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Increased Intensities of YOYO-1–labeled DNA Oligomers Near Silver Particles[¶]

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Abstract

DNA detection is usually performed using fluorescence probes. Using a DNA oligomer stained with the widely used dye 1,1'-[1,3-propanediylbis[(dimethylimino)-3,1-propanediyl]]-bis[4-[(3-methyl-2(3H)-benzoxazolylidene)methyl]]-quinolinium tetraiodide (YOYO-1), we show that a substrate containing silver particles can lead to a greater than 10-fold increase in the fluorescence intensity. Proximity to silver particles also increases the photostability of YOYO-1–DNA. These results suggest that substrates or gels containing silver particles may be used for increased sensitivity in DNA detection.

INTRODUCTION

The intrinsic fluorescence from DNA is extremely weak. As a result, DNA detection is often performed using fluorescent probes. The TOTO and YOYO series are a valuable class of probes (1–3). These dyes contain multiple positive charges, bind strongly to DNA and display almost no fluorescence in water. As a result, these dyes are useful for the observation of DNA in electrophoretic gels.

During the past 2 years we have investigated the effects of metallic silver particles on fluorescence (4–7). We found that the positioning of fluorophores 50–100 Å from a metallic silver surface (8) results in increased emission intensities, decreased lifetimes and modest increases in photostability. These effects appear to be due to interactions of the excited fluorophore with the surface plasmon resonance (SPR) displayed by subwavelength size silver particles (9). The SPR is due to electron oscillations on the metallic surface induced by an incident field. For clarity, we note that our studies are not the usual SPR assays, which depend on a decreased reflectance from a continuous gold surface at specific angles of incidence.

In the present article, we examine the effects of silver particles on DNA oligomers labeled with 1,1'-[1,3-propanediylbis[(dimethylimino)-3,1-propanediyl]]bis[4-[(3-methyl-2(3H)-benzoxazolylidene)methyl]]-quinolinium tetraiodide (YOYO-1) (Scheme 1). Binding to a surface coated with silver particles was found to result in dramatically increased emission intensities of YOYO-1–DNA.

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MATERIALS AND METHODS

Biotinylated oligonucleotide 5'-GAA GAT GGC CAG TGG TGT GTG GA-3'-biotin and complementary oligonucleotide 5'-TCC ACA CAC CAC TGG CCA TCT TC-3' were obtained from the Biopolymer Core Facility at the University of Maryland, School of Medicine (Baltimore, MD). YOYO-1 and avidin (egg white) were purchased from Molecular Probes (Eugene, OR). Bovine serum albumin (BSA)-biotin (bovine albumin-biotinamidocaproyl labeled) was from Sigma (St. Louis, MO). Other compounds used for silver island film (SIF) and buffer preparation were from Sigma-Aldrich (St. Louis, MO).

SIF are particles of silver on inert substrates formed by chemical reduction. Such films are widely used in surface-enhanced Raman scattering. Our SIF were deposited on clean quartz slides (Starna Cell Inc., Atascadero, CA) using an ammonia solution of silver nitrate and D-glucose as a reducing agent as described previously (5,10). The size distribution of the SIF based on atomic force microscopy (AFM) image is described by Lakowicz *et al.* (5).

We used protein-coated surfaces to bind DNA to quartz or SIF (Scheme 1). Each slide (12.5 × 45 mm, half coated with SIF) was covered with 250 µL of 10 M BSA-biotin aqueous solution and placed in a humid chamber for 20 h (5°C, cold room). After washing three times with water, slides were placed again in the humid chamber and 250 µL of 5 µM avidin in 0.1× phosphate-buffered saline (PBS) was deposited on each BSA-biotin-coated surface for 40 min at room temperature. The slides were then washed three times with 0.1× PBS buffer, and 250 µL of YOYO-1-DNA-biotin (3:1) solution was deposited for 60 min at room temperature.

Double-stranded (ds)-DNA sample (DNA-biotin) was prepared by mixing complementary oligonucleotides in 5 mM *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid) (HEPES; pH 7.5), 0.1 M KCl and 0.25 mM ethylenediaminetetraacetic acid (EDTA) solution to a final concentration 1 µM and very slow cooling after incubation at 70°C for 2 min. To the above solution, 1 mM YOYO-1 solution in dimethyl sulfoxide (DMSO) was added to a final concentration of 3 µM, yielding a YOYO-1/DNA ratio of 3:1. After YOYO-1-DNA-biotin deposition, the slides were washed three times with hybridization buffer (5 mM HEPES, pH 7.5; 0.1 M KCl; 0.25 mM EDTA) and covered with one part of a 0.5 mm demountable cuvette filled with the same buffer. For titration, we used a 1 µM DNA-biotin solution in the hybridization buffer and an appropriate amount of 1 mM YOYO-1 in DMSO.

To check for the presence and possible emission of unbound YOYO-1 on one of the above SIF slides coated with a monolayer of BSA-biotin-avidin instead of YOYO-1-DNA-biotin solution, we deposited a similar sample but without DNA-biotin.

The titration experiment was performed in a 1 cm² cuvette using a Cary Eclipse fluorometer (Varian, Walnut Creek, CA) and an excitation wavelength of 470 nm. Emission spectra of YOYO-1-DNA-biotin on the protein layer were measured in front face geometry on a SLM 8000 spectrofluorometer with 470 nm excitation from a xenon lamp. Lifetimes were measured on a 10 GHz frequency-domain (FD) fluorometer using a mode-locked argon ion laser (514 nm, 76 MHz repetition rate) (11). Excitation and emission polarizers were in the magic angle orientation. Emission was collected with a combination of a 520 nm long pass filter and a 540 nm interference filter.

