane and reduced the possibility of receptor-mediated endocytosis.

As well as other polycationic compounds, dendrimers can exhibit cytotoxic effects, which increase with generation growth [50, 51]. However, Fischer *et al*. [51] showed that PAMAM dendrimers display lower toxicity than the analogous linear polymer. The cationic PAMAM and PPI dendrimers have comparable cytotoxic effects [52, 53]. CPPDs are not toxic in the concentration of 1 µM; further growth of the toxicity depends on the dendrimer generation. It should be noted that the toxicity of CPPD is lower than those of PAMAM and PEI dendrimers, presumably, owing to the quaternization of the nitrogen atoms which is a well-known method for a reduction of the toxicity [54]. Furthermore, the experiments showed that the toxicity of CPPD essentially reduces upon interaction with the proteins [40]. Whereas the addition of 5 µM of the third generation dendrimer led to the death of 50% of cells, the addition of the dendrimer in the complex with the prion protein did not affect the viability of neuroblastoma cells (100% live cells). Nevertheless, further studies must focus on the surface modification of CPPD, which allows one to reduce the toxicity. The modification with compounds such as poly(ethylene glycol), pyrrolidone, or biocompatible molecules affords an essential reduction in the toxicity to the levels acceptable for the modern pharmaceutical industry [50].

Hence, dendrimers are promising compounds that can significantly affect the processes of protein amyloid transformation. They possess a broad spectrum of antiamyloid activity, which is caused, first of all, by the structural features of their molecules: a great charge and its high density, nanometer sizes, and a large number of functional groups. The introduction of constantly charged groups is a promising strategy for strengthening of the antiamyloid action of dendrimers since it allows one to retain their activity independent from the medium conditions. The hydrophobic interactions ensure the high stability of dendrimer complexes with proteins, which is an important criterion in the synthesis of antiamyloid compounds.

Conclusions

Dendrimers are often called as nanomaterials of future that have already found application in some fields of medicine [55]. A striking example is the antiviral drug VivaGel (Starpharma, Australia) which active substance is the polylysine dendrimer modified with naphthalenedisulfonic acid. Some drugs based on dendrimers, for example, DEP docetaxel and DEP cabazitaxel (Starpharma, Australia) exhibiting antitumor activity are now in clinical trials. Nevertheless, the successful application of dendrimers in medicine, especially in the fields where there are no efficient methods for treatment, is directly connected not only with the investigation of their toxicity, bioavailability, and biodistribution, but also on the elucidation of the fundamental principles of interaction of dendrimers with biomolecules, investigation of the influence of a dendrimer structure on the achievement of the desired effect, and complete physicochemical characterization of the resulting complexes. In this context, elucidation of the fundamental principles of dendrimer interaction with amyloidogenic proteins plays an important role in understanding the development of pathologies associated with the conformational transformations of proteins and contributes to the investigation of the mechanisms of control over protein aggregation *via* exogenic ligands. Dendrimers are not simply the compounds that exhibit high activity; they represent an excellent model for the detailed exploration of the structure–activity relationships. Subsequently, this will enable the directed synthesis of chemical compounds featuring the antiamyloid activity. The use of dendrimers as such compounds has undoubtedly much room for further development.

Acknowledgements

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