МЕДИЦИНСКАЯ БИОФИЗИКА

siRNA COMPLEXATION BY CARBOSILANE DENDRON MICELLES

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Nucleic acid therapy is an emerging field of modern medicine. Therapeutic nucleic acids, in particular, siRNA, are widely studied as prospective drugs for targeted modulation of gene expression. However, the problem of efficient intracellular delivery of siRNA is still a challenge. To overcome it, as well as to protect therapeutic oligonucleotides during the transfection, supramolecular delivery systems (micelles and liposomes) are used.

Amphiphilic dendritic molecules are novel and promising precursors for the supramolecular assemblies. The examples reported [1, 2] evidence high efficiency of siRNA delivery by micelles and liposomes made of dendrons bearing fatty acid residues, with particles being biocompatible and biodegradable.

In this work, siRNA delivery systems based on micelle-forming carbosilane dendrons are designed. First, a series of cationic carbosilane dendrons of generation 1-3 bearing hexanoic (HAGn) and palmitic (PAGn) acid moieties in focal point (fig.1) was synthesized and characterized.

The presence of aliphatic fragment along with cationic groups gives the amphiphilicity to the molecules provoking the association in the micelles in water solutions. The critical micelle concentration (CMC) values in PBS were estimated for HAGn and PAGn dendrons by standard technique using pyrene as a fluorescent marker. The ratio of intensities of the two peaks in the fine structure of fluorescence (I_{373}/I_{383}) was measured. The CMC values found are presented in fig 2.

The ability of cationic micelles to bind siRNA was then tested. As a model, pro-apoptotic siRNA Mcl-1 was used. The siRNA was mixed with cationic micelles in different charge ratio, and the samples were analyzed by agarose gel electrophoresis. The free siRNA was visualized by staining with ethidium bromide.

