THERMAL DENATURATION OF DNA AND DNA:POLYPEPTIDE COMPLEXES.[†] SIMULTANEOUS ABSORPTION AND CIRCULAR DICHROISM MEASUREMENTS.

Richard Mandel[*] and Gerald D. Fasman

Graduate Department of Biochemistry
Brandeis University
Waltham, Mass. 02154

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Summary: A method for observing the thermal denaturation of DNA and DNA:polypeptide complexes by the simultaneous monitoring of the absorption and circular dichroism at a single wavelength is outlined. Previously it was known that both the extinction coefficient and the ellipticity were sensitive probes of the denaturation of the DNA double helix and herein it is shown that the same melting temperature is obtained by these two methods. In addition, the ellipticity at 280 nm versus temperature shows additional features not seen in the absorption curves. At temperatures less than the melting temperature of DNA, the ellipticity increases (premelt), and at temperatures higher than the melting temperature of DNA the ellipticity decreases (postmelt). A complex of poly-(L-Lysine$^{84.5}$, L-Valine$^{15.5}$):DNA shows two distinct thermal transitions, one corresponding to free DNA and the other to bound complex. In addition the slope of the premelt with respect to temperature is different from that of pure DNA.

Thermal denaturation has been used extensively to study the helix to coil transition in DNA, and as a probe of the stability, composition and structure of DNA in solution (1, 2, 3). In addition, the same technique has been applied to complexes of polypeptides and proteins with DNA (4, 5, 6, 7), and to nucleohistone (8, 9). Information about the extent and relative strength of binding between polypeptides or proteins and DNA has been obtained in this manner. In the majority of these studies the thermal transitions have been monitored by observing the increase in absorption accompanying the helix to coil transition.

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Circular dichroism (CD) spectroscopy has also been of value in observing conformational changes in DNA (10), in nucleohistone (11), in chromatin (12) and in complexes (13). In addition, the CD of DNA has been shown to change prior to melting (known as a premelt) (14) which may reflect subtle changes in the asymmetry of the DNA helix. There have also been studies of CD changes prior to and during the denaturation of DNA (15, 16) and nucleohistone (17). In order to further investigate the structural changes and the mode of binding of polypeptides to DNA, a simple modification of the Cary-60 spectropolarimeter, with the Cary-6001 CD attachment was made, which allows for the simultaneous measurement of absorption and CD changes during thermal denaturation. This technique provides a more sensitive probe of such transitions. Examples are given for the thermal denaturation of DNA and for a poly-(L-lysine, L-valine):DNA complex.

Experimental

Thermal denaturation measurements were made on the Cary-60 recording spectropolarimeter with the Cary 6001 CD attachment with the following simple modification. The connection between the low voltage side of the CD dynode voltmeter and ground were severed and an external connection to either a 100 or 150 ohm resistor in series with the voltmeter was made. During denaturation the resulting potential across the resistor was monitored with a Texas Instrument Servo Riter II, dual channel recorder with a full range of 10 mv and a 6:1 zero suppression. At each wavelength and slit opening there is a monotonic relationship between the dynode voltage and the sample optical density. This relationship is non-linear and a calibration curve was obtained using a set of neutral density filters (Baird Atomic) in the CD sample compartment. In this way both CD and absorption changes can be simultaneously recorded. A comparable arrangement has been previously reported (18).

The temperature and linear thermal gradient are controlled with

a Tamson TE-3 circulating bath (Neslab, Durham, N. H.) and Neslab TP2 thermal programmer. The bath circulates ethylene glycol through a 1 cm pathlength jacketed quartz cell (Optical Cell Co., Beltsville, Md.). The temperature is monitored with a linear thermal probe (# 702X, Yellow Springs Instrument Co., Yellow Springs, Ohio) at the cell jacket exit. The temperature was recorded on the other channel of the Texas Instrument chart recorder from the output of the thermal probe coupled to a Wheatstone bridge circuit, powered by a mercury cell. In order to record CD changes during denaturation the Cary 60 was run at full slit (2. 4 mm) in synchronous mode at a rate of one inch per 250 seconds, with a time constant of 10 seconds and a full range setting of 0. 02°. A bakelite cell holder was constructed to retard heat loss from the cell.

