Interaction of Indium(III) with DNA: Prelude to Fabrication of Low Melting Point Conductive Nanowires

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ABSTRACT

The interaction of indium(III) ions with double-stranded DNA in aqueous solution has been studied by several methods. UV-vis spectroscopy (UVS), circular dichroism spectroscopy (CD), extrinsic fluorescence emission and gel electrophoresis were used to characterize the binding of native calf thymus DNA (CT DNA) and of bacteriophage lambda phage DNA (λ DNA) to indium(III) chloride. The two DNA species were found to interact similarly with indium(III) ions. Spectroscopic titration showed an initial increase in absorbance at 260 nm followed by a sharp decrease in absorbance on increase of indium(III) concentration. Indium(III) also induced significant quenching of the fluorescence of DNA–ethidium bromide (EtBr) complexes, suggesting competitive binding. Consistent with this interpretation, addition of ethylenediaminetetraacetic (EDTA), a compound known to chelate indium(III), increased the absorbance at 260 nm and the EtBr fluorescence for a given indium(III) concentration. This study provides a foundation for a DNA template-based approach to the fabrication of conductive nanowires made of indium or an alloy thereof. The relatively low melting point of indium is advantageous for this purpose, as it should permit annealing and reduce grain boundaries under conditions that will not destroy the template for nanowire fabrication.

1. INTRODUCTION

DNA is widely regarded as a promising molecule for nanotechnology development, and more specifically for nanoelectronics. Given their high aspect ratio and self-assembly properties, DNA molecules are attractive building blocks for "bottom-up" manufacturing [1]. Electrical interconnects are a major requirement of the electronics industry. A reliable means of miniaturizing both electrical devices and interconnection is sought.

The possibility of using DNA molecules in semiconductor electronics has been explored for quite some time [2,3]. Investigation of electrical properties of DNA molecules began shortly after Watson and Crick elucidated the double-helical structure of DNA in 1953. The intrinsic conductivity of double-stranded DNA was a matter of controversy until relatively recently. It is now generally accepted that DNA is more an insulator than a conductor [4].

Efforts are underway to make use of extraordinary molecular recognition properties of DNA and to modify the molecule so as to increase its conductivity. The binding of metal cations to the molecule can play an important role in determining its conductivity. A type of DNA-metal ion complex known as M-DNA, with divalent metal ions (Zn^{2+}, Ni^{2+} or Co^{2+}) bound, has been described [5]. M-DNA consists of GC and AT base pairs in which the imino proton of guanine and thymine have been replaced by a Zn^{2+} ion, forming a wire of zinc ions sheathed

by DNA helix [6]. Various methods and steps involved in fabricating DNA nanowires have been studied extensively [7].

DNA-metal ion interaction studies have so far been mainly conducted for determining biological properties such as toxicity [8], carcinogenicity [9] and antitumour activity [10]. This work describes how certain well-established techniques of such studies have been adapted for exploration of the development of low melting point conductive DNA nanowires.

2. MATERIALS AND METHODS

2.1 Materials

λ DNA (10 mM Tris, 1 mM EDTA stock solution, 45% G-C, *E. coli* host strain W3110) and sodium salt of CT DNA (highly polymerized, type I, 42 % G-C) were from Sigma-Aldrich (USA) and used without further purification. The ratio of absorbance at 260 nm and at 280 nm (A_{260}/A_{280}) for λ DNA and CT DNA was 1.77 and 1.88, respectively, indicating that the preparations were mostly free of protein [11]. The DNA concentration per nucleotide (or phosphate) was determined by absorption (ϵ_{258} = 6600 M^{-1} cm^{-1}) [11]. Crystalline indium(III) chloride (98% pure), EDTA (anhydrous, 99%) and EtBr were also from Sigma-Aldrich and used as received. All solutions were prepared with 18 MΩ-cm water (Milli-Q, Millipore, USA). CT DNA stock solution was prepared by dissolving the

DNA in 10 mM Tris pH 7.5 to a final concentration of 1 mg/mL and gently mixing overnight. DNA samples were stored at 4 °C for not more than a week for use in experiments. $InCl_3$ solutions were prepared fresh and used within 4 h. 25 mM EDTA solution was in water. All experiments besides thermal denaturation of DNA were carried out at 22-24 °C.

