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COMMUNICATION

Molecular recognition of Nucleic Acids by Nucleolipid-Dendrimer surface complexes

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We show for the first time that 1,2-dilauroyl-sn-glycero-3-phosphatidyladenosine nucleolipid surface complexes with cationic poly(amidoamine) dendrimers can be used to selectively bind DNA including oligonucleotides. This molecular recognition has high potential for applications involving biomedical and bioanalytic devices as well as drug delivery systems based on nucleic acids.

One of the large challenges in Soft Matter is to control the assembly of polymers, lipids and surfactant at interfaces. This can be achieved by controlling colloidal and interfacial forces as well as by providing means for molecular recognition. The latter often means elaborated synthesis. Here we show for the first time the non-covalent functionalization of dendrimers with nucleolipids to form surface complexes that can selectively direct the binding of DNA, including oligonucleotides.

Therapeutic applications of nucleic acids require vehicles that protects and delivers them to a specific target while being biocompatible i.e. non-toxic and non-immunogenic.[1] Viral vectors are frequently used since they have the highest known transfection efficiency but suffer under side-effects, such as unexpected immune responses.[2] Alternatively, complexes with non-viral agents like cationic lipids and polymers condense DNA, reduce risk of enzymatic degradation and specific immune response.[2] Poly(amidoamine) PAMAM dendrimers have been widely investigated for this purpose, as they exhibit improved condensation and translocation of nucleic acids compared with other polymers.[3, 4] This feature has been attributed to their well-defined hyperbranched architecture, offering high density of terminal groups allowing to interact intermolecular and with surfaces.[5] A drawback of the use of cationic PAMAM dendrimers with anionic nucleic acids, however, is that their association is primarily driven by electrostatic affinity and it therefore lacks chemical specificity. Thus, the dendrimer can attach to any anionic surfaces like common cell membranes as well as to other negatively charged biomolecules. Furthermore, the transfection efficiency increases as a function of the dendrimer size (or generation, G)[6], which is directly correlated with the number of cationic amine surface groups . Unfortunately, at the same time the toxicity also increases.[7] The efficiency of PAMAM as delivery vectors can be significantly improved by covalently attaching different molecules e.g. arginine[8], peptides[9, 10] or azides or alkynes conjugated to one DNA strand[11] to the dendrimer surface groups. The transfection efficiency of the modified PAMAM is improved and the toxicity is reduced, but the dendrimer synthesis, functionalization and purification procedures can be rather challenging and therefore other alternatives are needed. Nucleolipids are synthetic derivatives of phospholipids where the choline in the head group has been exchanged with a nucleoside.[12, 13] These amphiphiles have a net negative charge from the phosphate group at the same time as they contain the nucleotide bases of nucleic acids. They can therefore interact with nucleic acids by means of selective base pairing.[14-17] The present work represents the development of an alternative pathway for delivery of nucleic acids for therapeutic and other biomedical purposes through the combination of PAMAM dendrimer with oppositely charged nucleolipids. We demonstrate the ability of nucleic acid recognition in films formed through non-covalent attachment of the nucleolipid 1,2-dilauroyl-sn-glycero-3-phosphatidyladenosine (DLPA) to PAMAM dendrimers of generation 4 (PAMAM-G4). This approach opens up the possibility to tune the structure of the formed complex as well as to provide means for molecular recognition, without extensive synthesis procedures. The adenine-based nucleolipid was chosen since it has been found that it shows higher potential for selective recognition of nucleic acids, compared with nucleolipids based on the other nitrogenous bases, due to Watson-Crick and Hoogsteen base pairing.[15, 18]

