SPECTROSCOPIC CHARACTERIZATION OF DNA MODEL-SYSTEM

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ABSTRACT

This study reports the physical characterization of a spin-labeled DNA complex designed by our lab. This DNA complex is to be used for future modeling studies on spin-spin interactions between the unpaired electrons of a spin-label and a metal center using Electron Paramagnetic Resonance (EPR). The model complex is composed of a synthetic, 19-base pair DNA duplex, which acts as the scaffold for a nitroxide radical (spin-label) and ethylenediaminetetraacetic acid (EDTA) that are covalently bonded to complementary strands. This model complex and its two derivative complexes, DNA duplexes containing only the nitroxide radical or the EDTA, were examined using Ultra-Violet (UV) and Circular Dichroism (CD) spectroscopic methods to determine their stability and conformation relative to that of the native DNA duplex. The melting temperatures, T_m, of the chemically modified duplexes were very similar to those of the native DNA duplex, and the CD studies of the modified duplexes indicated they have conformations similar to those of the B-form native duplex.

INTRODUCTION

Many important biological and synthetic processes involve both a metal center and an organic radical. The interactions between the unpaired electrons of the metal and the radical can be closely examined using Electron Paramagnetic Resonance (EPR). The strength of these electron spin-spin interactions can elucidate the structure of the reaction sites in these biological or synthetic macromolecules, which can provide insight into their mechanisms of action.[1-2]   In the scope of our research on these metal-radical interactions, a macromolecular model system was constructed using the rigid and well-defined DNA double-helix as the scaffold for a nitroxide radical, a radical in which the unpaired electron spin is localized primarily within the N-O bond, covalently attached to one strand and an ethylenediaminetetraacetic acid (EDTA)-metal complex covalently attached to the complementary strand. This paper is concerned with the stability and structural characterization of this DNA-model system using Ultra-violate (UV)-monitored thermal denaturation and Circular Dichroism (CD) spectroscopic methods.

The stability of a DNA complex is often measured by a thermal denaturation experiment.[3-4] The DNA helical structure is stabilized through hydrogen bonding between base pairs and by base stacking. Thermal energy is able to break these hydrogen bonds, and therefore able to denature the helical structure of the DNA. As a result, with the increase in temperature, the double helix will unwind or melt into two separate strands. This process is reversible and the double-helix will re-assemble as the temperature decreases to room temperature. The extent of the melting can be monitored using Ultra-violet (UV) spectroscopy because as the DNA duplex separates into single strands, the UV absorbance increases.[5] This effect is known as hyperchromism. The temperature at which half of the DNA strands in duplex are "melted" into single strands is defined as the melting temperature, T_m. The melting temperature depends on the neighboring base pairs as well as the ratio of G≡C to A=T base pairs in the DNA[6], where DNA duplexes rich in G≡C base pairs will generally have a higher T_m, since there are three hydrogen bonds formed between each of these base pairs, but only two between A=T base pairs.[7] Thus, the T_m can be used as an indicator for the stability of the chemically modified DNA complex, relative to the native DNA complex.

A well-characterized structure/conformation of the DNA-model complex is of great importance to our research, for it holds the authority to the EPR modeling studies on the metal-radical spin-spin

interactions. It is critical first to determine whether the covalently bonded chemical labels perturb the native structure of the DNA helix in our model. Circular Dichroism (CD) spectroscopy is a very useful technique for DNA conformational studies, because each of the three common conformations of DNA helixes, A-form, B-form and Z-form[8], provides a unique CD spectrum.[9-10]

MATERIALS AND METHOD

Two synthetic complementary single-stranded oligonucleotides with 19 bases, 5'-GCATAGATACATAGATACG-3' and its complementary strand 3'-CGTAXCTATGTATCTATGC–5', were employed as the backbone for the model complex. In one of the strands, referred to as the A19mer, a nitroxide radical (i.e., the spin label) was attached to an amine chain tethered to the guanidine unit of the 5' end, (Figure 1a) this chemical modification was performed in our lab. The chemically modified strand will be referred to as the spin-labeled A19mer (SL-A19mer) from this point on. The complementary strand of the SL-A19mer is the chemically-modified T19mer and will be referred to as the EDTA-T19mer. It was commercially purchased from TriLink BioTechnologies and has an ethylenediaminetetraacetic acid (EDTA) covalently attached to the second thymidine base from the 3' end (Figure 1b).