2.2 Methods

2.2.1 UV spectroscopy

All UVS spectra were obtained with a Shimadzu 1650PC UV-vis spectrophotometer (Japan). Samples were analyzed in a quartz cuvette with a 1 cm path length. An attached Fisher Scientific Isotemp 3006 water bath with a circulator pump (USA) was used for spectral analysis of DNA melting in aqueous solution. Stock solutions of CT DNA and λ DNA were diluted in 5 mM Tris, pH 7.5 to a final DNA concentration of 50 μg/mL. 10 μL aliquots each of 5 mM or 10 mM $InCl_3$ were titrated against 2 mL DNA solution in the cuvette. The solution was stirred by repeated gentle pipetting with a 1000 μL micropipette for 30 s and then allowed to sit for 1 min before recording the spectrum for each indium concentration. The micropipette tips were unusually large to minimize shearing of DNA molecules. Incubation times of 1 min, 10 min, and 1 h were tested. The variation in spectra after 1 min was negligible, suggesting that equilibrium was reached on this time scale. Similarly, kinetic spectroscopic studies at 260 nm

revealed that spectral changes were insignificant after 30 sec-1 min of mixing $InCl_3$ solution with DNA solution. 25 mM EDTA was titrated against DNA solution to test the chelating effect of EDTA on indium ions. Absorbance readings were adjusted to account for the dilution of DNA by the titrant.

2.3.2 CD Spectroscopy

CD spectra of CT DNA solution in the presence and absence of $InCl_3$ were taken with a Jasco J-810 circular dichroism spectropolarimeter (Japan) in a 1 mm-path length quartz cuvette. The wavelength range was 200 nm-300 nm; step size, 0.1 nm; scanning speed, 0.3 nm s^{-1}; response time, 0.3 s; number of scans, 50. The results are presented below as differential absorption in milliabsorbance units.

2.2.3 Extrinsic Fluorescence Emission

Fluorescence quenching and recovery experiments were done with a TECAN GENois microplate reader (USA). The concentrations of $InCl_3$ and CT DNA were as in the titration experiments, and the concentration of EtBr was 0.1 μg/mL (ϵ_{478} = 5680 M^{-1} cm^{-1} [12]). A 96-well microplate was used for the experiment. 12 samples were prepared by varying the concentration of $InCl_3$ in DNA solution such that the molar concentration ratio, $[In^{3+}]$/[DNA base pairs] (**R**), varied from 0 to 3.5. The molar concentration of DNA base pairs was calculated by taking 660 Da as the average base pair molecular weight. 100 μL of each sample was

added to separate wells in the microplate. A 12-well gradient of EDTA was obtained at **R** = 3.5 by varying the concentration of EDTA from 0 to 2.5 mM. Three samples were prepared independently for each $InCl_3$ concentration. Excitation was at 360 nm, emission was measured at 610 nm. Fluorescence emission $\mathbf{F_i}$ for each well was calculated as the average of the three samples. The emission data are presented as $\mathbf{F_i/F_0}$, $\mathbf{F_0}$ being the $\mathbf{F_i}$ value for DNA-EtBr complex in the absence of indium(III).

2.2.4 Gel Electrophoresis

20 μL aliquots of 50 μg/mL CT DNA, 0.1 μg/mL EtBr with **R** = 0-2 were introduced into separate wells in a 0.75 % (w/v) agarose gel (40 mM Tris, 50 mM boric acid, 1 mM EDTA, pH 8.0) and incubated for 1 h at 25 °C. The DNA samples migrated in the gel on application of an electric field of 5 V/cm for 4 h. DNA mobility was assessed qualitatively by visualization with a Spectroline UV transilluminator (USA) equipped with a light shroud and an Olympus digital camera (Japan).