Calf thymus DNA, prepared as described (19), and a random copolymer (MW 100, 000) containing 84. 5% L-lysine and 15. 5% L-valine, were used for the denaturation studies. Complexes of the polypeptide and DNA, with an optical density of approximately 1. 0 at the wavelength of interest, were prepared by a gradient dialysis (20) of the DNA and polypeptide solution from 2. 0 M NaCl to 0. 1 M NaCl over a period of 8-10 hours with subsequent dialysis against 2.5×10^{-4} M EDTA, pH = 7. 0 solution, all at 4° C. The initial optical density of both the solution and the solvent versus air, at the desired wavelength, is determined by measurement in the Cary-14 spectrophotometer. Samples are heated at a rate between 0. 25 and 0. 75° C/min. and the absorbance, ellipticity and temperature are simultaneously recorded at the desired wavelength. Upon completion of a denaturation run, the DNA concentration is determined by the perchlorate digestion technique (21). The data is processed on a PDP-10 computer and plotted on a Calcomp plotter. Both the extinction coefficient and the ellipticity are plotted as a function of temperature as well as their derivatives with respect to temperature. The derivative at point i

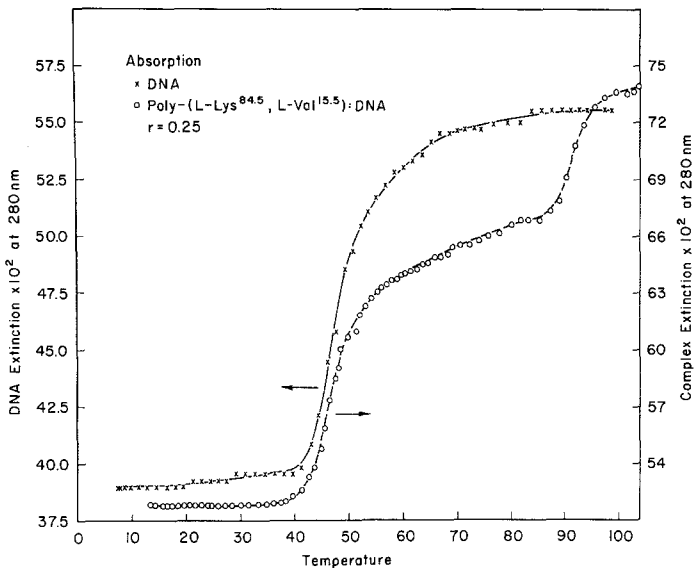

Figure 1 - Absorption thermal denaturation at 280 nm of: (x-x-x) DNA, and (o-o-o-o) a complex of poly-(L-lysine$^{84.5}$, L-valine$^{15.5}$): DNA, r = 0.25, both in 2.5 x 10^{-4} M EDTA, pH = 7.0.

with extinction ϵ_i, ellipticity $[\theta]_i$ and temperature T_i is:

$$\left(\frac{d\epsilon}{dT}\right) = \frac{\epsilon_{i+1} - \epsilon_{i-1}}{T_{i+1} - T_{i-1}} \quad \text{and} \quad \left(\frac{d[\theta]}{dT}\right) = \frac{[\theta]_{i+1} - [\theta]_{i-1}}{T_{i+1} - T_{i-1}}$$

The derivative plots are used to determine the melting temperature (T_m) which in this study is defined by the absolute maximum in the derivative of the extinction and of the ellipticity. These derivative plots more clearly determine the temperature of melting, the width, the total change in extinction or ellipticity for a transition, and the total number of transitions for a particular complex as previously demonstrated by Shih and Bonner (6).

