Melting of a DNA Hairpin without Hyperchromism†

Tina M. Davis, Lori McFail-Isom, Elaine Keane, and Loren Dean Williams*

School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia 30332-0400

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ABSTRACT: UV absorbance spectroscopy is the most common method for detecting nucleic acid structural transitions and obtaining thermodynamic parameters. UV-detected melting has been used to determine stabilities of nucleic acid hairpins, duplexes, triplexes, and higher order structures and to determine thermodynamic effects of unusual or modified bases and mismatched base-pairs. We report that in some cases UV absorbance spectroscopy is an inadequate analytical technique for these purposes. Some critical transitions are invisible to UV absorbance spectroscopy. For example, the conversion of dodecamer d(CGCAAATTCGCG) from hairpin to random coil is not accompanied by hyperchromism. Circular dichroism (CD) spectroscopy (263 nm) clearly detects two transitions for this dodecamer, each giving a pronounced change in ellipiticity. The concentration dependence of the low-temperature transition and the concentration independence of the high-temperature transition indicate that the predominant state converts from duplex to hairpin to random coil as the temperature increases. These assignments are confirmed by comparison to oligonucleotides of similar sequence that undergo a hairpin to coil transition only. In contrast to CD spectroscopy, UV absorbance spectroscopy shows only a single transition. The transition detected by UV absorbance spectroscopy corresponds to the low-temperature transition detected by CD. UV absorbance spectroscopy does not detect the second transition at any wavelength (from 218 to 310 nm) (by changes) in either absorbance or its derivative with temperature.

Optical properties of DNA and RNA are modulated by three-dimensional structure. The dependence of optical properties on structure has established ultraviolet (UV) absorption and circular dichroism (CD) spectroscopies as powerful techniques for determining nucleic acid structures and stabilities. Optical spectroscopies have been used to monitor melting of A-, B-, and Z-DNA (*1*−*3*), tRNA (*4, 5*), triplexes (*6, 7*), pseudoknots (*8*), quadruplexes (*9*), hairpins (*10*−*12*), and drug complexes (*13, 14*). Optical techniques have also been used to determine effects of covalently modified (*15*−*19*), bulged (*20, 21*), and mismatched bases (*22*−*25*).

UV absorbance spectroscopy exploits the hyperchromic effect, an increase in extinction coefficicent upon unstacking of nucleic acid bases (*26*). Extinction coefficients depend on both the magnitude of the intrinsic transition moment of each base and the relative orientations of adjacent bases. Theory predicts, and conventional interpretation assumes, that structural transitions that change the fraction of stacked bases also change the extinction coefficient.

We describe a DNA structural transition that significantly changes the extent of base stacking, yet is not accompanied by hyperchromism at any wavelength. Thus the transition is invisible to UV spectropscopy. Our observation challenges the use of hyperchromicity for monitoring nucleic acid structural transitions.

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* Author to whom correspondence should be addressed.

The UV-invisible transition we describe here is detected by CD spectroscopy. CD exploits ellipticity, the differential absorption of left- and right-handed circularly polarized light. Ellipticity depends on relative orientations of chromophores. Nucleic acid ellipticity is modulated by disorienting (melting) or reorienting (converting between structural forms) nucleic acid bases (*26*). The UV-invisible transition is clearly detected by ellipticity at 263 nm. However, at 253 nm, a wavelength commonly employed for monitoring nucleic acid transitions, CD is blind to another structural transition. In summary, our results show that structural transitions may occur without producing the expected signals. Therefore, lack of a signal by a single optical technique is not sufficient basis to conclude that a structural transition is absent.

EXPERIMENTAL PROCEDURES

Gel-filtration purified oligonucleotides d(CGCAAATTCGCG), d(CGCAATTTCGCG), d(CGCATTTTCGCG), d(CGCATATTCGCG), and d(CGCGAATTCGCG) were purchased from Midland Certified Reagent Company and desalted on G25 Sephadex columns. Oligonucleotide concentrations were determined by absorbance at 260 nm in denaturing conditions (92 °C).