3. RESULTS

3.1 Spectrophotometric Titration Experiments

Both λ DNA and CT DNA showed moderate hyperchromicity followed by significant hypochromicity on increasing the concentration of $InCl_3$. These changes were accompanied by a bathochromic shift of ~4 nm near 260 nm. Absorbance and the corresponding peak shift for λ DNA and CT DNA are plotted against **R** in Figure 1. **R** ranged from 0-2 (CT DNA experiments, 0-0.26 mM $InCl_3$) or 0-2.5 (λ DNA, 0-0.37 mM $InCl_3$). Absorbance near 260 nm increased steadily with **R** (up to 30 % for CT DNA and 20 % for λ DNA) before dropping (up to 20 % CT DNA and 30 % for λ DNA) and remaining steady thereafter (Figure 1, panels a and b).

CT DNA was also titrated against NaCl in the range 0-5 mM as a control for the possibility that Cl^- ions influence absorbance (data not shown). The concentration of NaCl in DNA samples was chosen to mirror the $InCl_3$ experiments (ionic strength of $InCl_3$ is 6x that of NaCl in dilute solution). NaCl did not influence absorbance at any concentration in the indicated range. Control experiments were also done at a lower DNA concentration (0.25 μg/mL), maintaining **R** and other conditions. The behavior of DNA was similar under both conditions.

R', defined as the **R** value at the mid-point of the hypochromic slope, represents the mid-point of the observed structural transition in DNA. It can be seen in Figure 1 that both λ DNA and CT DNA behave in a similar manner. The slight difference in **R'** values suggests that EDTA in the stock solution of λ DNA might chelate indium ions and therefore yield a higher apparent value of **R'** for λ DNA. The difference in hyperchromic and hypochromic effects between CT DNA and λ DNA can also be ascribed to the difference in counterions and buffer concentration in the respective stock solutions, as well as the difference in G-C content (42 % for CT DNA [13] and 45 % for λ DNA [14]).

The hypochromic effect due to interaction of DNA with indium ions was found to be largely reversible by addition of the metal ion chelator, EDTA (up to 80 % of the initial value for CT DNA and up to 60 % of the initial value for λ DNA). Panels c and d of Figure 1 show the effect of increasing EDTA concentration on absorbance of the CT DNA-In^{3+} solution (**R** = 2) and the λ DNA- In^{3+} solution (**R** = 2.5), respectively. These experiments suggest a 15-20 % recovery of absorbance for both species of DNA in the presence of EDTA. A hypsochromic shift of ~4 nm also occurred on addition of EDTA.

Figure 2 presents a family of UV spectra for λ DNA at different concentrations of $InCl_3$ and EDTA. The inset shows data in the 240-280 nm range. Absorbance changes in the far UV region, though significant, were not analyzed more thoroughly because many other chemical species, e.g., Tris, EDTA, and Cl^-,

absorb in this region, complicating interpretation. The spectral properties of CT DNA under the same conditions were found to be similar to those of λ DNA (data not shown).

The effect of counterions on In^{3+}-DNA binding was tested by varying the NaCl in the range 0-40 mM but keeping all other conditions same. Na^+ ions were found to affect the value of **R'** and the maximum hyperchromism and maximum hypochromism (Figure 3). The data suggest that the effect of counterions is particularly significant in the 0-10 mM range.

3.2 Melting Experiments

Thermal denaturation curves were obtained for CT DNA with **R** = 0, 0.8 and 2. As evident in Figure 4, T_m increased with increased in **R**, from 45 °C for **R** = 0 to 57 °C for **R** = 4. The increase in T_m is accompanied by a broadening of the transition and a reduction in final hyperchromicity (20 % for **R** = 0, 15 % for **R** = 1.5, and 5 % for **R** = 4). The results indicate that the interaction of CT DNA with In^{3+} ions stabilized the DNA double helix. That is, In^{3+} binds with greater affinity to the double-stranded molecule than to single strands. The spectra data would also suggest, however, that the binding process distorts the conformation of DNA.

