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Cylindrical Poly(oligo-DNA)

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Oligo-desoxyribonuleic acids (ODNs) having a sequence of 5′-TCC ATG ACG TTC-3′ were modified at the 5′ end by introduction of an amine group via a C6-amino linker. After subsequent reaction of the amine group with *N*-methacryloyloxysuccinimide, polymerizable ODNs were obtained. Free radical homopolymerization results in the formation of comb polymers, which possess an ODN side-chain at each repeating unit of the main chain. Mainly due to steric repulsion, the main chain has to adopt a semi-flexible wormlike shape instead of the otherwise preferred coiled structure. This leads to the formation of cylindrical poly(oligo-DNA) molecules. Characterization by static and dynamic light scattering of the poly(oligo-DNA) in aqueous solution gave a radius of gyration $R_{g,app} = 67.8$ nm, a hydrodynamic radius $R_{h,app} = 44.6$ nm, and a characteristic ratio of $\rho = R_g/R_h = 1.52$, indicating the cylindrical shape in solution. In addition, the cylindrical poly(oligo-DNA) molecules were adsorbed onto mica and visualized by atomic force microscopy.

Introduction

Nanoparticles equipped with DNA functionalities are of steadily growing interest mainly due to their promising potential application as markers or probes,[1,2] in gene delivery,[3] or for the stimulation of the immune system.[4] All of these DNA-containing nanoparticle systems under investigation have a basic spherical architecture in common. The influence of the architecture on the result for a specific nanoparticle−DNA hybrid system is an interesting research task. Especially in case of potential applications such as vehicles for gene delivery or as immune stimulant, a non-spherical architecture might induce a different, probably enhanced, effect.

Examples of nanoparticles having cylindrical shape are polymer combs, which have a flexible main chain with oligomeric side chains attached to each repeating unit of the main chain. This leads to a stretching of the main chain due to steric repulsion of the side chains, depending on the degree of polymerization of the side chains.[5] The polymer combs with such high grafting density of the side chains are obtained by the polymerization of macromonomers, which are oligomers carrying polymerizable end groups.[6] So far, DNA macromonomers have been widely used in copolymerizations, mainly in the synthesis of DNA-modified, cross-linked polyacrylamide for the characterization of complementary sequences.[7] If oligo-DNA macromonomers are homopolymerized as discussed above, poly(oligo-DNA) is obtained (Figure 1).

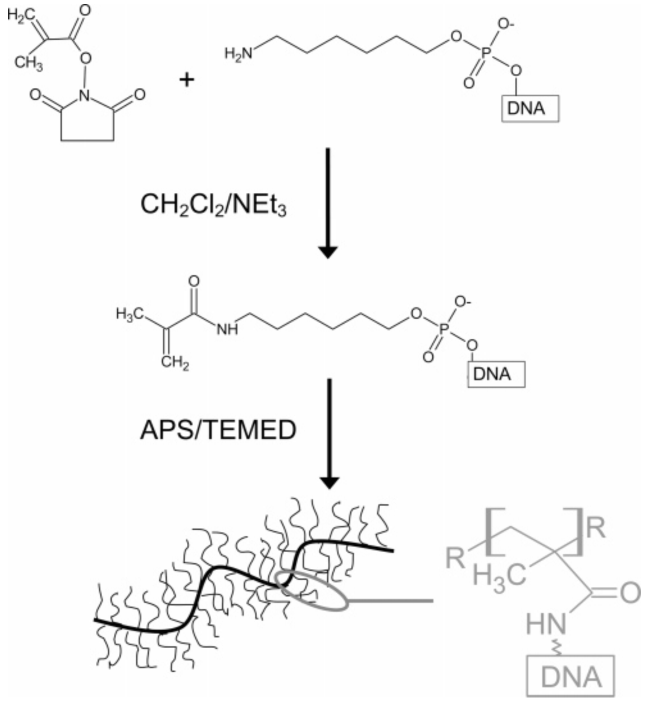

Figure 1. Scheme of the synthesis of poly(oligo-DNA). First step: reaction of *N*-methacryloyloxysuccinimide with C6-amino-linker modified oligo-DNA, attached to CPG. Second step: after cleavage and purification of the oligo-DNA macromonomer follows subsequent homopolymerization to poly(oligo-DNA).

