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The binding of phosphorothioate oligonucleotides to CdS nanoparticles

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Abstract

Protein-sized surface Cd^{2+}-rich, S^{2-}-rich and neutral CdS nanoparticles were used to imitate the binding of phosphorothioate oligonucleotides (PT-oligoG_{10}) to proteins. It is found that the binding of PT-oligoG_{10} to the surface neutral CdS nanoparticle shows positive cooperativity and the binding of PT-oligoG_{10} to surface S^{2-}-rich CdS nanoparticle shows negative cooperativity. In case of the surface Cd^{2+}-rich CdS nanoparticle, no cooperativity can be identified for the binding of PT-oligoG_{10}.

1. Introduction

In recent years, antisense oligonucleotides have been extensively studied since they can specifically down-regulate gene expression, and a number of first-generation antisense compounds have entered human clinical trials [1–3]. Phosphorothioate oligonucleotides (PT-DNA) are the most common modified version of antisense oligonucleotides, in which one of the nonbridging oxygen atoms of the phosphate backbone is substituted by a sulfur, which are better protected against cleavage by both exonucleases and endonucleases than other analogs [4,5]. There have been many reports in which PT-DNA were used to inhibit over-expression of cellular gene product implicated in cancer and inflammation, HIV-1 replication in HIV-1 infected cells and viral replication [6–8]. These inhibitions are primarily attributed to their sequence-specific activities by binding to the mRNA or nonsequence-specific activities by binding to proteins such as growth factors, serum protein, enzymes, etc. [9–11]. Although it has been known that their nonspecifically binding to proteins is

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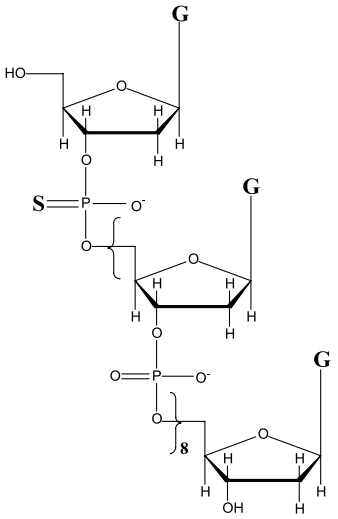
associated with the polyanionic characteristics of PT-DNA [12,13]. However, sometimes it is difficult to understand the effect of surface properties of proteins on the binding of PT-DNA since surface modifications on proteins usually will induce alteration of their folding pattern and introduce complications into the analysis of the binding process.

As a kind of inorganic nanosized species, CdS nanoparticle has been widely employed as fluorescent labels for biological targets [14–16]. In principle, CdS nanoparticles can also be used to analog of proteins for DNA-binding since not only it has the same size as natural protein, but also its surface can be readily modified as hydrophilic, hydrophobic, cationic, anionic or neutral, and in addition, its luminescence can be used as the signal of binding [17]. Murphy and coworkers have used CdS nanoparticles as luminescent probes to study the intrinsic structure and/or flexibility of double-stranded DNA and developed a minimalist model for nonspecific protein–DNA interactions [18,19]. In this work, we use three kinds of protein-sized surface Cd^{2+}-rich, S^{2-}-rich and neutral CdS nanoparticles to imitate the binding of PT-DNA (PT-oligoG_{10}, see Scheme 1) to proteins. It is found that cooperativities of the binding of PT-oligoG_{10} are greatly affected by the surface properties of the CdS nanoparticles.



Scheme 1. The molecular structure of PT-oligoG_{10}.

2. Experimental

2.1. Materials

PT-oligoG_{10} and oligoG_{10} were synthesized by Genemed Synthesis, Inc (USA). Cadmium chloride and sodium sulfide were analytical grade and recrystallized before use. Water with a conductivity of 18 MΩ cm was used in all the experiments.

2.2. Instrumentation

UV–visible spectra were recorded on a Shimadzu UV-1602 spectrophotometer. Photoluminescence spectra were acquired on a Shimadzu RF-5301 spectrofluorometer.