3.3 CD Experiments

Structural changes in DNA on addition of $InCl_3$ were studied by CD at different values of **R** (0, 0.15, 0.3, 0.5, 1, 1.5) (Figure 5). Figure 5 shows the differential absorbance for characteristic Cotton effects at 217, 245 and 275 nm as a function of **R**. In all cases, peak intensity decreased as **R** increased from 0 to 1.5. Moreover, changes in the peaks decreased in magnitude as **R** increased from 0 to 1; there was negligible change above **R** = 1, suggesting saturation. Below 210 nm, the differential absorbance increased considerably at high values of **R**.

3.4 Fluorescence Experiments

Relative fluorescence ($\mathbf{F/F_0}$) of 50 μg/mL CT DNA and 0.1 μg/mL EtBr was measured as a function of **R**. Fluorescence emission of EtBr-DNA was quenched by In^{3+} ions, reducing the fluorescence intensity by around 25 % at **R** = 3.5 (Figure 6). The quenching profile is closely related to the hypochromicity profile (Figure 1); the transition in fluorescence intensity, however, occured at a lower **R** value (**R'** = 0.25). The fluorescence recovery profile was obtained by adding EDTA to DNA-EtBr solution at **R** = 3.5, keeping all other conditions constant. The data show that EDTA was able recover the fluorescence to about 60 % of its initial value. A control experiment with NaCl (analogous to the Na^+ control in UVS

experiments) showed that increases in Na^+ ion concentration did not quench the fluorescence emission (data not shown).

3.5 Gel Electrophoresis Experiments

Lanes 1-7 in Figure 7 represent CT DNA solution with **R** = 0, 0.2, 0.4, 0.8, 1.5, 2.0, and 0, respectively. The concentrations of the various chemical species were same as in the fluorescence studies. The fluorescence of EtBr-stained DNA at **R** = 0 steadily decreased as **R** increased to 2, where the fluorescence intensity was low. This decrease in band brightness is a result of fluorescence quenching due to In^{3+}-DNA interaction. It is evident from the figure that there is no significant difference in electrophoretic mobility between the samples, despite the differences in $InCl_3$ concentration and fluorescence, and no apparent shearing of the DNA.

4. DISCUSION

A molecular model of 20 base-pair of mixed-sequence, right-handed B-DNA surrounded by 20 In^{3+} ions (**R** = 1) is shown in Figure 8. The van der Waals radius of In^{3+} is 0.93 Å [15]. The model gives an idea of the relative size of the interacting species.

The UVS titration results presented here suggest that the DNA-indium interaction occurs in at least in two stages: low metal ion concentration (**R** < 0.5), where absorption increases, and high metal ion concentration (**R** > 0.5), where absorption decreases. There was no evidence of DNA aggregation up to **R** = 20. The initial hyperchromicity can be attributed to the electrostatic interaction between In^{3+} and the phosphate groups in DNA backbone [16]. A parallel analysis of UVS data and CD data indicates that DNA undergoes a structural transition on interacting with In^{3+} [17, 18]. The CD spectra for **R** = 1 and **R** = 1.6 closely resemble that of left handed Z-DNA [19]. This suggests the possibility that the usual right-handed B-form shifts to the left-handed Z-form as the In^{3+} concentration increases. The decrease in the absorbance peak at 275 nm and 245 nm, together with the hypsochromic shift of the peak at 275 nm and the bathochromic shift of the 245 nm peak, is consistent with a conformation change from the B-form to Z-form [20, 21].