For the benefit of high surface sensitivity techniques, that allow precise structural determination, the investigation was performed
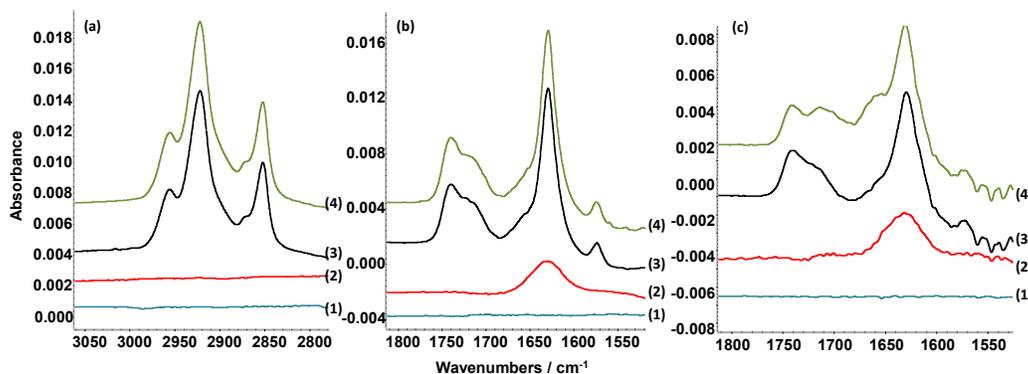
FIGURE 1. ATR-FTIR spectra showing (a) C-H stretching region and (b,c) C=O stretching and nucleic base vibration regions of the spectra arising from the clean silicon crystal surface (1) with sequential additions of 100 ppm PAMAM-G4 (2), 0.1 mM DLPA (3) and 200 ppm 20dA (4a,b) or 200 ppm 20dT (4c). The measurements were done in D_2O with 10 mM NaCl. Note that the y-axes of the spectra have been shifted for clarity.

using a solid support. The binding selectivity towards dendrimer/nucleolipid films was monitored using two different 20-mer oligonucleotides containing adenosine (20dA) and thymidine (20dT). Thymine and adenine are DNA complementary nitrogenous bases and therefore adsorption of 20dT and not 20dA to the PAMAM/DLPA layers could be attributed to selective base-pairing at the interface. The adsorption of 4331 bases single stranded DNA (ssDNA) was also investigated to show that even longer nucleic acids have the potential to attach to the PAMAM/DLPA layer. Attenuated total reflectance Fourier transform infrared (ATR FT-IR) spectroscopy was employed to identify the oligonucleotide interactions with the PAMAM/DLPA layers, while the structure and composition of the layers perpendicular to the interface were obtained by neutron reflectometry (NR).

Figure 1 shows the ATR-FTIR spectra of the dendrimer and dendrimer/DLPA films before and after the addition of 20dA or 20dT. The assignment of each specific absorption band is presented in the Electronic Supplementary Information. To avoid aggregation in bulk solution, the different components were added sequentially as: (i) pre-adsorption of the dendrimer on a silicon substrate, (ii) rinsing with pure solvent (aqueous solution of 10 mM NaCl or 10 mM Tris-HCl pH 7.6 buffer), (iii) addition of DLPA, (iv) rinsing again with buffer, (v) addition of nucleic acids and (vi) final rinsing with buffer. The rinsing process with pure solvent results in dilution of the surface active agents, i.e. dendrimer or nucleolipid, out of the subphase after the adsorption process. Rinsing steps are necessary in such studies so that subsequent exposure of different species in the bulk solution to the interfacial layer is not affected by intermolecular complexation in the bulk; note that the dendrimer/nucleolipid films were exposed to aqueous solvent throughout the experiment and never pass through the air/liquid interface. The current study was performed at physiological pH but at lower ionic strength compared to the biological buffers commonly employed since the dendrimer molecules can be partially desorbed from the silica substrate upon rinsing under such conditions.[19] However, it is expected that the interfacial structure of the dendrimer/nucleolipid complexes to be similar because the structure of the dendrimer layer[20] and the aggregation behavior of DLPA[21] are similar at higher ionic strengths.