(a) **SL–A19mer: 5'–SL–GCATAGATACATAGATACG–3'   SL= Spin Label**

Nitroxide spin label:
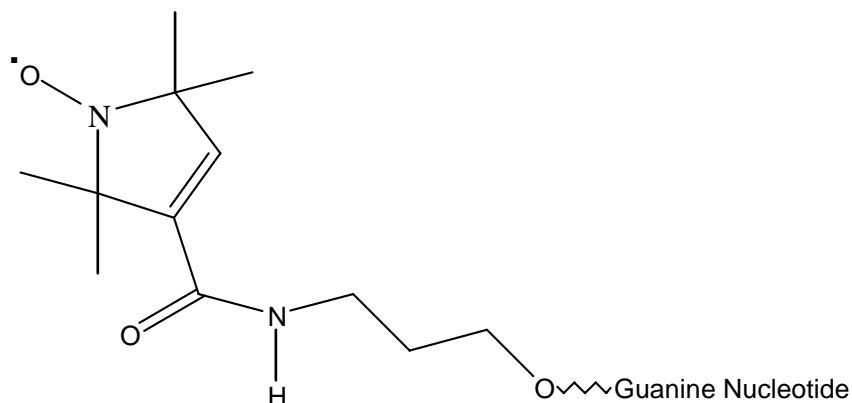
(b) **EDTA-T19mer: 3'–CGTAXCTATGTATCTATGC–5' X= dT–EDTA**
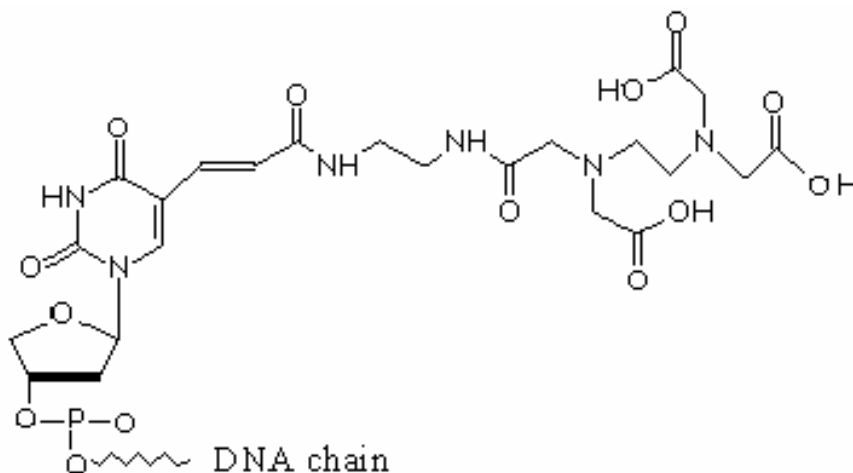
dT-EDTA:

Figure 1: (a) Showing the 19 base sequence of SL-A19mer and the position of the attachment of the spin-label (SL) and the chemical structure of the nitroxide spin-label. (b) Showing the 19 base sequence of the EDTA-T19mer and the position of the attachment of the EDTA. The chemical structure of the EDTA-modified thymidine base (dT-EDTA) is shown as well.

DNA duplex solutions used in this study were prepared by combining the appropriate complementary single strands of DNA suspended in cryoprotectant solvent in a 1:1 mole/mole ratio for each strand. Because impending EPR studies on these DNA duplex solutions will be conducted at temperatures as low as 4 K, all these solutions were suspended in cryoprotectant solvents to protect and stabilize the DNA from the low temperatures. All the DNA solutions in this study were suspended in two types of cryoprotectant solvents. The basic composition of the cryoprotectant solvent was made up of 30% polyethylene glycol, 15% ethylene glycol and 55% buffer (w/w/w). The buffer component of the two different cryoprotectant solvents used in the experiments was 55% 100 mM phosphate buffer, pH 7.0 (**POB**) or 55% 50 mM PIPES-buffer w/ 85 mM NaCl (**PIB**), pH 7.0.

