Molecular Recognition

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Molecular Recognition Drives Oligonucleotide Binding to Nucleolipid Self-Assemblies**

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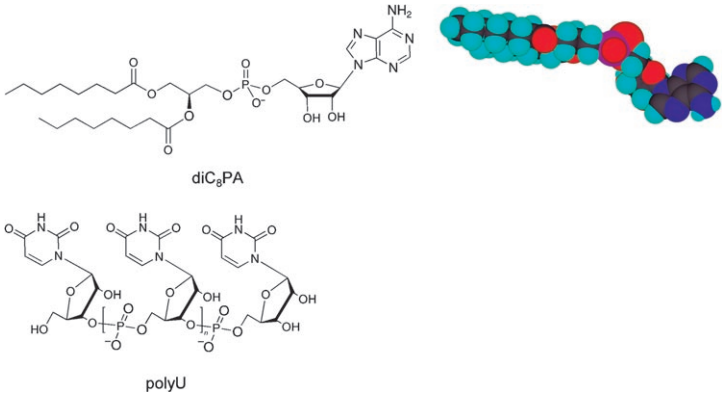
Molecular-recognition strategies can be used to engineer supramolecular constructs with a desired architecture through the assembly of preprogrammed building blocks.[1] Nature can provide a molecular toolbox, as in the case of nucleotides, to accomplish high levels of specificity and control.[2] Our group has pioneered the conjugation of amphiphilic building blocks to nucleotides;[3] this combination provides a powerful approach for the preparation of materials with complex and tunable properties and has therefore attracted considerable interest in view of the possible applications in materials chemistry and nanotechnology.[4] An extremely promising and practically unexplored topic is the conjugation of nucleolipid assemblies with natural nucleic acids. A major reason for the interest is practical and is connected to the confinement, delivery, and transfection of nucleic material for therapeutic purposes. Most current DNA–amphiphile assemblies rely on electrostatic compensation between the cationic amphiphiles and anionic DNA[5] and are therefore related to aspecific coulombic interactions. In some other examples, DNA complexation with neutral amphiphilic assemblies is mediated by divalent cations.[6] A real advancement would be the control and modulation of interactions through molecular-recognition strategies. Recently Shimizu and co-workers[7] reported the formation of DNA-like nanofibers, which result from the complementary oligonucleotide-templated self-assembly of a thymidine-appended bolaamphiphile with a series of short oligoadenylic acids.

The present paper reports the first evidence of selective supramolecular association of oligonucleotides with like-charged amphiphilic self-assemblies in aqueous solution in a process that is not driven by the presence of divalent cations.

The anionic self-assemblies are globular micelles of dioctanoylphosphatidyladenosine (diC_8PA; Scheme 1) and dioctanoylphosphatidyluridine (diC_8PU).[8] Deviations from an ideal behavior have been found in the critical micelle concentration (cmc) value and in the area per polar head of a 1:1 amphiphilic mixture of these compounds.[9] Selective and preferential interactions between the adenine and uracil moieties, according to a pattern resembling molecular recognition in nucleic acids, have been identified by NMR, UV/Vis, and CD spectroscopies.[8,9] Therefore, these systems represent excellent candidates to test possible interactions with polynucleotides.



Scheme 1. Structure and space-filling model of dioctanoylphosphatidyladenosine (diC_8PA, top); structure of polyuridylic acid (polyU, below).

Dynamic light scattering (DLS) experiments on polyuridylic acid (PolyU, 3.45 mg mL^{-1}, 0.01 M on a monomer basis) in tris(hydroxymethyl)aminomethane (Tris)-buffered saline (TBS, pH 7.5) yield autocorrelation functions of bimodal decay, similar to the results with many polyelectrolyte solutions.[10] An angular scan reveals a diffusive nature[11] for both modes (see the Supporting Information), the corresponding diffusion coefficients of which are reported in Table 1. The DLS data for micellar aggregates (lipid concen-

Table 1: Apparent diffusion coefficients (D) for the systems under investigation. For micellar binary systems, the hydrodynamic radii (R_h) are also reported.

