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Rigid DNA chains near nanoparticles

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Abstract

Two-photon excitation time-resolved fluorescence anisotropy and lifetime measurements were used to study the conformation of DNA near oppositely charged nanoparticles. Negatively-charged rhodamine-labeled DNA was allowed to adsorb onto positively-charged nanoparticles in deionized water and surface-induced fluorescence quenching was observed by measuring the fluorescence lifetime. Fluorescence quenching decreased with the addition of NaCl to the DNA-nanoparticle complex, which implies that the separation of DNA from the nanoparticles increased. However, comparison of fluorescence lifetime decays between free labeled-DNA and the DNA-nanoparticle complex shows that the adsorbed DNA remained less flexible than free labeled-DNA.

Keywords: DNA; Nanoparticle; Fluorescence lifetime

1. Introduction

Polyelectrolyte adsorption onto nanoparticles is an important theme not only in fundamental science [1] but also in numerous applications such as stabilization of colloidal particles [2–4] and various templates for microcapsules and immunoassays [5,6]. While the structure and conformations of adsorbed polyelectrolytes have been extensively studied mainly by using light scattering [7–9], the conformation of free polyelectrolytes near nanoparticle solid surfaces have been more problematical, as they are more difficult to measure by direct experiment.

Tohver et al. [10] reported interesting results related to stabilization of colloid suspensions using nanoparticles. When negligibly charged microspheres and strongly (but oppositely) charged nanoparticles were mixed, nanoparticles built up near the microspheres and stabilized them by electrostatic repulsion between microspheres. It is interesting to consider the possibility that an analogous situation might occur when colloidal particles are mixed with oppositely charged polyelectrolytes. Strong electrostatic interaction binds them tightly in deionized water. However, the addition of salt reduces the attractive force and the salt concentration can be controlled to vary the separation. While there is no concern about conformation in the case of hard nanoparticles, a flexible polyelectrolytes may adapt its conformations.

Here, we studied the conformation of rhodamine-labeled DNA oligomers near positively charged polystyrene (PS) latex particles using two-photon excited time-resolved fluorescence technique. Since both the DNA backbone and PS particles are quenchers of the rhodamine dyes [11,12], the conformation of DNA oligomers governed by electrostatic interaction can be deduced by measuring the fluorescence lifetime.

2. Experimental details

2.1. Materials

Amidine-terminated polystyrene latex particles were purchased from Interfacial Dynamics Corporation (Tualatin, Or) and used as received without further purification. They are positively-charged in deionized water, with surface charge density 3.2 $\mu C/cm^2$. The dry diameter of the latex particle is stated by the manufacturer to be 40 ± 10 nm. Rhodamine-labeled DNA oligomer, 5′-RhG-GATGATGA-GAAGAAC-3′, was custom-synthesized and purchased from TriLink BioTechnologies Inc. (San Diego, CA).

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Rhodamine-G dye was separated from the main chain by a sequence of six methylene units (six backbone carbons). The final concentrations of DNA and nanoparticles in the suspension were fixed at 1.7 μM and 330 nM, respectively.

2.2. Instruments

Time-resolved fluorescence depolarization is a method by which to quantify rotational relaxation times on the nanosecond time scale. In this way, rotational motions of a fluorescent molecule are used to probe the local microenvironment within which it resides. Two-photon excitation of the fluorescent probe molecules was induced using a femtosecond Ti:Sapphire laser (Mai Tai, Spectra-Physics) whose FWHM (full width at half maximum) pulse was measured to be 100 fs. The repetition rate was 80 MHz and the wavelength was 800 nm. The experiments were performed within a homemade microscope and a perfusion chamber (Sigma) with 1.3 mm total thickness.

In the design that we employed, the vertically-polarized laser beam was first split into two beams and one of them was introduced into an objective lens (Mitutoyo, numerical aperture NA = 0.55) and focused onto the sample. The other beam was used as a trigger signal for the single photon counting system (Becker & Hickl GmbH, Berlin, Germany). The emitted fluorescence was collected by the same objective lens and focused again by a tube lens in order to increase the response of the photomultiplier tube (PMT) detectors. A fast PMT (Hamamatsu, R5600) and a photodiode were used to detect the fluorescence and the trigger signal, respectively. The PMT signal was input to the time-to-amplitude converter as a start signal followed by a constant fractional discriminator (Becker & Hickl GmbH, TCSPC730). In this setup the total instrument response function was around 150 ps.