2.3. Synthesis of CdS nanoparticles

The CdS nanoparticles were synthesized according to the literature [20], with reagent weights based on a final concentration of 2×10^{-4} M. First, cadmium chloride and 2-mercaptoethanol were added to 100 ml of H_2O degassed with N_2, and the pH was adjusted to 10.5 with 0.5 M NaOH. Sodium sulfide was then added dropwise under vigorous stirring. When the molar ratio of Cd and S ions was 1:1, the surface of the CdS nanoparticles was neutral. The surface-rich Cd^{2+} or S^{2-} CdS nanoparticles were obtained by adding excessive Cd (Cd:S = 2:1) or S (Cd:S = 1:2) ions. The CdS nanoparticles were precipitated by ethanol, and then centrifuged and washed with ethanol. The precipitates can be re-suspended in water with good stability.

2.4. DNA titration

In a typical titration experiment, 200 μl of 2×10^{-4} M CdS solution was diluted into 3 ml by water. One micron aliquots of 10^{-3} M PT-oligoG_{10} and oligoG_{10} solution were added to dispersions of the nanoparticles every 30 min. The photoluminescence of the CdS nanoparticles alone was not

found to change significantly over the time course of this experiment.

3. Results and discussion

Fig. 1 show the UV–visible absorption and luminescence spectra of the surface neutral, surface Cd^{2+}-rich and surface S^{2-}-rich CdS nanoparticles. The diameter of nanoparticles can be calculated from the band gap (E_g) obtained from the absorption spectra by the effective mass approximation (Fig. 1A) [21]. The CdS nanoparticles with different surfaces have almost the same band gaps and all of their diameters are determined to be about 4 nm, which are also in consistent with the transmission electron microscopic observations. All the CdS nanoparticles show emission maximum around 525 nm with approximate emission intensity (Fig. 1B). The emission of these protein-sized CdS nanoparticles is very sensitive to the binding of DNA and luminescence quenching of the CdS nanoparticles can be used as the signal of binding [22]. The fractional change in luminescence is proportional to the fraction of DNA (Y) bound to the CdS nanoparticles [17]. The variation of the fraction of DNA (Y) bound to the CdS nanoparticles versus the concentration of DNA added (C) is defined as binding curve of DNA to CdS nanoparticle.

Fig. 2 showed the binding curves of PT-oligoG_{10} to the surface neutral, surface Cd^{2+}-rich, and surface S^{2-}-rich CdS nanoparticles. It can be seen that the binding curve of PT-oligoG_{10} to the surface neutral CdS nanoparticle is sigmoidal-like, indicating the binding of PT-oligoG_{10} to the surface neutral CdS nanoparticle is a cooperative process [23]. The fraction of PT-oligoG_{10} bound to the surface S^{2-}-rich nanoparticle increases rapidly in the range of low concentration of PT-oligo$G_{10}$and slowly in the range of high concentration of PT-oligoG_{10}. The curve looks like hyperbolic, but is not a hyperbola in fact. The binding curve of PT-oligoG_{10} to the surface Cd^{2+}-rich CdS nanoparticle is more approached to a hyperbola than to the sigmoid. The bound fraction

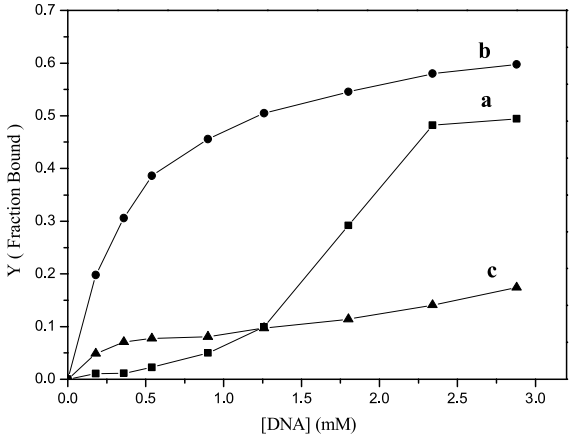


Fig. 2. The binding curves of the PT-oligoG_{10} to (a) surface neutral, (b) surface Cd^{2+}-rich and (c) surface S^{2-}-rich CdS nanoparticles.

