Temperature- and Salt-Dependent Binding of Long DNA to Protein-Sized Quantum Dots: Thermodynamics of "Inorganic Protein"−DNA Interactions

Rahina Mahtab, H. Hydrick Harden, and Catherine J. Murphy*

Contribution from the Department of Chemistry and Biochemistry, University of South Carolina, Columbia, South Carolina 29208

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Abstract: The adsorption of calf thymus DNA to 45 Å nanoparticles of Cd(II)-rich CdS has been examined by photoluminescence spectroscopy as a function of temperature. The resulting van't Hoff plot suggests that the driving force for adsorption is entropy, and the enthalpic contribution to DNA−surface binding is slightly unfavorable. A likely source of the increase in entropy upon binding is release of solvent and/or counterions from the interface, analogous to what has been observed for nonspecific protein−DNA interactions. Reverse salt titrations suggest that counterion release is a substantial component of the nanoparticle−DNA interaction.

Introduction

Semiconductor quantum dots, long of interest for their physical and materials properties, are becoming favored for robust luminescent probes in biological applications.[1−7] In our laboratory we have deployed these nanomaterials as luminescent probes of intrinsic DNA structure and/or flexibility.[1−3] These materials are in the size range of proteins, and their surfaces can be modified to present hydrophilic, hydrophobic, cationic, anionic, or neutral faces to the surrounding environment. To gain insight into sequence-directed DNA structure, we have considered the quantum dots to be generic curved surfaces that the DNA can wrap about, with any specificity in the binding process coming from the DNA.[1−3] Thus, the nanoparticles can be thought of as inorganic nonspecific DNA-binding "proteins" in this biophysical application.

The working model we have had of these DNA−nanoparticle interactions has been one in which electrostatic forces between negatively charged phosphate groups from the DNA backbone and the cationic nanoparticle surface dominated the binding process. This has been suggested by titration experiments in which the binding constants of certain DNAs to net cationic 40 Å particles were found to be higher by roughly a factor of 4 compared to net neutral 40 Å particles.[1−3] Here we examine the temperature-dependent and salt-dependent binding of DNA to nominally cationic particles to estimate the enthalpy and entropy changes associated with the adsorption process. Reverse salt titrations support the notion of counterion release as a dominant mechanism in the DNA−nanoparticle interaction.

Experimental Section

Materials. All common chemicals were obtained from commercial sources. Calf thymus DNA (sodium salt, Sigma) was purified by phenol−chloroform extraction. The DNA was dissolved in buffer (5 mM Tris, 5 mM NaCl, pH 7.2) and concentrations were determined spectrophotometrically using $\epsilon(260\ \text{nm}) = 6600\ \text{M}^{-1}\ \text{cm}^{-1}$ (nucleotide). CdS nanoparticles, size-stabilized by sodium polyphosphate and surface-enriched with Cd(II), were prepared as described in ref 1 to yield solutions that were ∼1 μM in nanoparticles. During the course of the activation, NaOH was added to achieve a basic solution; overall, the concentration of Na^+ in the nanoparticle solution was ∼6 mM.

Instrumentation. Electronic absorption spectra were obtained on a Perkin-Elmer Lambda 14 ultraviolet−visible spectrophotometer. Photoluminescence spectra were acquired on a SLM-Aminco 8100 spectrofluorometer. CdS particle size was determined from transmission electron microscopic data acquired on a Hitachi H-8000 instrument. Circular dichroism data were taken on a JASCO system courtesy of J. H. Dawson (USC). Temperature control for the optical spectra was provided by a Lauda circulating water bath that was calibrated in the cuvette with a thermometer. The melting temperature of calf thymus DNA under the conditions of the titration experiments was >70 °C; thus the DNA was largely double-stranded over the temperature range examined.