Experimental Section

Materials. Dichloromethane (p.a.) and triethylamine (p.a.) were purchased from Fluka and dried over calcium hydride (Fluka). Methacryloylchloride, *N*-hydroxysuccinimide (both Fluka), methanol (Aldrich), ammonium hydroxide (25%, Aldrich), disodium hydrogenphosphate (Merck), sodium dihydrogenphosphate (Merck), ammonium peroxodisulfate (APS, Sigma-Aldrich), and *N*,*N*,*N*′,*N*′-tetramethylethylendiamine (TEMED, Carl Roth) were used as received. The oligo-DNA (ODN, sequence 5′-TCC ATG ACG TTC-3′, modified with a C6-amino linker, CPG bound, protected) was purchased from Biomers. Water was purified with a MilliQ system (Waters).

Methods. Static light scattering experiments were performed at 20 °C using an ALV-SP-86 goniometer, an ALV-3000 correlator, an 25 mW Uniphase HeNe laser ($\lambda_0 = 632.8$ nm), and an ALV/High QEAPD avalanche diode fiberoptic detection system. Measurements were carried out from 30° to 150° in angular steps of 5°. Dynamic light scattering was performed with a SpectraPhysics SP2080 argon-ion laser ($\lambda_0 =$ 514 nm), an ALV SP-125-5/N-39 goniometer with single photon detector SO-SIPD, and an ALV-5000 Multiple-Tau digital correlator. Measurements were carried out at 20 °C from 20° to 150° in angular steps of 10°. All solutions for light scattering were filtered before

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measurement (Millipore Millex LG, 0.2 µm). AFM micrographs were recorded in tapping mode, using a Nanoscope IIIa multi-mode instrument (Digital Instruments). The solutions were deposited onto freshly cleaved mica.

Synthesis. *N*-Methacryloyloxysuccinimide was prepared according to ref 8. To 1.125 g of ODN-coated CPG was added a solution of 56.1 mg (0.31 mmol) of *N*-methacryloyloxysuccinimide in 4.5 mL of dichloromethane and 0.5 mL of triethylamine under argon, and the sample was shaken for 48 h.[9] The sample was washed two times with methanol, and subsequently ammonium hydroxide solution was added. The sample was kept at 47 °C overnight. The resulting supernatant was removed, and the residual sample was washed with water two times. This water and the former removed supernatant solution were mixed. This solution was dialyzed with MilliQ water (SpectrumLab dialysis tubing with cellulose-ester membrane, 500 MWCO) and subsequently freeze-dried. The yield was 27.5 mg of ODN macromonomer.

Molecular weight (MALDI TOF MS, Biomers) $M = 3831$ g/mol ± 15 g/mol ($M_{\text{theory}} = 3845$ g/mol). For comparison, molecular weight of the deprotected, C6-amino-linker modified ODN 5′-TCC ATG ACG TTC-3′: $M = 3769$ g/mol ± 10 g/mol ($M_{\text{theory}} = 3777$ g/mol).

To 27.5 mg of the ODN macromonomer was added 10 µL of APS in water (2 mg/mL) under argon in a 5 mL Schlenk vial. After 2 h, 4 µL of TEMED in water (4.5 µL/mL) was added. The reaction was kept at room temperature for 7 days. Subsequently, the sample was dialyzed against MilliQ water (SpectrumLab dialysis tubing with regenerated cellulose membrane, 10 000 MWCO) and freeze-dried. The yield of the polymer was 6.5 mg (24%).