Five different DNA solutions were prepared and they were the Native Duplex (A19mer/T19mer). One sample was suspended in pure buffer and another in buffer-cryoprotectant, the SL-Duplex (T19mer/SL-A19mer) in buffer-cryoprotectant, the EDTA-Duplex (combined A19mer with EDTA-T19mer) in buffer-cryoprotectant, and finally the model complex EDTA/SL-Duplex (combined SL-A19mer with EDTA-T19mer) in buffer-cryoprotectant. The EDTA-T19mer single strand samples were prepared at a higher concentration than the other single strand samples, since a solution of iron (III) was added to the samples to provide the source of metal for the EDTA to chelate to; this in turn diluted the EDTA-T19mer solutions to the same concentration as the other single DNA strands samples.

Thermal Denaturation

The melting of DNA duplexes was monitored by using a Varian Cary 1 Bio Ultraviolet/Visible spectrophotometer, measuring the absorbance at 260 nm in 1 mm path length quartz cuvettes while heating from 10°C to 70°C at a rate of 0.8°C per minute. Ultraviolet absorbance readings were taken every 0.8°C during the melting.

Circular Dichroism

The CD spectra of the various DNA duplexes were collected by using a JASCO J-810 CD spectropolarimeter with a Peltier temperature control attachment over the wavelength range 200 nm to 350 nm. CD spectra were collected for each duplex at 15°. The same samples in the 1 mm path length quartz cuvettes used in the UV-monitored thermal denaturation experiment were used for the CD experiment as well.

RESULTS AND DISCUSSION

Thermal Denaturation

The melting curves collected for the native duplex, SL/EDTA-duplex and its two derivative duplexes in PIB-cryoprotectant are shown in Figure 2. The three chemically modified duplexes exhibit similar melting profiles as the native duplex in both cryoprotectant solutions. The melting curves of the duplexes suspended in POB-cryoprotectant (not shown in this paper) are nearly identical to the ones in PIB-cryoprotectant. The melting temperatures and hyperchromicities determined from the melting curves for the three labeled-DNA duplexes, Table 1, are similar to that of the native duplex, suggesting that the chemical modification (the addition of the labels) did not significantly diminish the stability of the DNA helical structure.

Table I: The hyperchromicity and melting temperatures determined from the melting curves of the native duplex and the three chemically modified duplexes.

Sample Name *(PIB-cryoprotectant)*:	T_m (Numerical Derivative) (°C)	Hyperchromicity %
Native Duplex	54.1	24.5%
SL-Duplex	55.7	24.2%
EDTA-Duplex	55.6	21.1%
SL/EDTA-Duplex	56.4	20.9%

Circular Dichroism:

 The CD spectra collected for the native duplex in PIPES buffer and in PIB-cryoprotectant at 15°C, (Figure 3) indicate a B-form helical conformation for the native duplex in both solvents. The CD spectra of the three chemically-modified duplexes (Figure 4) suggest these modified-duplexes are in the B-form helical conformation as well. Nonetheless, small perturbations on the structures of the DNA helixes did result from the attachment of the labels, which can be are observed in the slight difference in the ellipticity of the CD spectra observed between the chemically modified and the unmodified DNA duplexes.