The DNA-indium absorption titration experiment revealed a hypochromic shift (20 %) and bathochromic shift (4 nm) of the absorbance peak at 260 nm upon saturation of indium binding sites. These data suggest that the binding of indium ions to DNA is more likely to induce some intercalation between bases [22] or other disruption of regular structure than simple non-specific electrostatic binding to phosphate [23]. This interpretation is supported by the fluorescence quenching profile, where the binding of indium ions inhibits the intercalaction of EtBr molecules and leads to increased quenching with increases in **R** [5, 6]. There are

obvious changes in 190 nm–210 nm range of the absorption spectrum (Figure 2) with changes in $InCl_3$ concentration.

EDTA is widely known to chelate most divalent and some trivalent metal ions by forming coordination compounds. A proposed structure of the indium-EDTA complex is shown in Figure 9 [24]. The observed recovery in absorbance and fluorescence on addition of EDTA might therefore be attributable to the formation of EDTA-In complexes, reducing the concentration of free In^{3+} ions and allowing the DNA molecules to return to their original confirmation in the absence of In^{3+}.

The two common sources of high and non-linear resistance of DNA-metallized nanowires are non-ideality of nanowire shape [25] and inter-grain boundaries [25, 26]. It has been observed that annealing metallized nanowires reduces electrical resistance by up to a factor of four [27]. Indium, having a relatively low melting point (156 °C), will enable low temperature annealing of indium nanoparticles. We have therefore adopted this metal for study of means to simplify the nanowire fabrication process and avoid damage to the DNA template and other components. Investigation of the interaction of DNA and indium thus opens new opportunities for technology development in the realm of DNA-nanowire fabrication.

5. CONCLUSION

We have shown that indium(III) ions bind to DNA molecules in aqueous solution, leading to a significant change in spectral and extrinsic fluorescence properties of DNA. Indium-bound DNA has a higher melting point than free DNA. In 5 mM Tris, pH 7.5, indium(III) ions saturate binding sites on DNA at a molar concentration ratio of around 1.25 for CT DNA and around 1.75 for λ DNA. EDTA chelates the indium(III) ions bound to the DNA molecules in aqueous solution, reversing the conformational changes that occur on increasing the concentration of In^{3+}. As indium(III) ions have a confirmed affinity to DNA molecules, it will be possible to fabricate DNA-templated indium nanowires by established metallization techniques [25,27,28] or by novel methods.