The adsorption of PAMAM at the silica-water interface is identified by the absorption peak of the C=O stretching from the amides at 1645 cm^{-1}. DLPA adsorbs on PAMAM monolayers by means of the electrostatic attraction between the cationic primary amine groups of the dendrimer and the anionic head group of the nucleolipid. Since DLPA is anionic, it does not adsorb on the bare silica surface. The adsorption of DLPA to the dendrimer layer is highlighted by the

occurrence of peaks between 3000 and 2850 cm^{-1} that correspond to the C-H stretching region. Additionally, the region between 1800 to 1550 cm^{-1} in D_2O is sensitive to the in-plane double bond stretching of the nucleic bases (C=C, C=N and C=O) and it reflects the effects of base pairing and/or base stacking.[22] The spectra do not show measurable changes resulting from the rinsing process, which indicates that the adsorption of the nucleolipid is irreversible. This may be attributed to the strong self-association of the DLPA, in part due to the head group base pair stacking reported previously.[21] The addition of 20dA shows no notable changes in the absorbance of any of the bands corresponding to adenosine, while the addition of 20dT shows a broad peak around 1712 cm^{-1}. This suggests that a triple-helical structure is formed by T*A-T base triplets with an antiparallel orientation of the third strand (Hoogsteen type).[23] Interestingly, evidence of Hoogsteen pairing has also been found in mixed micelles of the shorter-chain diC$_8$P-nucleoside based in adenosine and uridine.[24] The specific conformation of the nucleolipid-oligonucleotide structure cannot be resolved with this technique since the appropriate wavenumber range where the phosphate- and base-sugar vibrations characteristic of each conformation are determined is dominated by the absorption of Si bonds (below 1500 cm^{-1}).[25] However, the ATR FT-IR spectroscopy measurements indicate that the adsorption of the oligonucleotide is driven by the base pairing with the nucleolipid, but this technique cannot show if it is able to interact with the dendrimer.

The interfacial structure of the layers formed by the addition of DLPA to PAMAM layers on silica and their interactions with the oligonucleotides were further examined using NR. The reflectivity profiles for the adsorption of hydrogenous DLPA (hDLPA) after diluting the bulk system with solvent (rinsing) and the effects of the addition of the oligonucleotides in D_2O are shown in Figure 2a. The complete set of data and parameters obtained from the fittings together with the data recorded in other isotope contrast conditions can be found in the Electronic Supplementary Information. PAMAM-G4 adsorbs at the silica-water interface as a very thin layer (14 ± 2 Å) and the surface coverage corresponds to a dendrimer fraction of approximately 0.3 of the layer volume. DLPA adsorbs on the PAMAM monolayer forming a layer with an approximate thickness of 35 Å, which is likely to comprise self-assembled aggregates as the material does not desorb upon rinsing. These surface aggregates may resemble the "threadlike" micelles found in bulk solution as the layer thickness matches well the aggregate cross-section diameter.[21] The aggregates correspond to 0.6 ± 0.1 volume fraction of the layer surface coverage and an approximate dendrimer/DLPA charge ratio of 0.5, which indicates that the film is

negatively charged. Thus, one would expect that no electrostatically driven sequential adsorption of nucleic acids would occur.
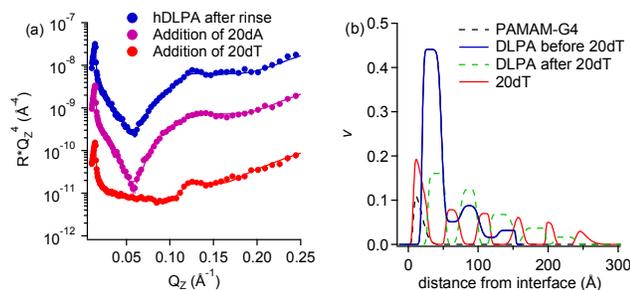

FIGURE 2. (a) Neutron reflectivity profiles recorded in D_2O, using INTER, and the best fits for the additions of 0.1 mM hDLPA to a pre-adsorbed PAMAM-G4 monolayer on silica after rinsing with solvent as well as subsequent addition of 200 ppm 20dA or 200 ppm 20dT. The data are offset in the y-axis for clarity. (b) Volume fraction (v) of PAMAM-G4, DLPA and 20dT as a function of the distance to the surface (SiO$_2$). The solvent was 10 mM NaCl.