Results

The absorption and CD thermal denaturation of DNA at 280 nm (Figs. 2 and 4) yields an average melting temperature (T_m) of 46.6±1.5. However, there are substantial differences between the extinction and the ellipticity curves. The ellipticity curve (Fig. 3) exhibits an increasing

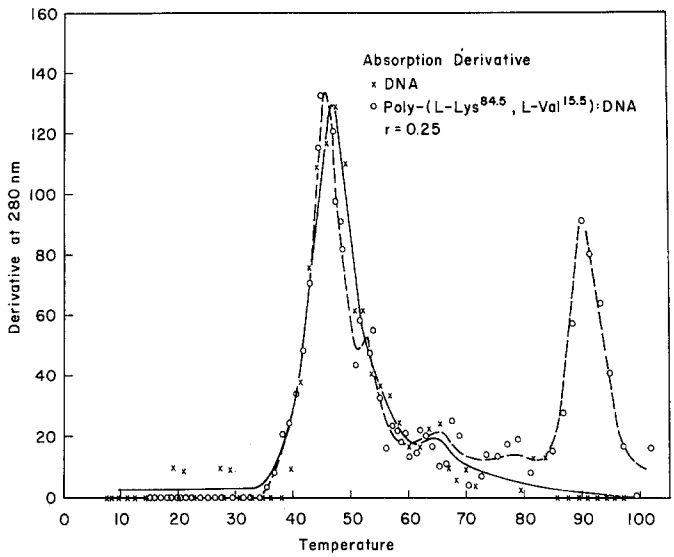

Figure 2 - Derivative with respect to temperature of the absorption denaturation curves (Figure 1): (x-x-x) DNA, and (o-o-o-) a complex of poly-(L-lysine$^{84.5}$, L-value$^{15.5}$): DNA, r = 0.25, both in 2.5 x 10^{-4} M EDTA, pH = 7.0.

[θ] at temperatures below T_m (premelt) and a decreasing [θ] at temperatures above T_m (postmelt). This is in contrast to the extinction curve (Fig. 1) which only shows a change of absorption centered at T_m.

The premelt at 280 nm is significantly smaller than that obtained by Gennis and Cantor (14), though it should be noted that the temperature range examined herein is smaller and the salt concentration much lower than in their study. The $[\theta]_{280}$ thermal denaturation, at the solvent conditions herein, also differs in detail with that of Usatyi and Shlyakhtenko (16) performed at 275 nm in 0.01 SSC (where 1 x SSC equals 0.15 M NaCl + 0.015 M Na citrate pH = 7.0). The study herein (Fig. 3) shows a much larger ellipticity change around T_m, and a levelling off of the CD melt prior to the postmelt. In the previous study (16) it was difficult to determine T_m from the ellipticity at 275 nm due to the small CD change observed and the continuing slope at temperatures greater than T_m. The presence of the postmelt phenomena may be due to either the temperature