Results and Discussion

Polymerizable oligodesoxyribonucleic acids (ODNs) with a sequence of 5′-TCC ATG ACG TTC-3′ were obtained by reaction of C6-amino-linker-modified 5′-TCC ATG ACG TTC-3′ with *N*-methacryloyloxysuccinimide.[9] After purification, this macromonomer was employed in a free radical polymerization in water to obtain poly(oligo-DNA) (Figure 1). Ammonium peroxodisulfate (APS) together with *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) were used as the initiator system. The poly(oligo-DNA) possesses a methacrylic main chain with ODN side-chains attached to each repeating unit. Nonreacted monomer was removed by dialysis, and the purified poly(oligo-DNA) was analyzed by static (SLS) and dynamic (DLS) light scattering in dilute aqueous solution containing 5 mM sodium phosphate as buffer system. The results are shown in Figure 2.

According to Zimm's equation,[10,11]

$$\frac{Kc}{R_q} = \frac{1}{M_w} + \frac{\langle R_g^{\,2}\rangle}{3M_w}q^2 + 2A_2c$$

the slope of Kc/R_q against q^2 (K is the optical constant, c is the concentration, R_q is the Rayleigh ratio, q is the absolute value of the scattering vector, M_w is the weight average of the molecular weight, and A_2 is the second virial coefficient) observed in SLS is proportional to the *z*-average of the apparent radius of gyration $\langle R_g\rangle_{z,\text{app}}$. The data analysis yields $\langle R_g\rangle_{z,\text{app}} =$ 67.8 nm. The determination of the correct refractive index increment (d*n*/d*c*) needed for the determination of M_w was not possible due to the limited amount of sample.

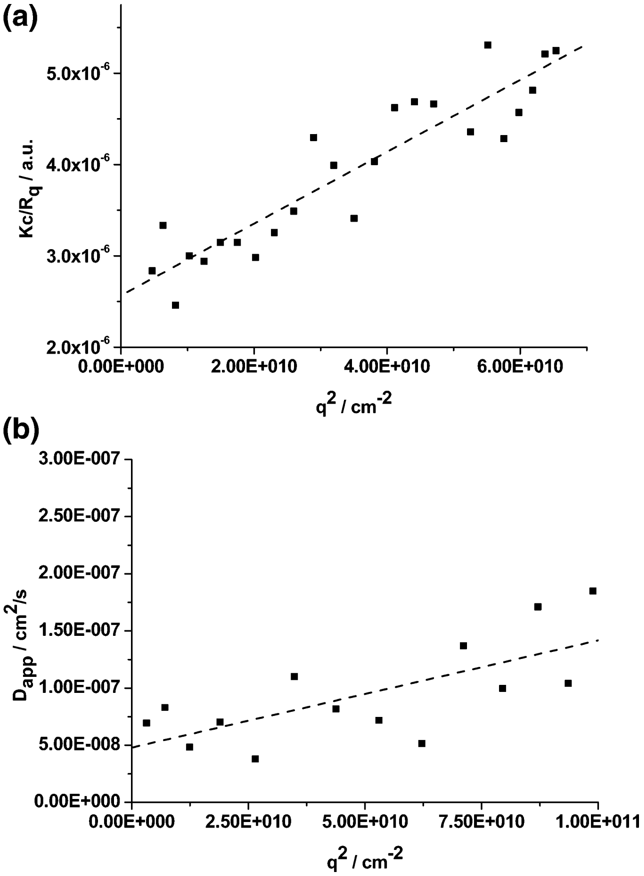

Figure 2. (a) SLS of poly(oligo-DNA) in 5 mM sodium phosphate buffer ($c = 0.1$ g/L); (b) extrapolation of the (at different scattering angles determined) apparent diffusion coefficients (DLS) to $q^2 = 0$ to determine $\langle R_h\rangle_{\text{app}}$ of poly(oligo-DNA) in 5 mM sodium phosphate buffer ($c = 0.1$ g/L).