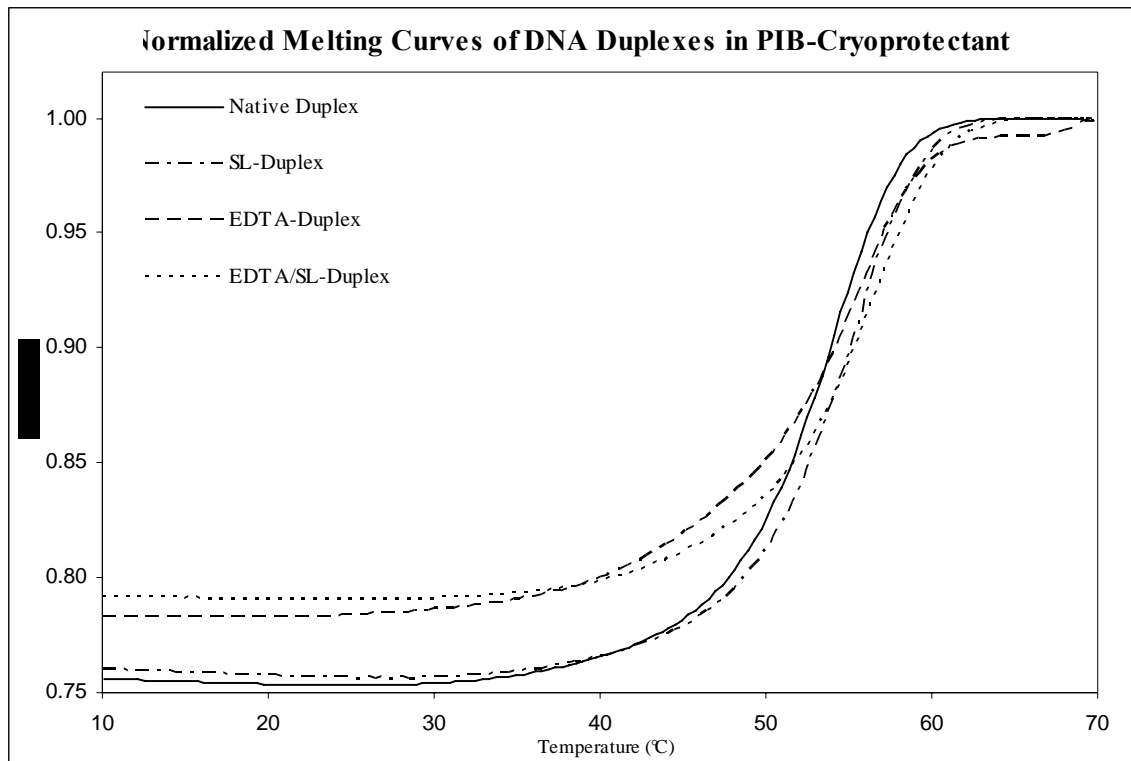**Normalized Melting Curves of DNA Duplexes in PIB-Cryoprotectant**

Figure 2: Melting curve of the native duplex and the DNA-model system (SL/EDTA-duplex) suspended in PIB-cryoprotectant with its two derivatives models collected from 10°C to 70°C at 0.8°C per min and monitored at 260 nm.
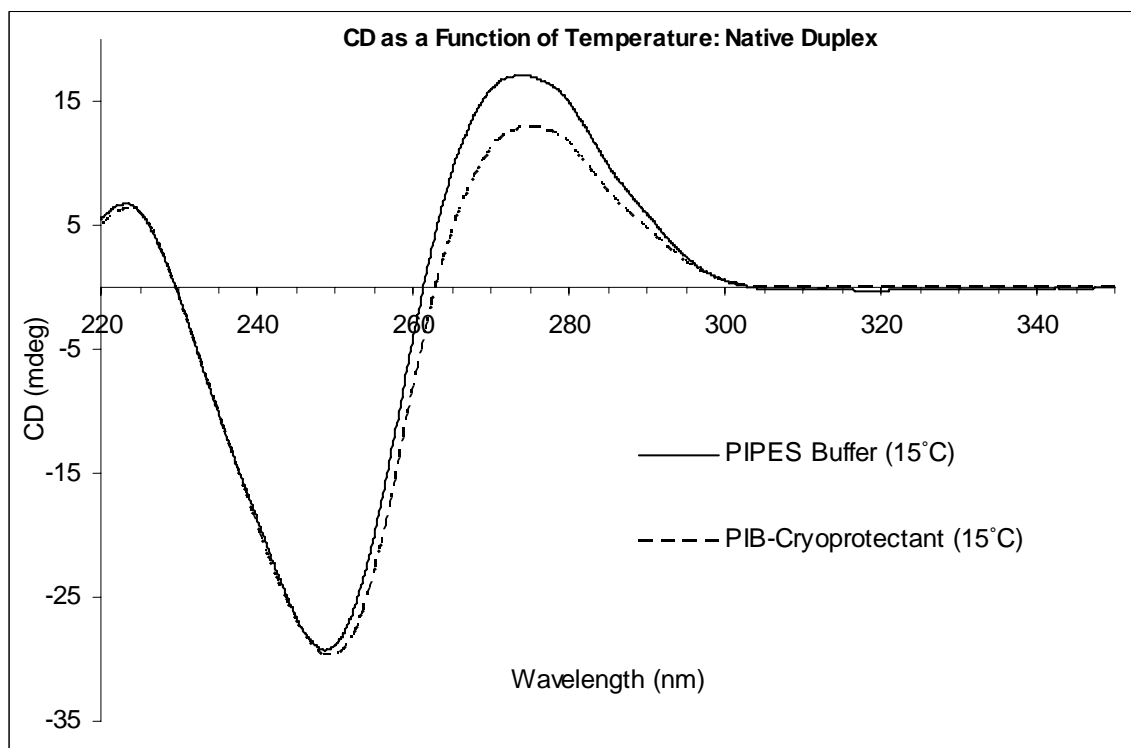
CD as a Function of Temperature: Native Duplex