UV experiments were performed with a Cary 1E UV−vis spectrophotometer equipped with a temperature controller and thermostated cell holder. Two-dimensional data (absorbance, temperature) were collected at 260 nm from 0 to 95 °C at 0.5 or 1 °C increments with a temperature ramp of 0.5 or 1 °C/min. Three-dimensional data (absorbance, wavelength, temperature) were collected from 310 to 218

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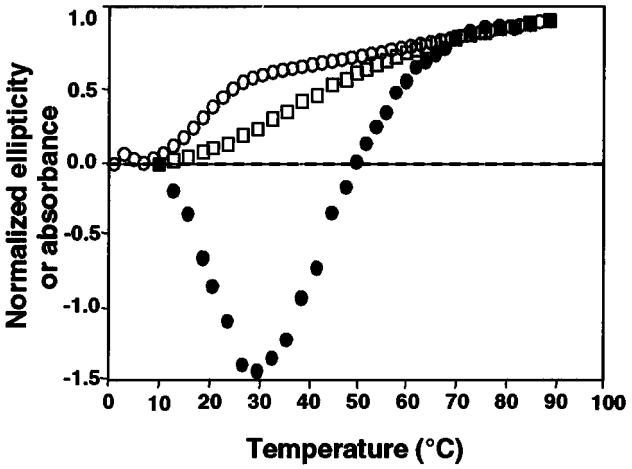

FIGURE 1: Normalized A_{260} (open circles), θ_{253} (squares), and θ_{263} (solid circles) as a function of temperature for AC-oligo (7.5 μM strand). A_{260} and θ_{253} detect different transitions. The low-temperature transition detected by θ_{263} corresponds to the A_{260} transition. The high-temperature transition detected by θ_{263} corresponds to the θ_{253} transition.

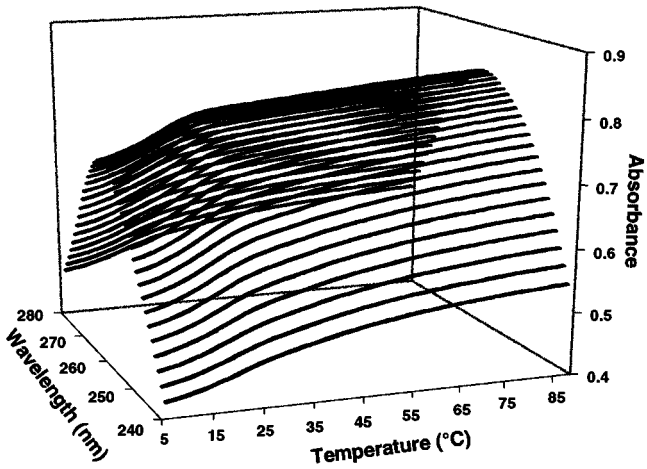

FIGURE 2: Absorbance as a function of temperature at several wavelengths for AC-oligo (7.5 μM). UV spectra were collected from 310 to 218 nm every 2 °C from 0 to 89 °C. For clarity, only data from 240 through 280 nm are shown. UV detects only the low-temperature transition at all wavelengths.

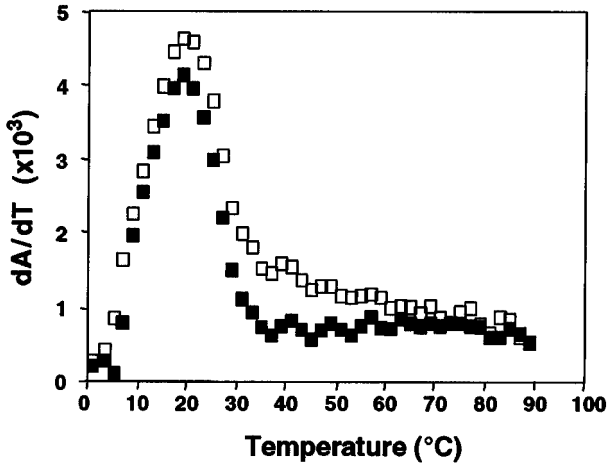

FIGURE 3: UV melting derivative curves for AC-oligo (7.5 μM) at 260 nm (solid squares) and 268 nm (open squares). First derivative curves of the melting profiles shown in Figure 2 are monophasic at all wavelengths.

nm with the program ADL NEWS number 27 (Varian). Spectra were collected every 2 °C from 1 to 98 °C with a temperature ramp of 0.5 °C/min.