Procedures. In a typical titration experiment, 200 μL of 2×10^{-4} M Cd(II)-rich CdS solution (∼1 μM nanoparticles) was placed in a thermostated cuvette in the spectrofluorometer, and the emission spectrum was recorded. Aliquots of a millimolar stock CT DNA solution were added to the nanoparticle solution (maximum dilution 10% of original volume) and emission spectra were recorded after 30 min of equilibration time per aliquot. For the reverse salt titrations, CT DNA was added to a 200 μL sample of 2×10^{-4} M Cd(II)-rich CdS solution to achieve ∼75% quenching of the emission, followed by subsequent additions of aliquots of a 0.25 M $NaNO_3$ stock solution. The intensity of the emission increased upon salt addition, smoothly reversing the

* Author to whom correspondence should be addressed.

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quenching observed with DNA. Sodium nitrate was preferred to sodium chloride because of the tendency of Cd(II) to make complex ions with Cl^- (e.g., $[CdCl_4]^{2-}$). Sufficiently high salt concentrations caused precipitation of the nanoparticles.

Fractional changes in luminescence as a function of DNA concentration were fit to the Frisch−Eirich−Simha (FES) adsorption isotherm[11] to extract relative binding constants at each temperature (25−50 °C):

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

where θ, the fractional surface coverage, was equated to fractional change in luminescence ($\theta = (PL - PL_{init})/(PL_{final} - PL_{init})$, where PL is the intensity of photoluminescence at an arbitrary point in the titration, PL_{init} is the initial photoluminescence intensity before DNA is added, and PL_{final} is the photoluminescence intensity at which no further changes take place as DNA is added); C is the DNA concentration in nucleotides; K_1 is a constant that is a function of the interaction of adsorbed polymer segments (set equal to 0.5 here, which we have empirically found to give best fits to the data); K is the equilibrium constant for binding; and ν is the average number of segments attached to the surface. The Frisch−Eirich−Simha model was originally derived for adsorption of a long polymer in short segments on a locally flat surface. Our definition of θ implicitly assumes a two-state model for the nanoparticle−DNA interaction; the nanoparticle is either bound to DNA (and emits with intensity PL_{init} to PL_{final} depending on DNA concentration) or free of DNA (and emits with intensity PL_{init}). For extracting equilibrium constants as a function of salt at room temperature at constant DNA concentration C, θ was calculated from the emission spectra as described above, and $1/\nu$ was set to the room temperature value from the FES adsorption isotherm.

Results and Discussion

Temperature Studies. CdS nanoparticles 45 ± 5 Å in diameter were prepared as previously described, and characterized by transmission electron microscopy and electronic absorption spectroscopy. Addition of excess Cd(II) was performed to "activate" the particles.[1] This procedure results in particles surface-enriched with Cd(II). The photoluminescence of these aqueous nanoparticle solutions was monitored to ensure complete activation; activation results in a high-energy band centered at ∼480 nm that is significantly more intense than the "defect" emission at ∼600 nm, as previously observed.[8] Calf thymus DNA (CT DNA; ∼10000 base pairs long) was used for the temperature study because of its higher thermal stability compared to oligonucleotides. One possible difficulty with temperature experiments is that the nanoparticles may aggregate or dissolve as a function of temperature unless they are covalently capped. As judged by electronic absorption spectroscopy, the particle size change upon temperature increases was small; the average size of the Cd(II)-capped CdS nanoparticles was reduced by ∼10% from room temperature to 50°C, slightly less than the inherent size distribution of the particles at room temperature.

Binding of the DNA to the cationic quantum dots was monitored by luminescence spectroscopy. Figure 1 shows a representative raw data set; the DNA quenches the emission of the nanoparticle solution. While the origin of the quenching is debatable,[9] it is nonetheless a useful empirical signal of adsorption of molecular species to the quantum dot surface. One trivial reason for luminescence quenching is removal of the activating Cd(II) ions by ion-exchange with the adsorbing DNA. However, because the same quenching effects are observed even when the surface is nominally neutral,[2] we favor the hypothesis that luminescence quenching (or enhancement in some cases) is a signal of simple adduct formation between the polymer and the colloid.[10]