5. ACKNOWLEDGMENT

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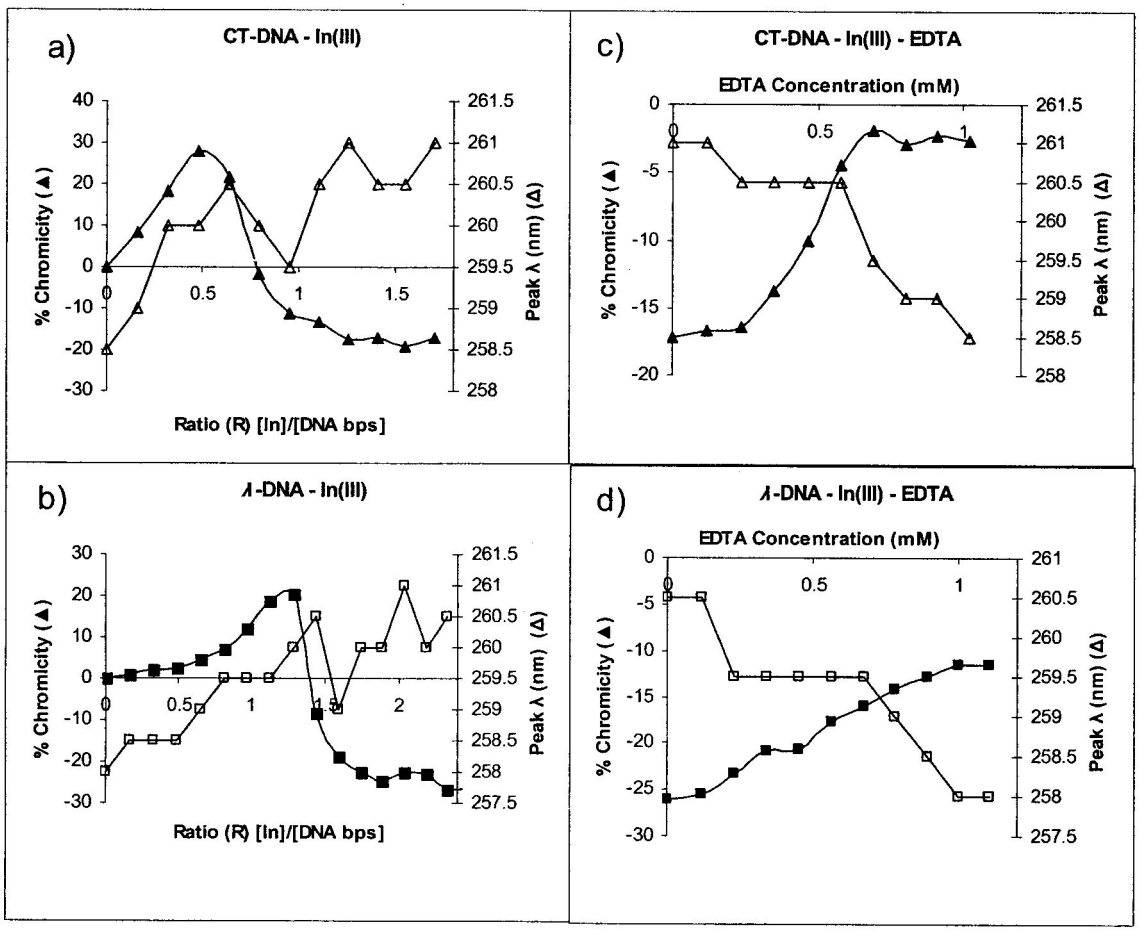

Figure 1. Indium(III) binding to DNA. a) CT DNA. b) λ DNA. c) **R** = 2, [CT DNA] = 50 μg/ml. d) **R** = 2.5, [λ DNA] = 50 μg/ml. Filled symbols, % chromicity. Open symbols, λ_{max}.

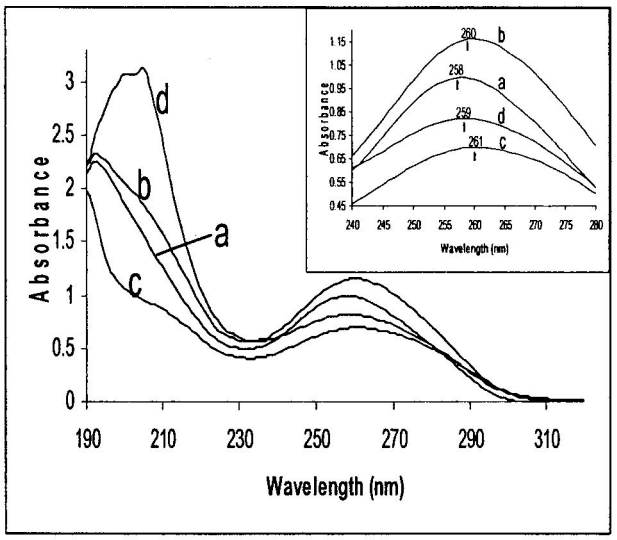

Figure 2. Ultraviolet spectrum for λ DNA. a) **R**=0. b) **R** = 1.2. c) **R** = 2.5. d) **R** = 2.5 and [EDTA] = 0-1.5 mM. Inset: Expanded view of the spectra in the 240 – 280 nm range.