Upon injection of nucleolipid-free buffer, the amount of DLPA does not change but the conformation of the layers does. The DLPA has now reorganized at the surface to form a layer adjacent to the dendrimer with a lower coverage (0.35 ± 0.18 volume fraction) and thickness (~ 30 Å), and 4 sparse layers of DLPA aggregates with the same thickness and a volume fraction of < 0.1 are bound. Other models such as a swollen DLPA layer were tested but failed to describe consistently the data in multiple isotopic contrasts which confirms that this model was the most appropriate one. This suggests that the DLPA is no longer rigidly bound to the dendrimer monolayer but forms some extended, stratified structure. It follows that these could be elongated micelles, as they form in the bulk solution, which protrude into the bulk solution.

The addition of 20dA shows no significant effect on the reflectivity profile, in keeping with the ATR FT-IR spectroscopy measurements. This proves that although the dendrimer is cationic, the lack of specific base pairing with the nucleolipid coupled with the electrostatic repulsion between the phosphates of the nucleolipid head group and the oligonucleotide backbone hinders the adsorption process. For the addition of 20dT, the data reveal significant changes at the interface, so several structural models were tested including adsorption of the oligonucleotide to: (i) the DLPA aggregate layers only, (ii) the PAMAM monolayer beneath the DLPA aggregate layer only and (iii) the PAMAM monolayer beneath the DLPA aggregate layers and in between the DLPA aggregate layers. The reflectivity profiles for the addition of 20dT fit well only to the third model. The oligonucleotide and nucleolipid layers had a low surface coverage ($\sim$ 0.10 volume fraction). However, the results from fitting of the layer model to data recorded in different isotopic contrasts indicate that the structure is unequivocally formed by multiple oligonucleotide layers (thickness ~ 13 Å) intercalated with DLPA layers (thickness ~ 30 Å) adsorbed onto the dendrimer monolayer (Figure 2b). The formation of these multiple layers may be attributed to the Hoogsteen pairing indicated by the ATR FT-IR spectroscopy measurements. Furthermore, the surface structure revealed by NR shows that the oligonucleotide adsorbs onto PAMAM-G4 only if it is able to interact with the nucleolipid.

Segments of ssDNA play an important role in the replication, recombination, repair, blocking, transcription and transposition of genes that are fundamentally important for any type of gene therapy treatments. Therefore, the ability of ssDNA with 4331 bases to molecularly recognize and base pair with formed PAMAM/DLPA layers were additionally investigated using NR (Figure 3a). A similar model to the adsorption of 20dT was applied successfully to fit the

reflectivity profiles with the longer DNA strand but with a higher volume fraction of the nucleic acid (~ 0.29) bound between the dendrimer and the DLPA layer.
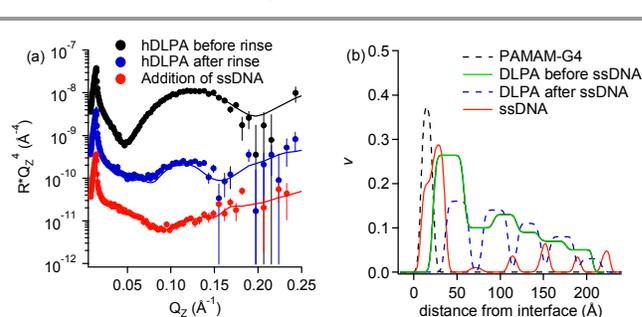

FIGURE 3. (a) Neutron reflectivity profiles recorded in D_2O, using FIGARO, and the best fits for the additions of 0.1 mM hDLPA to a pre-adsorbed PAMAM-G4 monolayer on silica, before and after rinsing with solvent as well as subsequent addition of 70 ppm ssDNA. The data are offset in the y-axis for clarity. (b) Volume fraction (v) of PAMAM-G4, DLPA and ssDNA as a function of the distance to the surface (SiO$_2$). The solvent was 10 mM Tris-HCl pH 7.6 buffer.