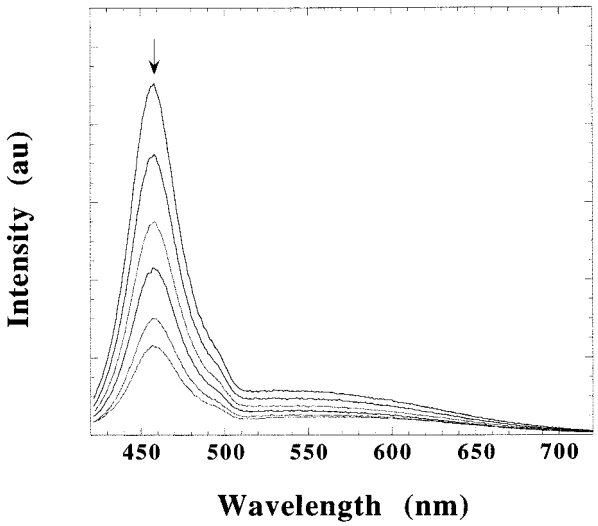

Figure 1. Photoluminescence spectra of 45 Å Cd(II)-rich CdS nanoparticles (2×10^{-4} M based on Cd, ∼1 μM nanoparticles) upon addition of (from the top) 0, 200, 400, 600, 800, and 1000 μM nucleotide calf thymus DNA (5 mM Tris, 5 mM NaCl, pH 7.2) at 30 °C.

Fractional changes in luminescence as a function of DNA concentration were fit to the Frisch−Eirich−Simha adsorption isotherm[11] to extract relative binding constants at each temperature (25−50 °C). Our data were corrected for any changes in the emission spectrum of CdS alone at each temperature with a buffer blank. The correlation coefficient of the adsorption isotherm curve fit to the data points is 0.94 at a minimum, more typically greater than 0.96. Equilibrium constants of $\sim 10^3$ M^{-1} (nucleotide) were obtained, similar to those found for "straight" oligonucleotides for this particle size and surface.[1] Using the relations $\Delta G_{obs} = -RT \ln K_{obs}$ and $\Delta G_{obs} = \Delta H - T\Delta S$ yields the van't Hoff equation

$$\ln K = -(\Delta H/RT) + (\Delta S/R) \quad (2)$$

where ΔH is the standard enthalpy change for the reaction, R is the gas constant, T is temperature, and ΔS is the standard entropy change for the reaction. This equation assumes that enthalpy and entropy themselves are temperature independent over the temperature range examined.

Figure 2 shows the van't Hoff plot. From the best-fit straight line (correlation coefficient 0.992), $\Delta H \sim +30$ kJ mol^{-1} and $\Delta S \sim +150$ J mol^{-1} K^{-1}. Thus, the binding of DNA to the cationic, curved, protein-sized surface costs about two hydrogen bonds worth of enthalpy, and is entropically favorable.

A complicating feature of our system is the presence of the polyphosphate stabilizing agent, necessary to prevent particle growth. For DNA to bind to the nanoparticles, it must out-compete any polyphosphate associated with the nanoparticles. Thus, replacing polyphosphate with DNA is essentially exchanging one set of phosphate−cation interactions for another. This implies that the enthalpic portion of the free energy change upon DNA binding ought to be small and that entropy is favorable for DNA binding.

Circular Dichroism Studies. Release of solvent and counterions are probable mechanisms of increased entropy in our

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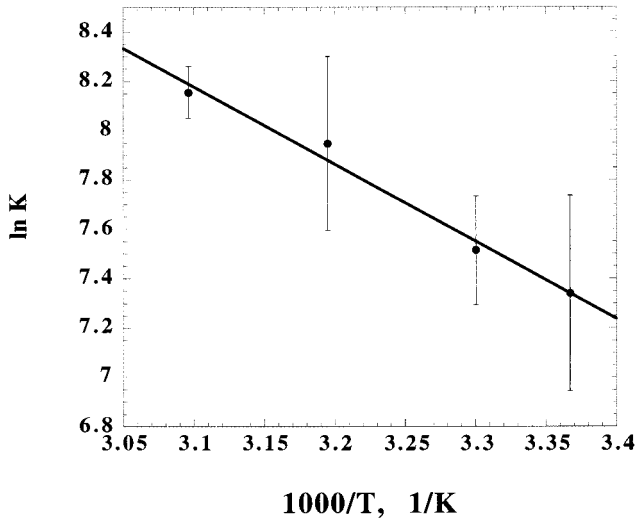

Figure 2. Van't Hoff plot for the adsorption of calf thymus DNA to 45 Å Cd(II)-rich CdS nanoparticles. Inverse temperature is given in inverse Kelvins. The points represent the average equilibrium constants from three separate temperature runs with three fresh batches of nanoparticles. The error bars are ± one standard deviation in equilibrium constant.

