Protein-Sized Quantum Dot Luminescence Can Distinguish between "Straight", "Bent", and "Kinked" Oligonucleotides

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There is increasing evidence from X-ray crystal structures, gel electrophoresis, enzyme cyclization, and electron microscopy experiments that DNA sequence can dictate overall DNA structure.[1-3] In particular, double-stranded DNAs that contain 5′-A_n-3′ (where $n = 3-7$) phased one full double-helical twist apart appear to induce curvature in the context of a long strand ($\sim 10^2$ bp) of DNA in gel mobility and enzyme cyclization experiments.[1-3] The sequence 5′-d(CATGGCCATG)-3′ has been crystallized as a self-complementary duplex and shows a definite kink of 23° across the central 5′-GGCC-3′.[4] However, packing forces and the presence of highly-charged counterions might also influence the DNA structure in the solid state.[5] Here we describe a novel method for probing DNA structure in dilute solution: the adsorption of oligonucleotides of defined sequence to protein-sized particles. The particles are "quantum dots" of the semiconductor CdS,[6] and we find that the surface-sensitive luminescence of these particles can discriminate between "straight", "bent", and "kinked" oligonucleotides in dilute solution.

Oligonucleotides were synthesized by standard phosphoramidite chemistry: 5′-GGGTCCTCAGCTTGCC-3′ and complement as a straight duplex; 5′-GGTCCAAAAAATTGCC-3′ and complement as a bent duplex; and the self-complementary 5′-GGTCATGGCCATGACC-3′ as a kinked duplex.[7] CdS quantum dots were synthesized by standard methods[6] and were surface-enriched with Cd^{2+} [8] to present a cationic surface to the DNAs. The average size, determined by transmission electron microscopy, was 40 Å (~15% standard deviation). The Cd^{2+} enrichment procedure leads to bright yellow photoluminescence that is very sensitive to the nature and amount of adsorbates.[9]

Luminescent titrations were performed[10] with 2×10^{-4} M CdS (~1 μM particles) and up to ~20 μM duplexes. Figure 1 illustrates how the kinked GGCC-containing DNA quenches the emission much more efficiently than the straight oligonucleotide.[11] Stern–Volmer plots of these systems are nonlinear and are upward-curving, indicating that the normal assumptions of collisional quenching are not valid. Other workers have shown that long (10^4 bp) DNA quenches the emission of CdS particles and have fit their data to a Perrin sphere of action quenching model;[12] our data, with much smaller biopolymer fragments, do not fit this model either.

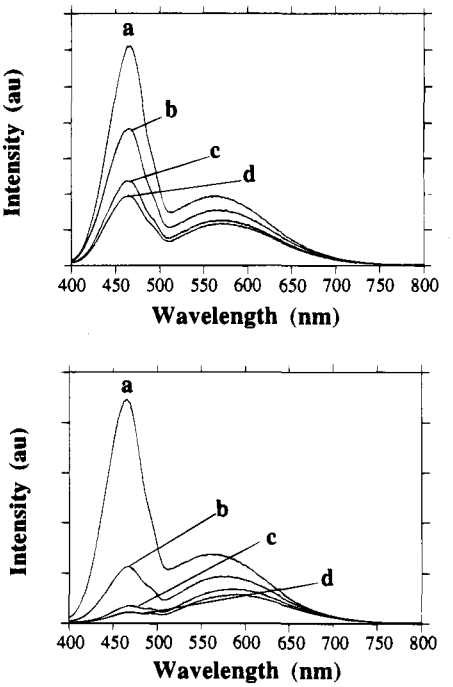

Figure 1. (Top) Luminescence quenching of 2×10^{-4} M, 40 Å, Cd^{2+}-rich CdS particles by (a) 0, (b) 4, (c) 18, and (d) 20 μM (duplex) concentrations of the straight oligonucleotide. (Bottom) Luminescence quenching of 2×10^{-4} M, 40 Å, Cd^{2+}-rich CdS particles by (a) 0, (b) 4, (c) 20, and (d) 23 μM (duplex) concentrations of the kinked oligonucleotide.

We can, however, fit changes in luminescence to an adsorption isotherm model to extract relative binding constants for adsorption of the three duplexes. We have used the Frisch–Simha–Eirich theory of a long polymer adsorbing in segments onto a locally flat surface as a starting point:[13]

$$[\theta \exp(2K_1\theta)]/(1-\theta) = (KC)^{1/\nu} \quad (1)$$

where θ, the fractional surface coverage, is equated to fractional change in luminescence;[14] K_1 is a constant that is a function of the interaction of adsorbed polymer segments; K is the equilibrium constant for binding; and ν is the average number of segments attached to the surface. ν is also related to chain

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(10) In a typical procedure, 5 μL aliquots of approximately millimolar (nucleotide) DNA solutions (5 mM Tris, 5 mM NaCl buffer, pH 7.2) were added every 30 min to 200 μL of a 2×10^{-4} M activated colloidal CdS solution. Emission spectra were acquired on an SLM 8100 spectrofluorometer with excitation at 350 nm, where the oligonucleotides themselves do not absorb. The luminescence intensity was integrated over the wavelength range of 400–480 nm and corrected for buffer effects. The photoluminescence of the activated CdS solution alone does not change significantly over the time course of this experiment.