3. Results and discussion

As polystyrene nanoparticles were added to the DNA solution, strong electrostatic interaction attracted DNA onto the surface since amine groups on polystyrene nanoparticles carry positive charges and DNA has a negatively-charged phosphate backbone. The ratio of the number of DNA molecules to the number of nanoparticles was fixed at 5 throughout this paper. Fig. 1 compares the fluorescence anisotropy decays of free DNA and adsorbed DNA on the nanosecond time scale. Both decays were best fitted by double exponential functions and two characteristic rotational correlation time constants were obtained. While the shorter time constant reflected the relatively fast rotation of the carbon linker for both cases, the longer time constants for free DNA and adsorbed DNA reflected the slower rotations that included the DNA backbone alone and the DNA-nanoparticle complex.

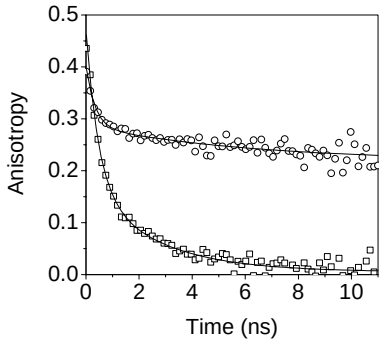

Fig. 1. Fluorescence anisotropy plotted against time on the nanosecond time scale, for 5′-RhG-GATGATGAGAAGAAC-3′ at 1.7 μM concentration in deionized water: (□) free DNA; (○), adsorbed DNA on amidine-terminated polystyrene latex particle. Lines through the data are fitted using double exponential functions.

Since the hydrodynamic volume of the DNA oligomer was small, the anisotropy of the free DNA completely depolarized within 10 ns. However, the anisotropy of the adsorbed DNA on the nanoparticles was slower and did not depolarize fully over the experimental time. Fluorescence lifetime measurements presented in a prior paper from this laboratory [11] showed that the fluorescence lifetime of the DNA-nanoparticle complex was almost constant independent of temperature while that of free labeled-DNA depended strongly on temperature. These findings support the view that the attraction between DNA and nanoparticles was so strong that DNA backbone was immobile on the nanosecond time scale.

Fig. 2 compares the fluorescence lifetime decays of free rhodamine G, free labeled-DNA and the DNA-nanoparticle complex in deionized water. While the lifetime decay of free rhodamine dyes follows a single exponential function, the lifetime decay of the DNA-nanoparticle complex was best fitted by double exponential functions. This is because

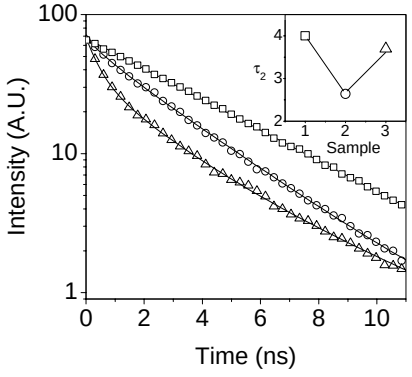

Fig. 2. Fluorescence intensity plotted logarithmically against linear time for: (□), free rhodamine G; (○), free 5′-RhG-GATGATGAGAAGAAC-3′; (△), RhG-DNA adsorbed on colloidal particles of opposite charges in deionized water. In the Inset, the longer lifetime constant of each sample is plotted. Error bars are smaller than the size of symbols.

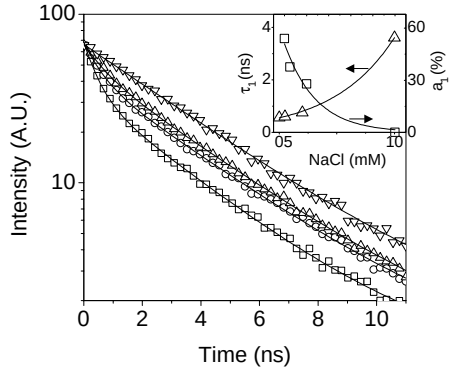

Fig. 3. Fluorescence intensity plotted logarithmically against linear time on the nanosecond time scale, for 5′-RhG-GATGATGAGAAGAAC-3′ adsorbed onto colloidal particles of opposite charge as described in the text, at various NaCl concentrations: (□), 0 mM; (○), 0.5 mM; (△), 2 mM; (▽), 10 mM. In the Inset, the shorter lifetime constant and its fractional contribution to the total lifetime decay are plotted against NaCl concentration. Error bars are smaller than the size of symbols.