Figure 3: CD spectra of the native duplex in PIPES buffer and PIB-cryoprotectant at 15°C.
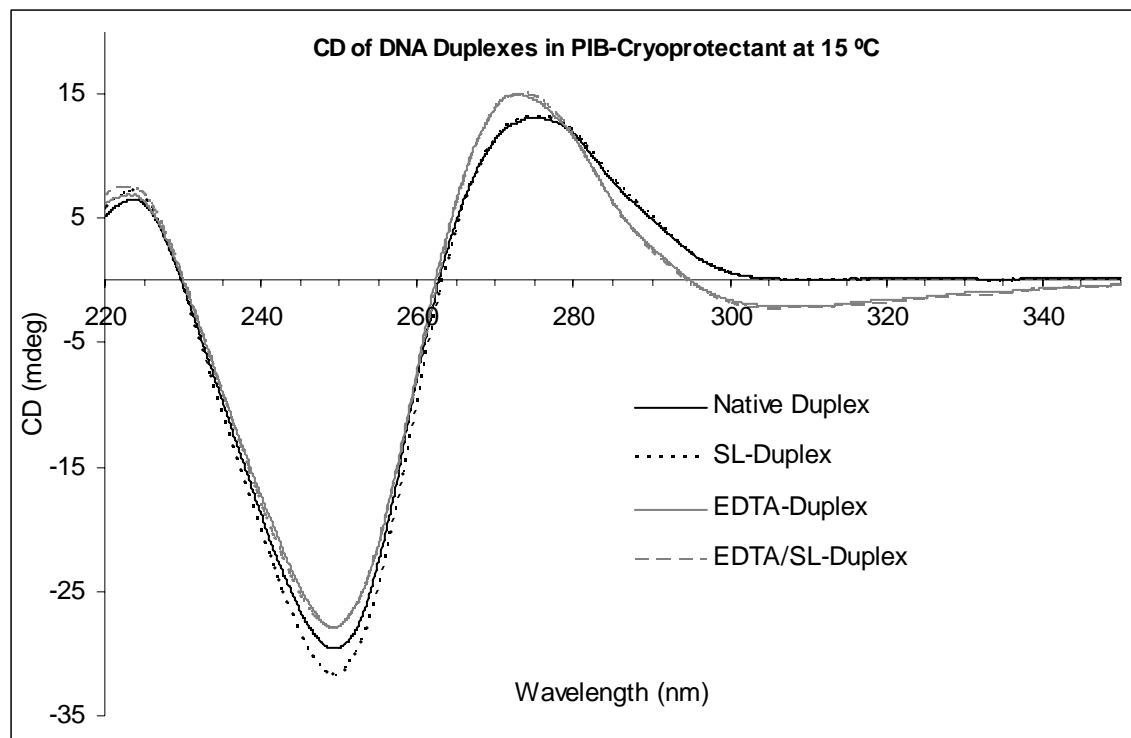
Figure 4: CD spectra of the native duplex with the three chemically-modified DNA duplexes suspended in PIB-cryoprotectant at 15˚C.