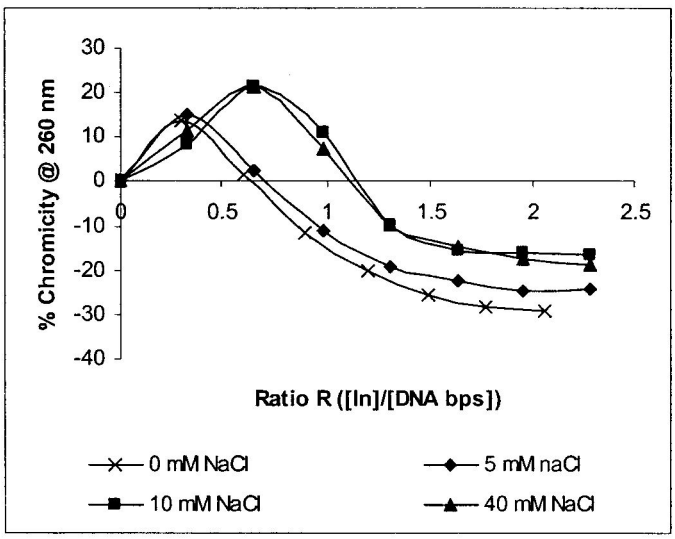

Figure 3. Change in absorbance for CT DNA at 260 nm in terms of % chromicity versus **R** in presence of NaCl.

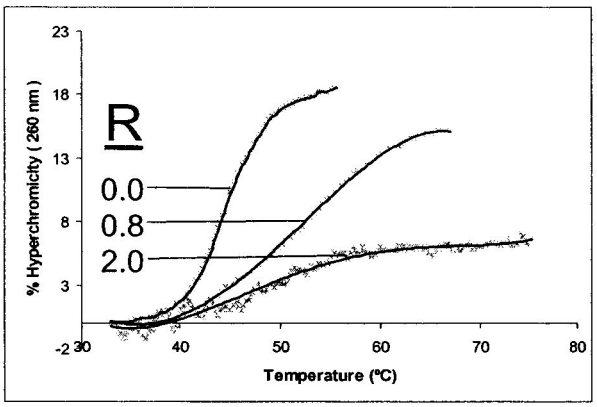

Figure 4. Thermal denaturation of CT DNA at **R** = 0, 0.8 and 2. Change in absorbance at 260 nm is shown as %hyperchromicity.

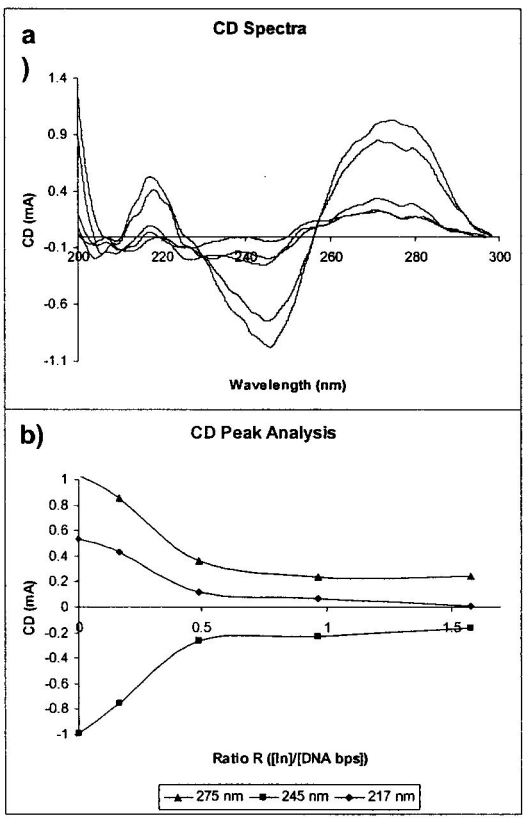

Figure 5. CD analysis. a) Spectra of CT DNA at various concentrations of $InCl_3$ (R = 0 – 1.6) plotted as milliabsorption units versus wavelength. Each spectrum is the average of 50 scans. B) $\Delta\varepsilon$ values for peaks (275 nm, 245 nm, 217 nm) for various concentrations of $InCl_3$ (R = 0 – 1.6).