CD melting experiments were performed with Jasco 720 and 715 spectropolarimeters equipped with Neslab RTE-110 and RTE-111 temperature control units, respectively. Three-dimensional data (wavelength, temperature, ellipticity) were collected from 310 to 210 nm in 1 nm increments with a 50 nm/min scan rate and 1 s signal averaging time. Spectra were collected from 3 to 95 °C. Samples were equilibrated for 10 min at each temperature prior to data acquisition. Spectra collected in water-jacketed cells were equilibrated at each temperature for 2 min. A thermocouple probe inserted into the sample showed that these hold times are sufficient for thermal equilibration. Five or 10 spectra were collected and averaged at each temperature. Reciprocal noise reduction was applied. We use the symbol θ_{nm} to represent ellipticity at the specified wavelength.

For both CD and UV experiments, cuvette path lengths of 1.0, 0.2, or 0.1 cm were used as appropriate to ensure linearity of the detectors. Melting experiments were conducted in 100 mM NaCl, 20 mM NaH_2PO_4, pH 6.9, with DNA concentrations from 6.5 μM to 57.8 μM (strand).

RESULTS

The information provided by UV and CD spectroscopies is thought to be redundant. Nucleic acid structural transitions are most commonly detected by absorbance at 260 nm (A_{260}) or by ellipticity at 253 nm (θ_{253}). For d(CGCAAATTCGCG) (AC-oligo), the melting curve obtained by A_{260} is not consistent with that obtained by θ_{253} (Figure 1). Variation of A_{260} with temperature indicates a single relatively sharp transition with a midpoint (T_m) of 19 °C. In contrast, under the same conditions, variation of θ_{253} with temperature indicates a single, broader transition with an apparent T_m of 37 °C. The θ_{253} melting curve differs from the A_{260} melting curve in both slope and T_m. These discrepancies suggest that A_{260} and θ_{253} detect different transitions with distinct thermodynamic parameters.

The apparent contradiction presented by these two melting curves is resolved by ellipticity at 263 nm (θ_{263}), which reveals two transitions (Figure 1). Both the low-temperature transition observed by A_{260} and the high-temperature transition observed by θ_{253} are detected by θ_{263}.

Absorbance at 260 nm fails to detect the high-temperature transition. The absorbance melting curves for AC-oligo at wavelengths other than 260 nm are uniformly monophasic. The high-temperature transition is not observable by absorbance (Figure 2) or by derivative at any wavelength (Figure 3).

AC-oligo is a symmetric dodecamer capable of forming mismatched duplex, hairpin, and random coil. The effects of concentration on the melting temperatures were used to assign transitions between these states. Both hairpin and random coil are monomolecular. Therefore, the conversion from hairpin to random coil should be independent of concentration. Conversely, duplex DNA is bimolecular. Thus, conversion of either monomolecular species to duplex should show a concentration dependence.

Concentration behavior of the transitions indicates that the low-temperature transition is conversion of duplex to hairpin