Figure 3b reports the volume fraction profiles of the dendrimer and the nucleolipid, after rinsing with solvent, before and after the adsorption of ssDNA. The data indicate that the adsorption is irreversible as the layers are stable after multiple rinsing with solvent. The DNA interacts electrostatically with the dendrimer, independently of the nucleotide, but it forms multiple layers with DLPA defined by the base selectivity. It is worth to note that the nucleolipids do not desorb due to the addition of nucleic acids and the formation of multiple DNA/DLPA layers can create an additional protection to the nucleic acid in the delivery vehicle.

From the structural information of the layers resulting from the interactions of PAMAM/DLPA films with single stranded nucleic acids obtained by NR, a schematic illustration of the layer structure is presented in Figure 4. The schematic representation shows the attractive interactions between the nitrogenous base of the nucleolipid (adenine, blue) and matching base of the DNA strand (thymine, orange) through e.g. hydrogen bonding. This attachment is responsible for the multiple DLPA/nucleic acid stacked layers as the dendrimer interacts electrostatically with the phosphate groups of the DNA backbone.
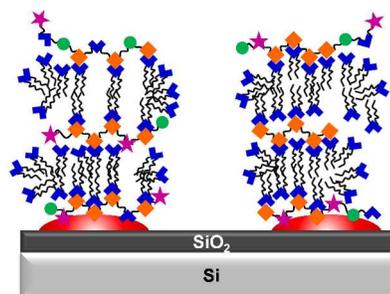

FIGURE 4. Schematic representation of a layer of PAMAM-G4 (red) and DLPA (blue) adsorbed at the silica-water interface interacting with single stranded nucleic acids by means of selective pairing to one of the four nitrogenous bases (blue, orange, green and purple).

In the present work we have shown that it is possible to achieve non-covalent functionalization of cationic PAMAM dendrimers with anionic nucleolipids. The formed PAMAM/DLPA complexes are stable and indeed increase the selectivity of nucleic acid binding. Although the interaction with the nucleic acid is opposed by the loss in configurational entropy upon adsorption and the electrostatic

repulsion to the nucleolipid layer, it is favored by selective base-pairing, i. e. hydrophobic interactions in combination with the matching hydrogen bonds. Therefore, the presence of the nucleolipid screens the electrostatic attraction of the cationic charges of the dendrimer towards negatively charged molecules that do not have nucleotides with specific base pairing. The proposed functionalization method is practically much easier than covalent conjugation and it could potentially be used to reduce the toxicity effects caused by the cationic charges of the dendrimer. It is noted that further development of dendrimer/nucleolipid complexes for gene delivery purposes requires further investigation of their bulk phase behavior. Preliminary results have shown that the size, charge and colloidal stability of these complexes in the bulk solution depend on the dendrimer/nucleolipid ratio. The information regarding the structure, composition and functionality of the surface complexes obtained in the present work will be used as a basis to elucidate the interactions of the complexes with nucleic acids in bulk solutions. Equally, our approach has potential to be used to form dendrimer/nucleolipid layers at solid interfaces for biomedical applications such as biosensors for DNA. This technology is extremely useful for analysis such as tissue matching, forensic examinations and environmental contamination monitoring, among others.[26-28] These biosensors are usually made by surface immobilization of oligonucleotides through attaching covalently the 5' or the 3' hydroxyl group to a solid support.[29] Additionally, dendrimers have been investigated previously as DNA biosensors by immobilizing these macromolecules covalently to the surfaces.[30] PAMAM/DLPA multilayers have chemical specificity towards the nucleic acid and, thus, they are able to immobilize the oligonucleotides without a synthetic procedure. Moreover, they are not sensitive to the electrostatically driven attachment of contamination like the current polymer matrices alternatives.