	D_1 [cm^2 s^{-1}]	D_2 [cm^2 s^{-1}]	D_3 [cm^2 s^{-1}]	R_h [nm]
diC_8PA	7.7×10^{-7}	–	–	3.2
diC_8PU	7.4×10^{-7}	–	–	3.3
polyU	4.5×10^{-7}	4.5×10^{-8}	–	–
diC_8PA/polyU	9.2×10^{-7} [17%][b]	2.8×10^{-8} [38%][b]	4.1×10^{-9} [45%][b]	–
diC_8PA/polyU[a]	8.9×10^{-7} [14%][b]	3.3×10^{-8} [25%][b]	3.2×10^{-9} [51%][b]	–

[a] Results after 4 h of mixing. [b] Relative abundance of the different populations contributing to decay, determined according to a three-exponential fitting to the curves.

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

tration = 0.01 M) show a single diffusive decay; the corresponding hydrodynamic radii are also reported in Table 1.

When diC_8PA micelles are mixed with polyU (1:1, on a monomer basis), dramatic variations in the DLS spectra are observed (Figure 1). The dominant feature is the appearance

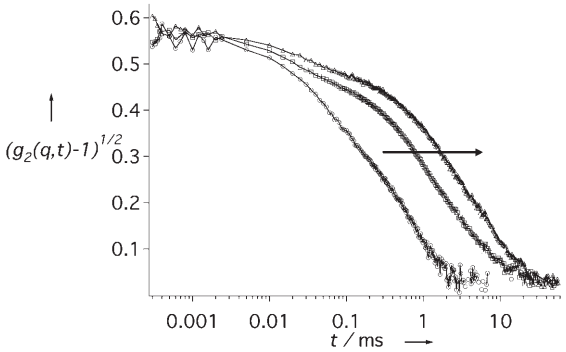

Figure 1. Dynamic light scattering autocorrelation functions of binary (polyU/TBS; ○) and ternary (polyU/diC_8PA/TBS) systems soon after mixing (□) and after 4 h of annealing (△). The direction of change upon annealing is indicated by the arrow.

of a third "ultraslow" mode, not originally present in the binary systems, which originates from the simultaneous presence of the nucleolipid micelles and the polynucleotide.

A CONTIN Laplace inversion[12] has confirmed the presence of three relaxation modes that can be quantified through a three-exponential function fitting. A fast mode, correlated both to the diffusion of diC_8PA micelles and to the fast relaxation of the polymer is observed; the micelle and polymer contributions cannot be reliably distinguished because they are not separated widely enough on the time axis. A second decay, corresponding to the slow mode measured for polyU, is also present.

Small-angle neutron scattering (SANS) and small-angle X-ray scattering (SAXS) measurements (see the Supporting Information) performed on the same system have also confirmed the persistence of micellar structures, while a low-q upturn indicates the growth of superstructures of length scales beyond those probed by the explored q range.

Moreover, the mixed polyU/diC_8PA system shows a temporal evolution, mainly involving the slowest relaxation mode, as shown by the relative contributions to the scattered intensity (Table 1).

These results indicate that the onset of the ultraslow diffusion occurs at the expense of the intermediate relaxation. If the slow relaxation for polyU is ascribed to multichain domains, where an increased polymer concentration is present,[13] the appeareance of the ultraslow mode is consistent with a model where nucleolipid anionic micelles template a size growth of these domains, thereby resulting in an "apparent" diffusion coefficient which is one order of magnitude lower than that of the starting multichain domains, as we have tentatively sketched in Figure 2. The additional ultraslow mode is therefore due to a reorganization of the population responsible for the slow relaxation of the polyelectrolyte; this reorganization takes place only in the presence of diC_8PA micelles.