Two interesting distinctive features arise in the EDTA-modified duplexes (SL/EDTA-duplex and EDTA-duplex) CD as well as UV absorbance spectra. In the CD spectra of the EDTA-modified DNA duplexes, a consistent negative band from 295 nm to 350 nm was observed, but not for the other two duplexes. Moreover, the UV absorbance spectra of these EDTA-modified DNA duplexes suggest that they absorb at longer wavelengths, between 295 nm to 350 nm, where DNA would normally have no absorbance. The UV absorbance spectra of the EDTA(Fe(III)) metal complex and of the native duplex in the presence of free EDTA(Fe(III)) showed roughly only one-tenth the absorbance observed for the EDTA-modified DNA duplexes at longer wavelength. The UV absorbance spectrum of another metal complex, EDTA(Ca(II)), showed no absorbance at all. The CD spectra of the two metal complexes and of the native duplex with free EDTA(Fe(III)) did not exhibit the unusual negative rotation at the longer wavelength region observed the EDTA-modified DNA solutions.

Further to investigate the causation of the unusual characteristics observed for the EDTA-modified duplexes, a EDTA(Ca(II))/SL-duplex was prepared in which the metal center used was calcium(II) instead of iron(III). Since it appeared that the metal center the EDTA is chelated to has some effect on its UV-absorbance, we wanted to investigate whether it affects the CD absorbance as well. We found that the CD and UV absorbance spectra collected for this duplex exhibit the same unusual characteristics as the other EDTA-modified duplexes. The thermal denaturation of the EDTA(Ca(II))/SL-duplex was also conducted at a rate of 1˚C per min, and after each temperature increment a CD spectrum was collected. The UV-monitored melting curve collected at 260 nm exhibited similar melting profile as the duplexes in Figure 2. The CD spectra collected throughout the melting of the duplex indicated that the negative rotation between 295 nm to 350 nm began to diminish as the duplex started to melt and completely disappears at 64˚C, Figure 5 (top). However, the unusual UV absorbance by the EDTA-modified duplex at longer wavelength (around 295 nm to 350 nm), remained unchanged throughout the melting process. A melting curve was constructed to monitor the degree of disappearance of the 295 nm to 350 nm negative CD rotation with respect to temperature. The CD-monitored melting curve uses the ellipticity (θ) measured at 320 nm for each temperature as the y-axis and temperature as the x-axis, Figure 5 (bottom). The melting temperature