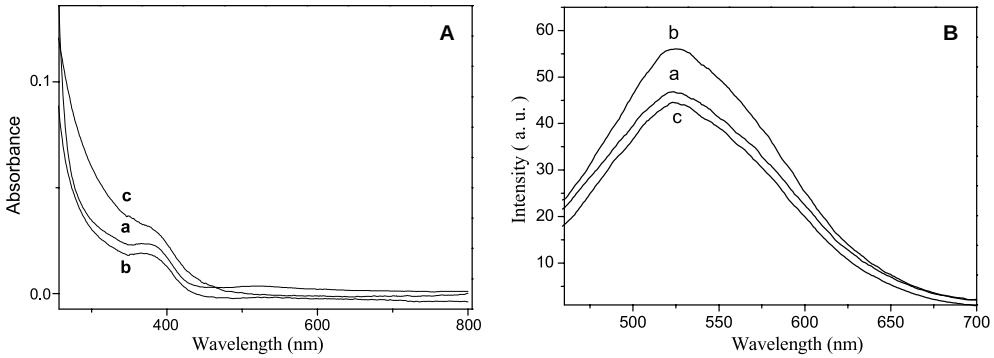


Fig. 1. UV–visible spectra (A) and fluorescence spectra (B) of the three kinds of CdS nanoparticles (a) surface neutral, (b) surface Cd^{2+}-rich and (c) surface S^{2-}-rich. (Excitation wavelength is at 370 nm).

is higher for the surface Cd^{2+}-rich CdS nanoparticle than for the surface neutral CdS nanoparticle. This means that the surface Cd^{2+}-rich CdS nanoparticle has a higher affinity for PT-oligoG_{10} than the surface neutral CdS nanoparticle [23]. The cooperativities of the binding processes are further diagnosed by the slope n of the Hill plot of $\log(Y/(1-Y))$ Vs $\log C$ (see Fig. 3) [23]. The value of n increases with the degree of cooperativity and the possible maximum value of n is equal to the number of possible binding sites. In the Hill plot, the slope for the binding of PT-oligoG_{10} to the surface neutral CdS nanoparticle is determined to be 3.62, indicating the binding of PT-oligoG_{10} to the surface neutral CdS nanoparticle shows positive cooperativity. The binding of PT-oligoG_{10} to one possible site of the surface neutral CdS nanoparticle facilitates its binding to other possible sites [24]. The binding of PT-oligoG_{10} to the surface S^{2-}-rich CdS nanoparticle shows a slope of 0.40, indicating negative cooperativity of the binding process [25]. For the surface Cd^{2+}-rich CdS nanoparticle, the slope of the plot is calculated to be 1, indicating no cooperativity be identified for the binding process. This means that the possible sites on the surface of the nanoparticle for PT-oligoG_{10} binding are independent of each other [24]. It is expected that the dominant interactions between the DNA and the nanoparticles are electrostatic forces between negatively charged phosphate backbone of the DNA and surface of the nanoparticles [22]. The positive charged surface of Cd^{2+}-rich CdS nanoparticle will interact favorably with hydrophilic groups such as OH, P=O and P=S of PT-oligoG_{10}, and thus the surface Cd^{2+}-rich nanoparticle show higher affinity for PT-oligoG_{10} than the surface S^{2-}-rich or neutral nanoparticle and the slope of the Hill plot is approached to 1 and no cooperativity can be identified for the binding of PT-oligoG_{10} to the surface positively charged nanoparticle. The electrostatic repulsion between the surface extra S^{2-} and the phosphate groups of PT-oligoG_{10} resists the binding of those hydrophilic groups of DNA to the negatively charged surface of the CdS nanoparticle, thus the Hill coefficient is lower than 1 and the binding of PT-oligoG_{10} to the negatively charged surface S^{2-}-rich nanoparticle shows negative cooperativity.