Notes and references

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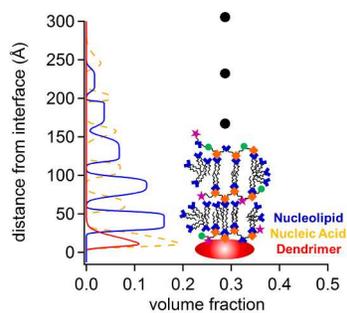
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Table of Content Graphic

Functionalization of dendrimer layers with nucleolipids allows selective molecular recognition of nucleic acids.
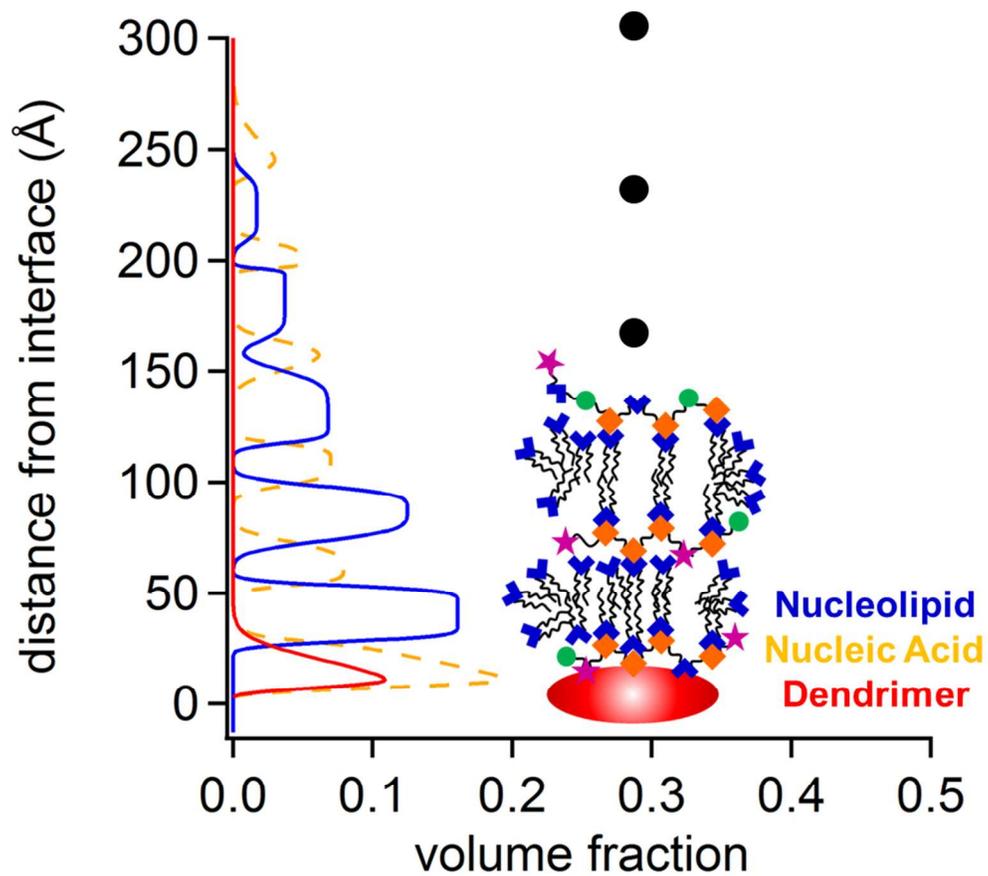
Table of Content Graphic: Functionalization of dendrimer layers with nucleolipids allows selective molecular recognition of nucleic acids.
171x150mm (150 x 150 DPI)