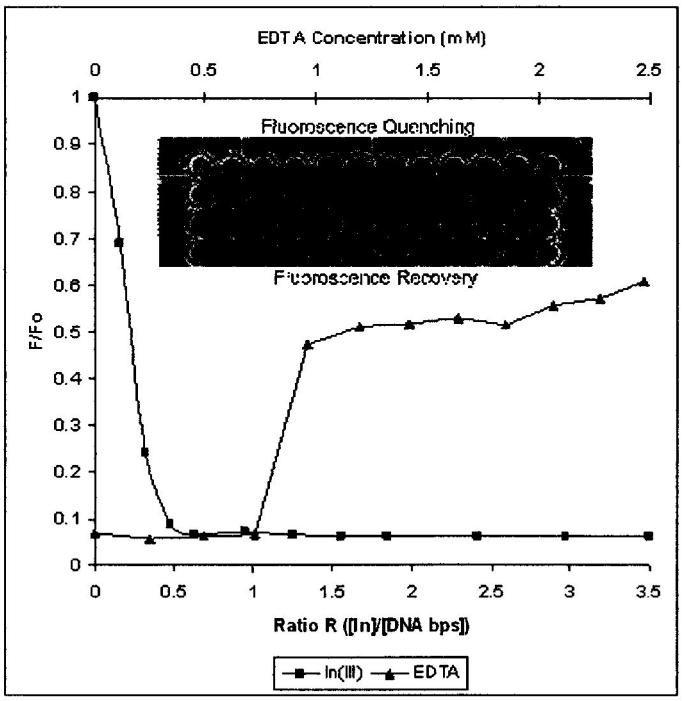

Figure 6. Ethidium bromide fluorescence quenching and recovery profiles. F/F_0 is shown as as a function of **R** (square) and as a function of EDTA concentration (mM) with **R** = 3.5 (triangle). Squares, quenching profile, [DNA] = 50 μg/mL, [EtBr] = 0.1 μg/mL, **R** = 0 – 3.5. Triangles, recovery profile, [DNA] = 50 μg/mL, [EtBr] = 0.1 μg/mL, **R** = 3.5, [EDTA] = 0 - 2.5 mM. Inset: Image of 96 well microplate, rows A and B show quenching, rows C and D; show recovery.

Figure 7. Gel electrophoresis. Lanes 1 – 7, **R** = 0, 0.2, 0.4, 0.8, 1.5, 2.0 and 0 respectively. (0. 50 μg/mL CT DNA, 5 mM Tris (pH 7.5), 0.1 μg/mL EtBr).

Figure 8. Molecular model of DNA. A mixed sequence of dsDNA is shown surrounded by In^{3+} ions at **R** = 1. The van der waal radius of indium in 3+ oxidation state is 0.93 Å [X].

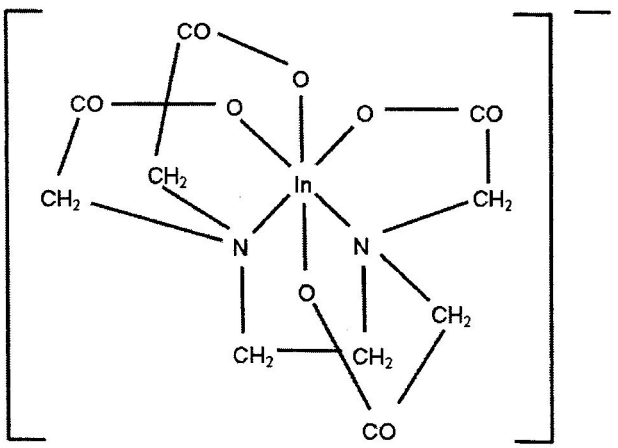

Figure 9. EDTA–In complex, EDTA can chelate indium [23]