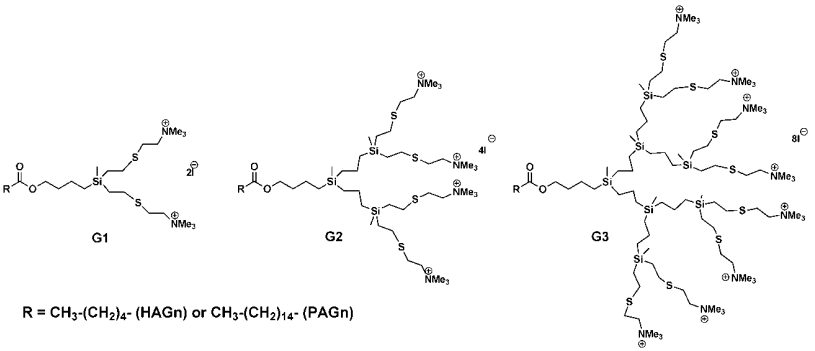

Figure 1 – Structures of pyrene-modified carbosilane dendrons

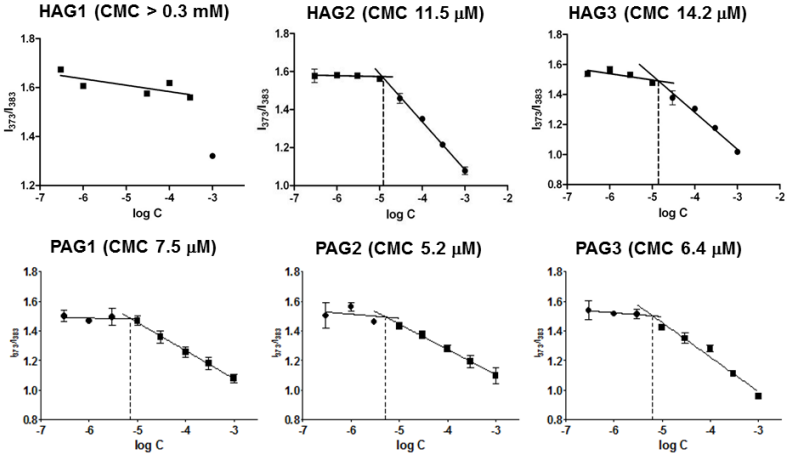

Figure 2 – Estimation of the CMC values of the carbosilane dendrons in PBS

The dendrons have been found to bind siRNA in different manner. Among the samples of HAGn, the positive dendritic effect on the binding (G1<<G2<G3) is clearly identified. The PAGn dendrons do not exhibit large difference in the siRNA binding, the siRNA is bound at charge ratio of 2. These observations correlate with the dynamics of CMC values found and suggest that the siRNA binding by dendrons is the result of micelle formation. Hexanoic acid moieties cannot cause micelle formation as efficiently as palmitic acid moieties, that results both in higher CMC values in the case of HAGn and in the inefficient siRNA binding by HAG1 and HAG2.

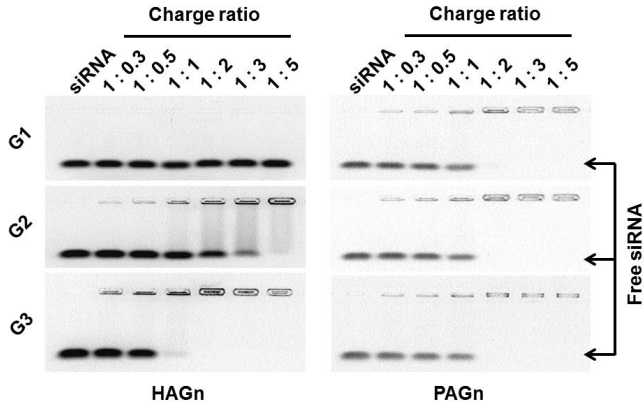

Figure 3 – siRNA binding by carbosilane dendron micelles

The results obtained can serve as a platform for the design of the supramolecular constructions for the siRNA delivery.

This work was supported by RFBR grant No.16-33-60152_mol_a_dk, by the Scholarship of the President of the Russian Federation (grant No. 882.2016.4) and by Marie Curie International Research Staff Exchange Scheme Fellowship within the 7th European Community Framework Programme, project No. PIRSES-GA-2012-316730 NANOGENE.

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CARBON NANOTUBES DECORATED WITH CATIONIC CARBOSILANE DENDRONS AND THEIR HYBRIDS WITH siRNA

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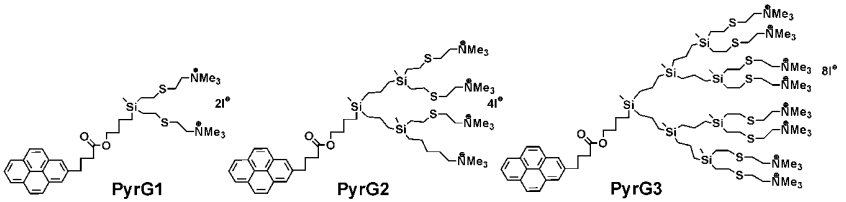
Carbon nanotubes (CNTs) are extensively used as carriers nucleic acid therapeutics, including small interfering RNA (siRNA) [1]. A versatile approach commonly used to construct hybrids of siRNA with single-walled and multi-walled carbon nanotubes (SWCNTs and MWCNTs) presumes the electrostatic interactions between nucleic acid backbone and cationic groups on the CNT surface. Cationic SWCNTs and MWCNTs can be easily obtained by grafting of amino-bearing compounds to CNT surface. The use of dendritic compounds to functionalize CNTs is highly promising due to the increase of the surface charge that leads to more efficient siRNA binding and better biocompatibility of hybrid constructions [2]. Due to their structure and properties, carbosilane dendrimers are prospective carriers for siRNA [3, 4]. In this work, multicomponent hybrid nanoparticles for siRNA delivery built of CNTs and carbosilane dendritic molecules were designed.

A series of cationic carbosilane dendrons of generations 1 to 3 bearing pyrene residues in focal point (fig. 1) has been synthesized and characterized. Pyrene is known as a good anchor group to immobilize macromolecules on the CNT surface. Meanwhile, cationic groups of dendrons can bind siRNA in the complex with nanoparticles.



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