(11) It is not obvious why the emission at 400–480 nm, more characteristic of shallow trap or excitonic emission, would be more sensitive to surface adsorbates than the deep trap emission at ~600 nm; see refs 6 and 8 for discussions of these states. The nature of the chemical bonds between DNA and the CdS surface have been postulated to be hydrogen bonds; see ref 12.

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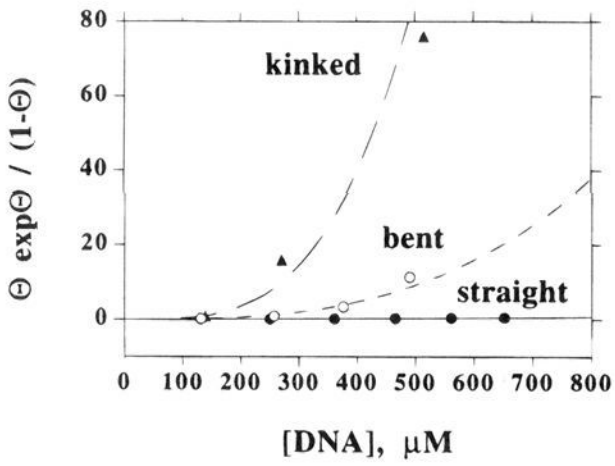

Figure 2. Frisch−Simha−Eirich plot for oligonucleotide adsorption to 40 Å, Cd^{2+}-rich CdS particles. K_1 is assumed to be the same for all oligonucleotides, reflecting the likelihood that surface-bound oligonucleotides interact with each other electrostatically in a sequence-independent manner. Best fits were obtained for $K_1 = 0.5$.

flexibility.[13] (For $K_1 = 0$ and $\nu = 1$, eq 1 would reduce to the simple Langmuir adsorption isotherm model.) In our treatment, we have implicitly assumed that all three duplexes adsorb "lengthwise":[15]

Figure 2 shows fits of our data to the Frisch−Simha−Eirich model.[13] We find reasonably good fits to the model (correlation coefficient is 0.98 or better), with $K \sim 7200 \pm 700$ M^{-1} (nucleotide) for the kinked GGCC oligonucleotide, $K \sim 4200 \pm 400$ M^{-1} for the bent A-tract, and $K \sim 1000 \pm 100$ M^{-1} for the straight DNA. These values are similar in magnitude to small molecule adsorption to CdS colloids, which are routinely fitted to the Langmuir adsorption isotherm model.[9,16] These values are also similar to those found for nonspecific protein−DNA interactions.[17] Calculation of the corresponding $\Delta\Delta G$ values reveals that the kinked oligonucleotide binding to the cationic surface is more favorable by ~0.3 kcal/mol (nucleotide) compared to the bent A-tract and by ~1.2 kcal/mol compared to the straight oligonucleotide, within the framework of this model. These values agree well with those obtained in nucleosome reconstitution studies, in which certain DNA sequences wrap about the protein core with differential bending energies of 1.2−1.9 kcal/mol compared to reference sequences.[18] It is important to note that our CdS is stabilized by sodium polyphosphate, and these binding energies of the DNAs for the surface are relative to that polyelectrolyte.

Irradiated CdS colloids generate electron−hole pairs which, if separated, can be used for redox chemistry on adsorbed species.[19] We performed snake venom phosphodiesterase/alkaline phosphatase digests on free and bound DNAs (in both dark and light) to look for any modified bases that were formed by electron or hole capture. From our HPLC detection limits, there appear to be <1% modified bases formed. (This, however, does not address any sugar modifications.) We have also monitored the kinetics of binding by measuring luminescence quenching as a function of time after manual mixing. We find that within 300 s, the substrate emission has decreased dramatically for the kinked DNA (by ~40% from its initial measured value, which is already only a third of that for the other DNAs), while the bent A-tract and straight DNAs are virtually constant on this time scale (data not shown).

Whether the oligonucleotides are "prebent" in solution, or whether they are all relatively rigid rods whose sequence imparts flexibility upon initial contact with the Cd^{2+}-rich substrate, is not unambiguous by these luminescence studies alone. Hydroxyl radical cleavage experiments, which provide information about the relative width of the minor groove, are in progress on bound and free oligonucleotides.[20]

These results uniquely show that it is possible to view these luminescent quantum dots as generic DNA-binding proteins. These particles are readily accessible in a range of sizes and the surface is easy to modify.[6] These experiments provide a "protein-free" background in which to examine intrinsic and/or induced DNA bending to a substrate, which is crucial for the regulation of transcription.[21] Even more generally, these experiments suggest that polymer shape influences adsorption to colloidal particles.

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(15) The 400−480 nm component of the emission is completely quenched by sufficient concentrations of the kinked and straight DNAs under our experimental conditions. However, titration experiments with even more concentrated solutions of the bent A-tract show that it cannot completely quench the emission, at least not in the same time frame. This might indicate that the A-tract adsorbs differently; further experiments are in progress to test this hypothesis. Base composition is known to influence the luminescence of small DNA-bound inorganic molecules; see, for example: Murphy, C. J.; Barton, J. K. *Methods Enzymol.* **1993**, *226*, 576.

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