In addition, DLS measurements were performed at different scattering angles. The *q*-dependence of the obtained apparent diffusion coefficients is shown in Figure 2b. The extrapolation to $q = 0$ gives the diffusion coefficient $D_{q\to 0,\text{app}} = 4.79$ µm²/s. According to the Stokes−Einstein relation,[10,11]

$$\langle R_h\rangle_{\text{app}} = \frac{k_B T}{6\pi\eta D_{q\to 0,\text{app}}}$$

where k_B is the Boltzmann constant, T is the absolute temperature, and η is the solvent viscosity, this leads to an apparent hydrodynamic radius of $\langle R_h\rangle_{\text{app}} = 44.6$ nm.[10,11] The ratio ρ of $\langle R_g\rangle_{z,\text{app}}$ to $\langle R_h\rangle_{\text{app}}$ is related to the shape of the molecules in solution.[10,11] The observed value of $\rho = \langle R_g\rangle_{z,\text{app}}/\langle R_h\rangle_{\text{app}} = 1.52$ is an indication for semi-flexible, wormlike molecules in solution. These results are in good agreement with those found for polymacromonomers having oligo-styrene-, oligo-2-vinylpyridine-, or oligo-(2-vinylmethylpyridinium bromide)- side chains.[5,12]

Measurements with an atomic force microscope (AFM) were performed to confirm the cylindrical shape of the molecules. The poly(oligo-DNA) was adsorbed onto mica and visualized by AFM as shown in Figure 3.

The individual, cylindrically shaped poly(oligo-DNA) molecules are easily identified. The broad length distribution is a result of the free radical polymerization. The results obtained from light scattering in solution and from AFM on mica consistently show the cylindrical shape of the poly(oligo-DNA).[12]

Conclusion

Poly(oligo-DNA) with a methacrylic main chain and oligomeric DNA side chains attached to each repeating unit was

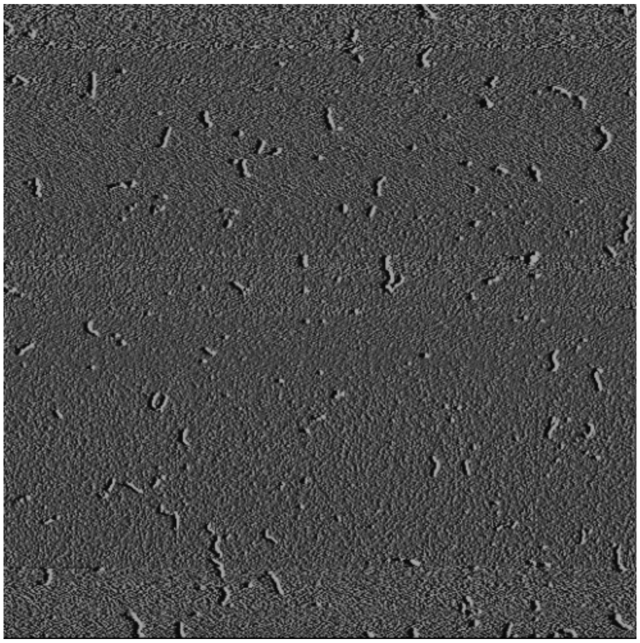

Figure 3. AFM pictures (height) obtained in tapping mode from poly(oligo-DNA) in aqueous solution adsorbed onto mica ($c = 0.35$ g/L, containing 5 mM $MgCl_2$). The size of the picture is (4 μm)^2.

successfully synthesized. Characterization in solution yields a $\langle R_g \rangle_{z,app} = 67.8$ nm and $\langle R_h \rangle_{app} = 44.6$ nm, which indicates the cylindrical shape in solution. This was confirmed by AFM, where single poly(oligo-DNA) molecules were successfully visualized. This synthetic approach can be employed in the synthesis of different ODN-containing polymer combs, and a structure−property relation regarding the impact of the cylindrical architecture as compared to spherical DNA-containing nanoparticles is the subject of ongoing research.

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