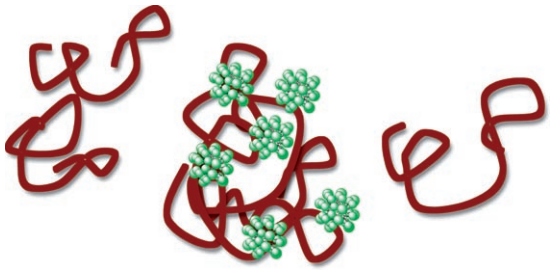

Figure 2. Proposed model for the superstructures: polyU multichain clusters are formed through mediation of diC_8PA micelles.

To demonstrate that the driving force for the onset of additional collective relaxation is due to the specific molecular recognition between the complementary bases of the polymer and the nucleolipid, we have performed a control experiment by adding globular sodium dodecylsulfate (SDS) anionic micelles to a polyU solution. The correlation decay is well described by a double exponential, as shown in Figure 3, and there is no evidence of an additional ultraslow decay mode or of time evolution.

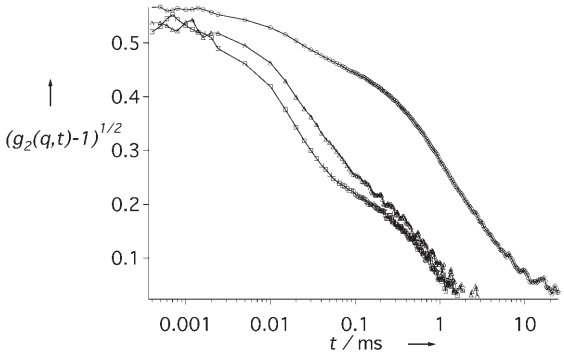

Figure 3. DLS autocorrelation functions of ternary systems polyU/SDS/TBS (□), polyU/diC_8PU/TBS (△), and polyU/diC_8PA/TBS (○) soon after mixing. For the former two systems, no time evolution is observed.

This evidence rules out a purely hydrophobic driving force due to the aspecific association of the polynucleotide backbone with the micelles. We may then speculate whether base-driven association follows a specific A–U pairing pattern, that is, with the same specificity as in RNA double-strand formation, where adenine and uracil form a complementary pair. Figure 3 also shows a DLS measurement performed on diC_8PU/polyU under the same experimental conditions as those used for the complementary pair. The resulting relaxation for the fast mode is similar to that with SDS, with a slower diffusion due to the larger size diC_8PU micelles. The slower decays due to "unperturbed" polyU are identical as far as amplitude and rate are concerned.

We can conclude that the mechanism of interaction is not simply hydrophobic but shows molecular specificity between complementary bases, similar to that in DNA/RNA, that is,

the micelles must be decorated by the complementary nucleic base with respect to the polynucleotide in order to form the polynucleotide–micelle adduct. The hydrogen-bonding pattern might not be strictly of the Watson–Crick type found between adenosine and uridine in RNA, since other binding modes, such as the Hoogsten mode, can occur. However, the selectivity of polyU for diC_8PA micelles resembles that observed between bases in natural nucleic acids.

Circular dichroism spectroscopy can further support the existence of molecular recognition between complementary bases acting in the polyU/diC_8PA "nucleolipoplexes".

Figure 4 shows the spectra of the mixed diC_8PA/polyU system compared with the sum of the single spectra. As the picture reveals, meaningful deviations from an ideal behavior