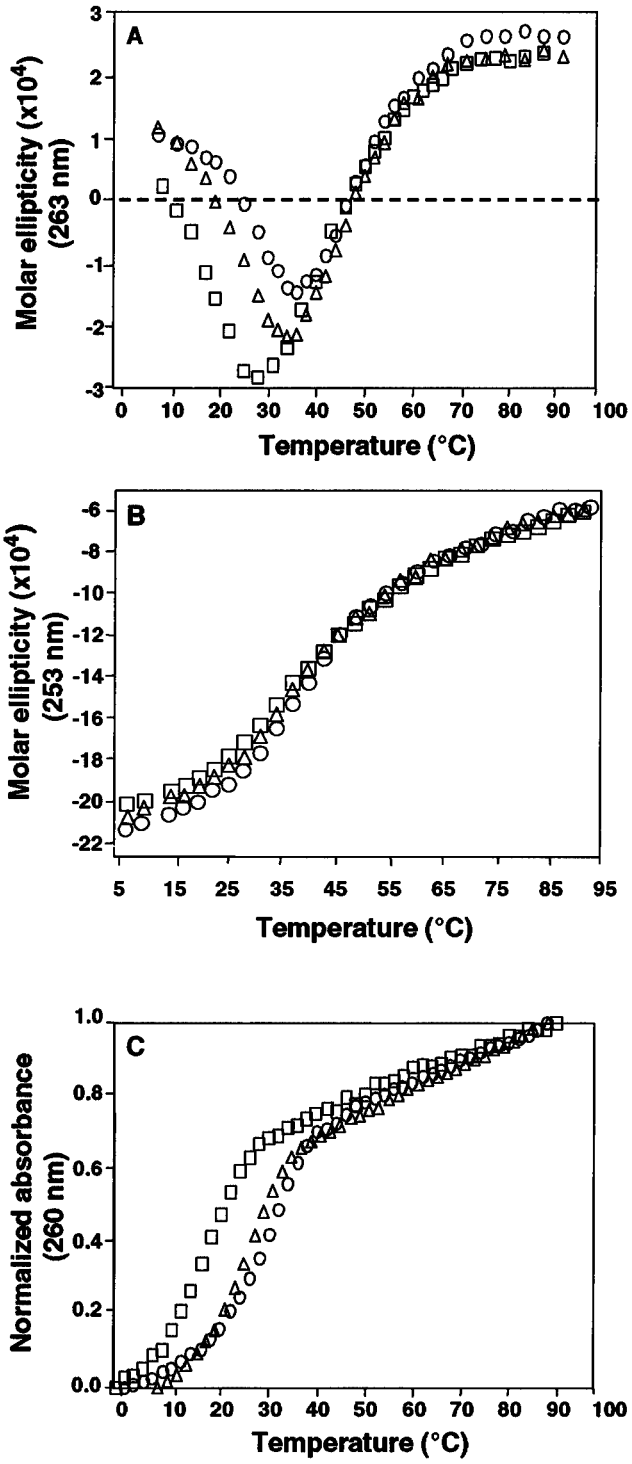

FIGURE 4: Effects of AC-oligo concentration on melting curves. (A) Melting monitored by θ_{263}. The low-temperature T_m is concentration dependent while the high-temperature T_m is not. Squares, 7.5 μM; triangles, 23.1 μM; circles, 57.8 μM (strand). (B) Melting monitored by θ_{253}. The transition detected by θ_{253} is concentration independent. Squares, 10 μM, triangles, 30 μM, circles, 50 μM. (C) Melting monitored by A_{260}. Each curve appears monophasic. The A_{260} transition is concentration dependent. Squares, 7.5 μM; triangles, 23.1 μM; circles, 57.8 μM (strand).

and the high-temperature transition is conversion of hairpin to random coil. The T_m of the low-temperature transition is concentration dependent. This concentration dependence is observable by θ_{263} and A_{260} (Figure 4A,C). The T_m of the high-temperature transition is concentration independent, as indicated by both by θ_{263} and θ_{253} (Figure 4A,B).