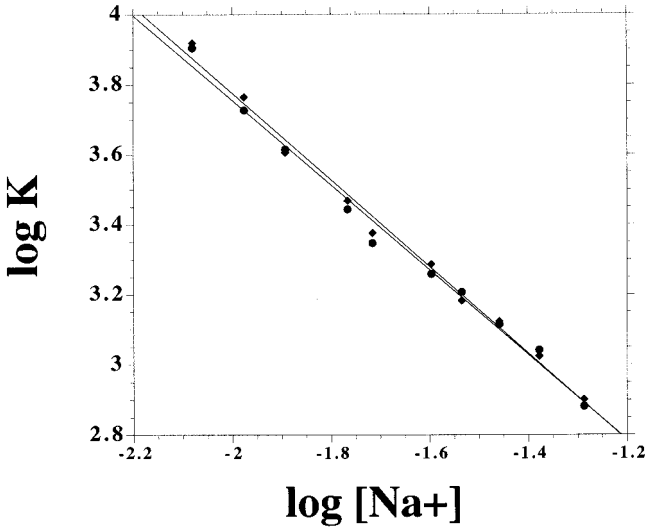

Figure 3. Salt dependence of calf thymus DNA−nanoparticle binding. The circles and diamonds represent the data for two separate reverse-salt titration experiments. The correlation coefficients of the least-squares best-fit lines are 0.99.

system (see below for salt studies). DNA melting is another process that might contribute to a net increase in entropy in our system. The nominal Cd(II) surface presented to the DNA by the nanoparticles is relatively soft, and the softer nitrogens of the bases may be preferred coordination partners compared to the harder phosphate oxygens. To accommodate base binding to the nanoparticle, which is far larger than a molecule, DNA melting may be necessary. To test the hypothesis that the double-helical structure of DNA was perturbed upon adsorption to the nanoparticles, circular dichroism (CD) spectra were acquired at room temperature. At concentrations comparable to the end of a titration experiment (2×10^{-4} M CdS in [Cd], 700 μM nucleotide DNA), the adsorbed DNA CD signal was marginally perturbed in magnitude but not in shape compared to free DNA in solution. Local melting events, however, will be masked in this experiment.

Salt-Dependent Studies. Previously published data from our laboratory[1−3] suggest that electrostatics are important for DNA binding to nanoparticles; for the same size nanoparticle, DNA adsorbs with significantly higher binding constants to cationic nanoparticles compared to nominally neutral nanoparticles.

We have now performed reverse-salt titrations to extract the electrostatic contribution to the free energy change upon binding.[12,13] The data are plotted in Figure 3. From polyelectrolyte theory, the slope of the best-fit line for a plot of log K vs log[Na^+] is related to counterion release:

$$\mathbf{SK} = \delta \log K/\delta \log[Na^+] = -Z\Psi \qquad (3)$$

where **SK** is equivalent to the number of counterions released upon binding of the nanoparticle to the DNA, Z is the charge on the nanoparticle per DNA phosphate binding site, and Ψ is the fraction of counterions associated with each DNA phosphate (= 0.88 for double-stranded B-form DNA).[12,13] The initial [Na^+] level at the beginning of the titration is not zero, since Na^+ is present in the DNA solution, the sodium polyphosphate, and from the NaOH step in the activation of the nanoparticles; we estimate ~6 mM Na^+ for the initial [Na^+]. From the slope of the lines in Figure 3, there are 1.2 counterions released per phosphate upon DNA binding to the Cd(II)-rich CdS nanoparticles; the net charge per site on the nanoparticle is then +1.4. This number is reasonable, given the heterogeneous nature of the surface and the significant covalent character of the Cd−S bond: one textbook estimates that the bonding in II−VI semiconductors is 50−60% covalent.[14] Thus the presumed Cd(II) surface site for phosphate adsorption could readily have an effective charge of +1.4 instead of the nominal +2.