The binding of normal unmodified DNA (oligoG_{10}) to CdS nanoparticles is further investigated for comparison. Fig. 4 shows the binding curves of the PT-oligoG_{10} and oligoG_{10} to the surface

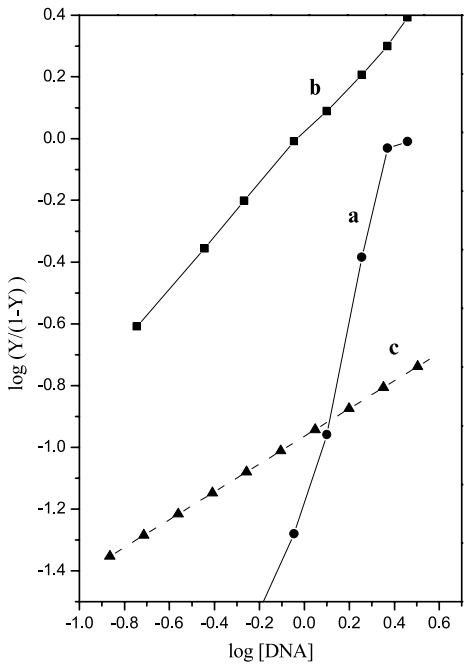


Fig. 3. The transformation of the binding curves by the Hill equation (a) surface neutral, (b) surface Cd^{2+}-rich and (c) surface S^{2-}-rich CdS nanoparticles.

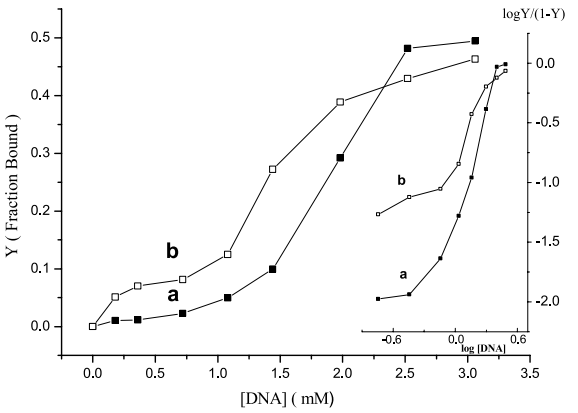


Fig. 4. The binding of the (a) PT-oligoG_{10} and (b) oligoG_{10} to the surface neutral CdS nanoparticles. The inset represents the respective Hill plots.

neutral CdS nanoparticles and the insert represents the respective Hill plots. It is found that the binding of oligoG_{10} to the CdS nanoparticles shows a Hill coefficient of 2.86, lower than that (3.62) for the binding of PT-oligoG_{10} to the surface neutral CdS nanoparticles, indicating the binding of PT-oligoG_{10} to the neutral nanoparticle shows higher cooperativity than oligoG_{10} [21]. The same as the PT-oligoG_{10}, no cooperativity can be identified for the binding of oligoG_{10} to the surface Cd^{2+}-rich CdS nanoparticles, too. For the binding of oligoG_{10} to the surface S^{2-}-rich CdS nanoparticles, the Hill coefficient is determined to be 0.70, indicating the binding process also shows negative cooperativity.

4. Conclusions

In summary, three kinds of protein-sized CdS nanoparticles with different surface properties were employed to imitate the binding of PT-oligoG_{10} to proteins. It is found that the cooperativities of the binding of PT-oligoG_{10} are greatly affected by the surface properties of the CdS nanoparticles. The binding of PT-oligoG_{10} to the surface neutral CdS nanoparticle shows positive cooperativity and the binding to surface S^{2-}-rich CdS nanoparticle shows negative cooperativity. In case of the Cd^{2+}-rich surface CdS nanoparticle, no cooperativity can be identified for the binding of PT-oligoG_{10}. These results may provide useful information to understand the clinical effects of PT-DNA.

Acknowledgements

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