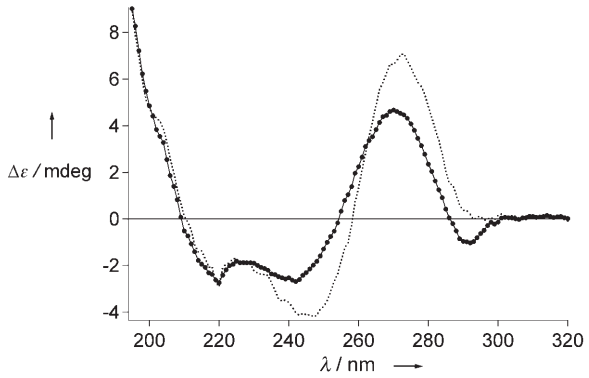

Figure 4. CD behavior (●) of the ternary system diC_8PA/polyU/TBS compared with the calculated spectrum (-----) obtained by averaging the spectra of the single components.

can be appreciated for diC_8PA/polyU (intensity reduction of the CD bands, slight blue-shift of the maximum of the positive CD band, and appearance of a negative band at 290 nm), consistent with the presence of a structure stabilized by the stacking of the bases and the formation of H-bonded base pairs.[13]

This effect is accompanied by a 20% hypochromism for the 260 nm absorbance band (not shown), which supports the existence of an enhanced nucleobase stacking interaction between diC_8PA and polyU mediated by a specific base-pairing mechanism between the complementary adenine and uracil nucleobases. The appearance of the negative CD band at 290 nm seems to be a peculiar feature of this system; a similar CD behavior was found in a previous work[14] for mixed liposomes made of 5'-(1,2-dioleoyl-*sn*-glycero(3)phospho)adenosine (DOPA) and 5'-(1,2-dioleoyl-*sn*-glycero(3)-phospho)uridine (DOPU) in TBS. This could possibly indicate conformational variations of adenine groups on the polar head of the assembled nucleolipid, induced by cooperative interaction with uracil bases.

The results above are consistent both with a monomer–polynucleotide interaction and with a micelle-mediated one. To address this point, we have measured the CD spectum for diC_8PA/polyU as a function of nucleolipid concentration, from the monomers to the self-assembled surfactant systems. The results, shown in Figure 5, indicate that deviation from

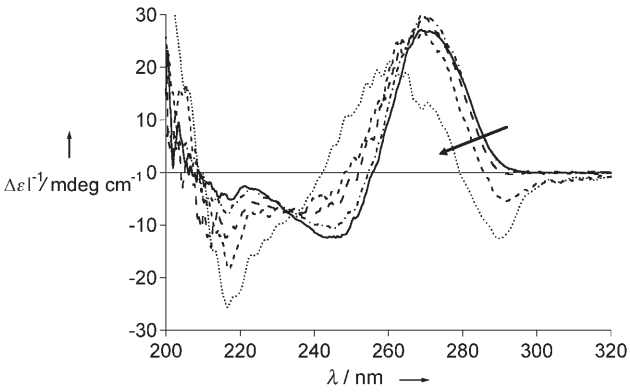

Figure 5. CD variation for diC_8PA/polyU/TBS (3.45 mg mL^{-1}) as a function of diC_8PA concentration in a range below and above the cmc value (2.0×10^{-4} M). The direction of change with concentration increase is indicated by the arrow. —— 7.9×10^{-5} M, –·– 1.5×10^{-4} M, – – – 2.4×10^{-4} M, ----- 3.8×10^{-4} M, ••••• 5.9×10^{-4} M.

ideality occurs only above the aggregation threshold, thereby indicating that micelles are necessary to activate the recognition process.

The model sketched in Figure 2 proposes a tentative picture of the early stages of association. The observed time evolution demands a study on longer timescales to investigate the formation of ordered structures of nucleolipoplexes; the formation of ordered DNA–calcium–zwitterionic-lipid complexes has, for instance, been found to proceed through long-term incubation at 277 K.[15]

After storage at 277 K for a week, the sample of diC_8PA/polyU shows a precipitate that is not present in the control systems (that is, polyU, diC_8PU/polyU, and diC_8PA). Figure 6 shows the SAXS spectrum of the precipitate, obtained after the storage. The observed reflections correspond to a well-defined hexagonal phase with a lattice spacing of 98 Å.