The polyelectrolyte contribution to the overall observed binding ΔG_{obs} can be estimated from

$$\Delta G_{elec} = \mathbf{SK}(\mathrm{RT}) \ln[Na^+] \qquad (4)$$

at a given salt concentration, and the nonelectrostatic contribution can be calculated by difference.[12,13] We find that ~65% of the binding of the DNA to the nanoparticle is due to this polyelectrolyte effect through the range of salt concentrations in Figure 3. The nonelectrostatic portion, still a significant component of the binding, is likely to be a combination of water release and van der Waals interactions, possibly between DNA bases and the nanoparticle surface.

Comparison to Nonspecific Protein−DNA Interactions. There is ample precedent in the literature for entropy driving nonspecific protein−DNA interactions via release of solvent and counterions from the interface.[12,15,16] The numerical results for enthalpy and entropy from such studies are the same order of magnitude that we obtain from our data. A specific example is given by the Cro protein−DNA interaction; Cro can bind DNA specifically, but pulsed-flow microcalorimetry experiments under nonspecific binding conditions find $\Delta H \sim +20$ kJ mol^{-1} and $\Delta S \sim +200$ J mol^{-1} K^{-1},[17] very similar to what we observe here.

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Another example is the nonspecific binding of the Sac7d protein to double-stranded DNAs.[18] The salt dependence of the Sac7d-DNA binding constants is consistent with the polyelectrolyte effect as a primary component (70%) of the overall driving force; the remaining 30% of the driving force was considered to be due to favorable interactions between DNA bases and the protein's tryptophan 24.[18] The 70% figure is very similar to the one we calculate for our inorganic nanoparticles.

Oligonucleotides Compared to Long CT DNA. Previously, we have observed shape-selective binding of oligonucleotides to neutral and cationic nanoparticles, in which intrinsically curved DNAs adsorbed more strongly to the curved nanoparticle surface.[1,2] In comparing 40 Å nanoparticles with a $-SCH_2CH_2OH$ surface to those with Cd(II) on the surface, we noted that a "kinked" oligonucleotide had a 4-fold decrease in affinity for the alcoholic surface, compared to the cationic surface.[1,2] Crudely, then, we could estimate that the binding of the oligonucleotide to the curved inorganic surface was ∼75% electrostatic and 25% "other". This estimate agrees surprisingly well with the salt-dependent data we present here for long DNA.

The degree to which the nanoparticle surface area is contacted by the DNA surely influences the number of solvent molecules and counterions that may be expelled at the interface, which is a probable function of the static shape and/or flexibility of the DNA.[19] Preliminary temperature studies in our laboratory, albeit over a much narrower range, support the notion that binding of oligonucleotides to cationic nanoparticles is also entropically favorable. However, there are additional subtle effects such as ion clustering within sequence-directed structures of oligonucleotide DNA[20] and cation-induced bending of DNA[21] that make a "shape alone" comparison more ambiguous. Other workers have noted the base composition dependence of DNA−nanoparticle interactions.[22] Another complicating feature of oligonucleotide−nanoparticle binding is that the polyelectrolyte effect may not be equivalent for oligoelectrolytes; for example, an oligolysine oligocation (+8 charge) binds to single-stranded poly(dT) with higher affinity per site and has a more pronounced salt dependence than its analogous binding to the decaanion $dT(pdT)_{10}$.[23] Detailed salt and hydration studies should be conducted with oligonucleotide−nanoparticle systems to sort out the details.

Conclusions

In summary, the adsorption of long calf thymus DNA to nominally cationic CdS nanoparticles appears to be entropically favorable, not net enthalpically favorable due to electrostatic interactions, based on the temperature dependence of the binding constants. Reverse-salt titrations suggest that the polyelectrolyte effect is largely responsible for this observation. Entropically driven nonspecific binding is frequently observed for protein−DNA interactions, and thus the inorganic nanoparticles in this sense are functioning as "inorganic proteins".

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