determined from this CD-monitored melting curve at 320 nm was 50.1°C, which is very similar to the melting temperature of 50.8°C for the UV-monitored melting curve collected at 260 nm for this duplex. This indicates that the structure that gave rise to the negative CD rotation from 295 nm to 320 nm is induced only when the EDTA-modified- DNA is duplexed.
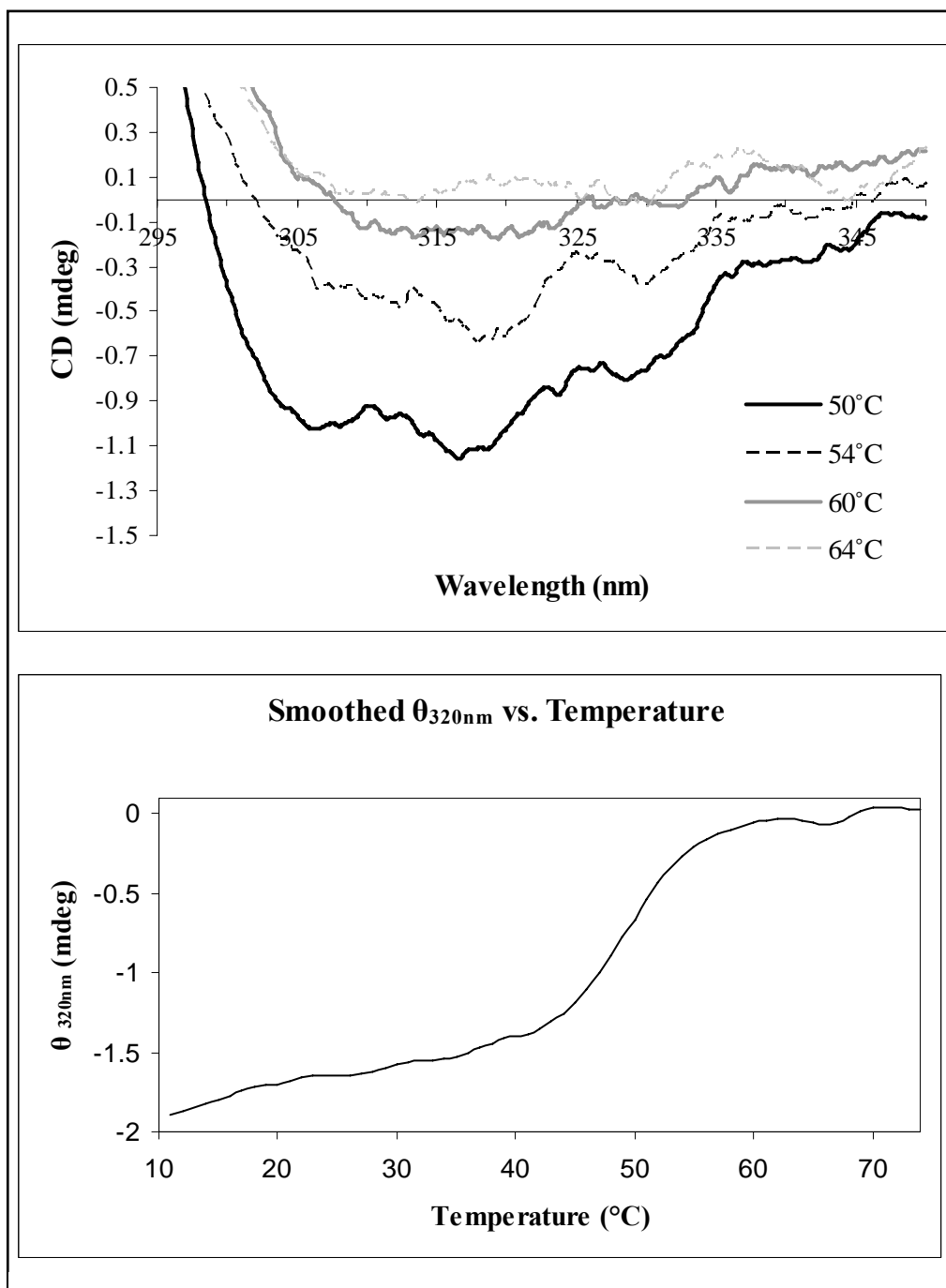

Figure 5: (top) The negative rotation observed between 295 nm to 350 nm for EDTA-modified duplexes starts to diminish around the melting temperature; (bottom) Smoothed CD-monitored "melting" curve measured at 320 nm.

Based on observations made on how the melting of the DNA affects the negative rotation at 295 nm to 350 nm in the CDs of EDTA-modified strand containing duplex, along with the observation that neither free iron-EDTA complexes nor native duplex in the presence of free iron-EDTA complexes CD and UV absorbance spectra display the distinctive characteristics for the EDTA-modified DNA duplex, it is reasonable to conclude that the unusual characteristics observed for the EDTA-modified duplexes in the CD and UV absorbance spectra are results of the covalent attachment of the EDTA to the DNA helix. Also, given that the CD spectra of these EDTA-modified duplexes remain fairly similar to those of other duplexes, we proposed that perhaps the EDTA-metal complex locally disturbs the conformation of the helix, enough to cause the unusual feature in the CD spectra and yet not so significantly that it would induce conformational change of the entire helical conformation.

In summary, the thermal denaturation spectroscopic characterization of our model study indicates that the chemical modifications made to the single-stranded DNA oligomers do not decrease the stability of the DNA helix significantly. The CD study indicates that even after the chemical modifications, the DNA duplex continues to exhibit the B-form helical conformation with small perturbations. Thus, the results of the spectroscopic studies all concurred that our DNA model-complex is a rigid and stable model that will allow considerable interactions between the metal and radical labels. Thus, it is a feasible model to be used for the EPR study of the metal-radical interactions.

ACKNOWLEDGEMENTS

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