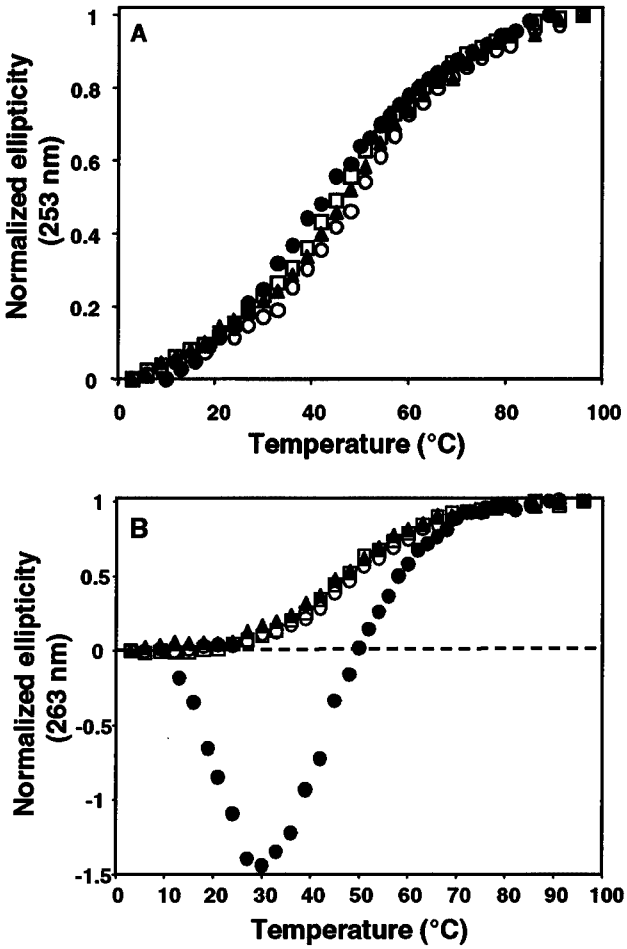

FIGURE 5: Normalized ellipticity as a function of temperature for AC-oligo (solid circles) and d(CGCAXXXXCGCG), where XXXX is TTTT (open circles), ATTT (squares), and TATT (triangles). Oligonucleotide concentrations are 6.5 to 7.5 μM (strand). (A) Melting monitered by θ_{253}. (B) Melting monitored by θ_{263}. All oligonucleotides exhibit similar slopes and melting temperatures for the hairpin to coil transition.

The assignment of the high-temperature transition as hairpin to coil is confirmed by comparison with similar oligonucleotides. Oligonucleotides d(CGCAXXXXCGCG), where XXXX = TTTT, ATTT, and TATT, are expected to form hairpin but not duplex due to the extensive number of mismatches in the duplex state. The A_{260}, θ_{263}, and θ_{253} melting curves are all consistent with a single transition from hairpin to random coil. The T_m and slope of the high-temperature transition of AC-oligo are similar to the T_m and slopes of these hairpin to coil transitions (Figure 5A,B) supporting the assignment made from concentration melting behavior.

DISCUSSION

We have investigated the capabilities of UV and CD spectroscopies for detecting nucleic acid structural transitions. Our comparison of these two spectroscopic techniques reveals a direct failure of UV absorbance spectroscopy to detect a nucleic acid structural transition. We also observe that at a commonly used wavelength (253 nm) CD spectroscopy fails to detect a different structural transition. At the appropriate wavelength, CD appears to successfully detect all transitions. Our results challenge the reliability of UV absorbance spectroscopy for investigating nucleic acid

structure and stability, and suggest that the wavelength of the CD experiment must be empirically chosen.

AC-oligo can occupy three states: (i) a mismatched duplex, (ii) a hairpin, or (iii) a random coil. The fractional occupancies of each state depend on salt, DNA concentration, and temperature. At the appropriate wavelength, CD spectroscopy (θ_{263}) clearly reveals that this oligomer undergoes two structural transitions. The T_m of the low-temperature transition is concentration dependent, while the T_m of the high-temperature transition is concentration independent. These results are consistent with conversion of the predominant state from duplex to hairpin to random coil with increasing temperature.

UV absorbance spectroscopy shows only a single transition for AC-oligo, corresponding to the low-temperature transition detected by θ_{263}. UV absorbance does not detect the second transition at any wavelength (from 218 to 310 nm) by change in either absorbance or its derivative with temperature (Figures 2 and 3). Thus the conversion from hairpin to random coil does not give a change in hyperchromism.

This lack of hyperchromism is not a general characteristic of hairpin melting. Melting of some highly characterized hairpins results in clear hyperchromism (*27*−*30*). Indeed, our control oligomer d(CGCGAATTCGCG) gives a pronounced change in hyperchromism upon conversion of hairpin to random coil (ref *28*, and data not shown). We are currently investigating whether the lack of hyperchromism is restricted to the DNA fragment described here or is common to many sequences and types of nucleic acid transitions. In addition, we are investigating the structure of the hairpin by NMR. We anticipate a hairpin with a three base pair stem and six base loop.

UV absorbance spectroscopy has been used extensively for investigating nucleic acid structure and stability and is often the sole technique employed for these purposes. Generally, it is assumed that the lack of a signal translates to the lack of a transition. Our results challenge this presumption and suggest that UV absorbance spectroscopy may be an inadequate technique for some nucleic acid systems. In addition, our results show deficiencies in CD spectroscopy. We argue that relying on a single optical technique may lead to incomplete characterizations and incorrect conclusions. Complete UV and CD absorbance−temperature−wavelength matrixes should be collected and analyzed for proper analysis of all nucleic acid systems.

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