The adduct can be modeled as being formed of PolyU micelles that arrange in a hexagonal array, thanks to the

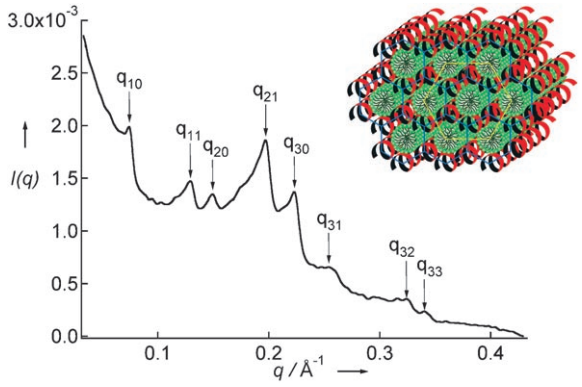

Figure 6. Small-angle X-ray scattering spectrum obtained from the precipitate formed by ageing a diC_8PA/polyU/TBS solution at 277 K for one week; the arrows highlight the scattering vectors where the reflections for a hexagonal phase with a lattice spacing of 98 Å should occur. The inset shows a tentative structural model consistent with the data in which the hexagonal arrangement is templated by the complementary polynucleotide (represented here by a helix).

cooperative action of the complementary single-strand oligonucleotide.

Stable liquid-crystalline phases (mainly H_{II} and L_a) of cationic lipids with DNA have been observed in the last few years. Recently Safinya and co-workers have shown that an H_I phase can be also observed for lipids having positive spontaneous curvature.[16] diC_8PA has a packing parameter of about 1/3, which means that it will adopt a positive curvature in water when aggregated.

We can conclude that diC_8PA micelles cooperatively interact with the complementary polynucleotide, thereby inducing novel mixed structures. The additional ultraslow mode observed by DLS is due to a reorganization of the population previously responsible for the slow relaxation of the polyelectrolyte. This reorganization takes place thanks to the presence of the diC_8PA micelles. The mechanism of interaction is not hydrophobic and shows molecular specificity, that is, micelles must be decorated by the complementary nucleic base with respect to the polynucleotide in order to form the polynucleotide–micelle adduct. Interestingly this effect is displayed only above the cmc value, a fact indicating that supramolecular structures are necessary to cooperatively promote the base–base interactions and recognition. Eventually, polyU templates the formation of a direct hexagonal network of micelles, where cylindrical amphiphiles are edged by the polynucleotide.

Experimental Section

diC_8PA and diC_8PU were synthesized as described elsewhere.[9] The lyophilized powder was dissolved in 0.05 M Tris-buffered saline (pH 7.5) prepared in MilliQ water. The critical micelle concentration, monitored through static surface tension with a KSV tensiometer, was 2.0×10^{-4} M for diC_8PA and 2.8×10^{-4} M for diC_8PU.

Polyuridylic acid (Na^+ salt) was purchased from Sigma–Aldrich; the weight-averaged molecular weight, determined through a Zimm plot (see the Supporting Information), was approximately 130000. Mixed polyU–diC_8PA/U solutions were prepared by mixing solutions of polyU and the appropriate amphiphile.

Dynamic light scattering was performed with the apparatus previously described.[10] Circular dichroism and UV absorption measurements were carried out with a Jasco J-715 spectropolarimeter. SAXS analysis was performed in a Kratky compact instrument provided by HECUS MBraun, Graz, Austria.

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[11] Under our experimental conditions (angular range and polyelectrolyte concentration), it has never been possible to observe a single decay behavior from polyU. The q^2 dependence ($q=(4\pi n/\lambda)\sin\theta/2$; q = scattering vector, n = refractive index, λ = wavelength, θ = scattering angle) of the nature of both modes does not imply that their origin is due to objects undergoing Brownian diffusion but that their appearance and disappearance is stochastic.

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