the rhodamine dyes were quenched by both DNA bases and nanoparticles. Considering that fluorescence quenching in the earliest decays of the lifetime is caused by the fast rotation of a carbon linker and affects both free labeled-DNA and the DNA-nanoparticle complex, [11] the longer lifetime time constants (τ_2) are compared in the inset of Fig. 2. As expected, the τ_2 of free labeled-DNA and DNA-nanoparticle complex are smaller than τ_2 of free rhodamine dyes ($\tau_1 = \tau_2$) due to the fluorescence quenching. However, note that τ_2 of the DNA-nanoparticle complex is larger than that of the free labeled-DNA while τ_1 shows the opposite tendency. This is because the DNA backbone in the DNA-nanoparticle complex does not rotate on the nanosecond time scale due to electrostatic attractions and the dyes are not quenched by the DNA backbone.

Fig. 3 shows that the fluorescence lifetime decays slowed as NaCl salt concentrations were increased from 0 to 10 mM. At concentrations above 10 mM, nanoparticles aggregated. While the lifetime decay at 10 mM concentration could be fitted by a single exponential decay ($\tau = 3.7$ ns), the others were best fitted by double exponential functions ($y = a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2) + y_0$). Since all the curves in Fig. 3 show the same rate of decay except at the earliest times, for subsequent analysis the longer time constant (τ_2) was fixed at 3.7 ns in order to consider the effect of fluorescence quenching on the earliest fluorescence decays.

The inset of Fig. 3 shows the short lifetime time constants (τ_1) and its fractional contribution to the total decay (a_1) plotted against salt concentration. As salt concentration increased, τ_1 increased because the screening effect of counter ions reduced the electrostatic attraction to the surface of opposite charge. The resulting increased separation between DNA and nanoparticles reduced the possibility of collisional quenching of the dyes. The gradual increase of τ_1 summarized in inset of Fig. 3 implies the separation between DNA and nanoparticles increased with NaCl concentration, and that this separation in 10 mM NaCl exceeded the length of six carbon linkers and rhodamine G (∼1.5 nm) and the fast decay completely disappeared. Most interestingly, the excellent fit of the data suggests that τ_2 was indeed constant (3.7 ns) regardless of the NaCl concentration, yet slower than that of free labeled-DNA (2.6 ns). Since τ_2 is inversely dependent on the degree of quenching by DNA backbone, the constant τ_2 implies that the mobility of DNA backbone is as slow as that on nanoparticles even though the separation increased with NaCl concentration.

4. Prospects

The main point of this study is that, although the separation between DNA and nanoparticles was increased by adding salt, the DNA molecules continued to be electrostatically affected by the nanoparticles, adopting conformations less flexible than those of unattached DNA. Note also that, in principle (if there were no fluorescence quenchers present apart from DNA bases and nanoparticles), the root mean square distance ($\langle\delta^2\rangle$) between the dye and the nanoparticles at various NaCl concentration could be calculated from $\langle\delta^2\rangle = (2D\tau)$, where D is the rotational diffusion coefficient and τ is the fluorescence lifetime [1]. Since the dyes were covalently connected to the DNA backbone and the DNA backbone does not move on the nanosecond time scale, the diffusion coefficient of the dyes corresponds to the rotational diffusion coefficient measured in Fig. 1. However, quantification of the distance between DNA and nanoparticles requires knowing the rotational diffusion coefficient from the short rotational correlation time constant, τ_1, with a suitable model that considers the geometry of a single-point fixed rotor.

In the largest sense, we note that the combination of fluorescence and nanoparticles showed several potential advantages to study the local environment of adsorbed molecules. First, fluorescence photobleaching (one of the main barriers to adapting fluorescence techniques to surfaces) was easily circumvented owing to the fast mobility of the nanoparticles. Second, various colloids with different size and functional group on the surface are commercially available. Third, the tiny focal volume produced by two-photon excitation makes possible to use the fluorescence technique inside small devices such as MEMS.

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