The FD intensity decay was analyzed in terms of the multiexponential model

$$I(t) = \sum_i \alpha_i \exp(-t/\tau_i), \quad (1)$$

where τ_i are the lifetimes with amplitudes α_i and $\sum \alpha_i = 1.0$. Fitting to the multiexponential model was performed as described previously (12). The contribution of each component to the steady-state intensity is given by

$$f_i = \frac{\alpha_i \tau_i}{\sum_j \alpha_j \tau_j} \quad (2)$$

The mean decay time is given by

$$\bar{\tau} = \sum_i f_i \tau_i \quad (3)$$

The amplitude-weighted lifetime is given by

$$\langle \tau \rangle = \sum_i \alpha_i \tau_i \quad (4)$$

RESULTS

Initially we examined the spectral properties of YOYO-1–DNA in a cuvette, not bound to a surface. The emission intensity increased with increasing amounts of YOYO-1 until the base pair to YOYO-1 ratio decreased to about 6, at which point the intensity remained constant (Fig. 1). The constant high intensity and constant anisotropy indicated that the dye is not quenched by energy transfer between the probes as occurs with fluorescein, rhodamines and 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene derivatives (BODIPY) probes.

We were aware about the possible fluorescence signal from YOYO-1 unbound to DNA. Therefore, instead of streptavidin we used avidin, which reveals positively charged YOYO-1, and washed the sample extensively. In fact, we did not observe any significant emission from YOYO-1 in the absence of DNA (Fig. 2). The insert of Fig. 2 shows the absorption spectra of a typical SIF.

We measured the emission spectra of YOYO-1–DNA when bound to quartz or silver *via* the protein layers (Fig. 3). The emission intensity is 15-fold higher on the SIF than on quartz. This dramatic intensity increase can be seen in the upper panels, which are photographs of equal amounts of YOYO-1–DNA spotted on a slide.

There are two mechanisms responsible for the increase of brightness near the SIF, an enhanced local field and an increase in radiative decay rate (4). Taking into account the quantum yield of YOYO-1 bound to ds-DNA of 0.38 (13), the increase in radiative decay rate can be responsible for an about three-fold increase in the brightness because the quantum yield of any fluorophore cannot exceed 1.0.

Figure 4 shows the FD intensity decays of YOYO-1–DNA on quartz and silver. The lower panel shows the time-dependent decays reconstructed from the FD data. The lifetime of YOYO-1–DNA on quartz is similar to that found in free solution (Table 1). The lifetime

decreases about four-fold when bound to the SIF. A decrease in lifetime, accompanied by an increase in intensity, indicates an increase in the radiative decay rate of the probe (4).

If all other factors are equal, decreased lifetimes are expected to result in increased photostability. By photostability we mean the number of excitation cycles before photobleaching. If the lifetime is shorter, there is less time for photoreactions. We tested photostability by measuring the steady-state intensity with continuous illumination (Fig. 5). When illuminated with the same incident intensity, the area under the curves is about 17-fold higher on the SIF than on quartz (top panel). When normalized to the same intensity at time zero, the photobleaching on quartz is faster than on the SIF (bottom panel).

CONCLUSION

YOYO-1-labeled DNA displays increased intensity and increased photostability when positioned near silver particles. These results suggest the incorporation of silver particles into gels or chromatographic systems used for DNA detection.

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Abbreviations

AFM	atomic force microscopy
BODIPY	4,4-difluoro-4-bora-3a,4a-diaza-s-indacene derivatives
BSA	bovine serum albumin
DMSO	dimethyl sulfoxide
EDTA	ethylenediaminetetraacetic acid, disodium salt
FD	frequency-domain
HEPES	<i>N</i> -(2-hydroxyethyl)piperazine- <i>N'</i> -(2-ethanesulfonic acid)
PBS	phosphate-buffered saline
SIF	silver island film
SPR	surface plasmon resonance
YOYO-1	1,1'-[1,3-propanediylbis[(dimethylimino)-3,1-propanediyl]]bis[4-[(3-methyl-2(3H)-benzoxazolylidene)methyl]]-quinolinum tetraiodide

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Figure 1. Emission spectra of the DNA oligomers labeled with increasing concentrations of YOYO-1. The inserts show the anisotropies and intensities with increasing concentrations of YOYO-1.

Figure 2.
Absorption spectra of an SIF and emission spectra of the protein-coated surfaces treated with YOYO-1 but without DNA.

Figure 3.

Emission spectra of YOYO-1-labeled DNA bound to the quartz (Q) and silver (S) surfaces. The upper panels show a real-color photograph of labeled DNA spotted on the silver (left) and quartz (right) surfaces.

Figure 4.
FD (top) and reconstructed time-domain (bottom) intensity decays of YOYO-1-DNA on quartz (Q) and silver (S).

Figure 5. Photostabilities of YOYO-1-DNA on quartz and silver with the same incident power (top) and at the same incident power but normalized at time zero (bottom).

Scheme 1.

Schematic of surface, sequence of the DNA oligomers, structures of YOYO-1 and biotinylated oligonucleotide and experimental geometry. Note that sizes in the surface schematic are not in scale. The BSA-avidin protein layer is about 80 Å thick, and the silver particles are about 400 Å high.

Table 1
Multiexponential analysis of YOYO-1-labeled DNA intensity decays

Conditions	$\langle \tau \rangle$ (ns)	$\bar{\tau}$ (ns)	a_1	f_1	τ_1 (ns)	χ^2_R
Cuvette	2.28	3.08	0.449	0.153	0.78	1.1
			0.551	0.847	3.50	
Quartz	1.98	2.85	0.503	0.166	0.65	2.1
			0.497	0.834	3.29	
SIF	0.44	1.26	0.516	0.108	0.009	1.4
			0.402	0.454	0.49	
			0.082	0.438	2.34	