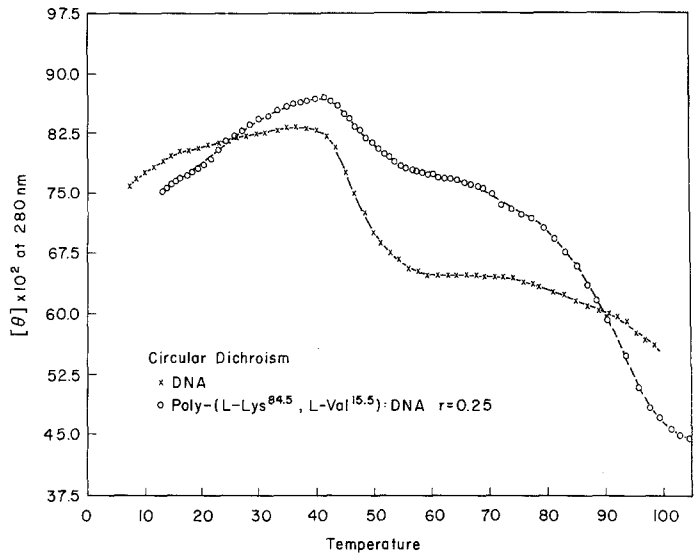

Figure 3 - Circular dichroism thermal denaturation at 280 nm of: (x-x-x) DNA, and (o-o-o-) a complex of poly(L-lysine$^{84.5}$, L-valine$^{15.5}$): DNA, r = 0.25, both in 2.5×10^{-4} M EDTA, pH = 7.0.

dependence of the ellipticity of the individual nucleotides or an increasing randomness of the single stranded structure at temperatures greater than the strand separation temperature.

The thermal denaturation curves of the annealed complex poly-(L-lysine$^{84.5}$, L-valine$^{15.5}$):DNA, r(lysyl/phosphate) = 0.25, is also seen in Figures 1-4. Examination of the extinction denaturation (Figs. 1 and 2) clearly reveals two transitions, the first at an average temperature of $T_m = 46.2 \pm 1.5$ corresponding to the unbound DNA in the complex, and the second at $T'_m = 92.6° \pm 2.0°$ corresponding to the bound segments. The ellipticity melting curve (Figs. 3 and 4) also shows two transitions at temperatures in agreement with the extinction melting curve. The percent of nucleotides unbound may be determined from the fractional change in extinction through the first transition divided by the fractional change in extinction for DNA at the same wavelength (7). On this basis, the percent of unbound DNA in the complex equals 68%. This corresponds to a value of r = 0.27 in good agreement with the nominal value of r = 0.25.

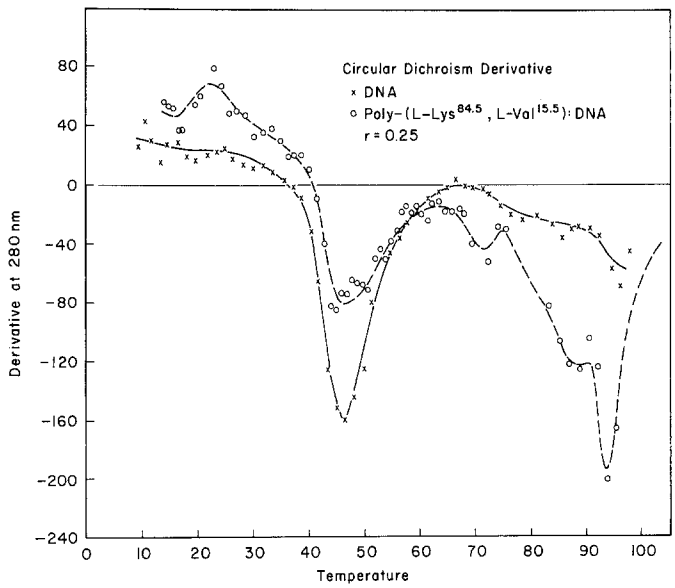

Figure 4 - Derivative with respect to temperature of the circular dichroism denaturation curves (Figure 3): (x-x-x) DNA and (o-o-o) a complex of poly-(L-lysine$^{84.5}$, L-valine$^{15.5}$): DNA, r = 0.25, both in 2.5 x 10^{-4} M EDTA, pH = 7.0.

The CD spectrum of the DNA:polypeptide complex at room temperature is similar to that of DNA, as can be seen by the large ($\sim$8000) $[\theta]_{280}$ value (Fig. 3). This is in marked contrast to similar complexes between poly-L-lysine and DNA which show that the positive ellipticity band has been replaced by a very large negative band (22, 23). The complex ellipticity denaturation curve shows a slightly larger premelt than DNA, a much smaller ellipticity decrease at T_m and a very large ellipticity change in the temperature range of the postmelt and near T'_m (Figs. 3 and 4). These differences may be due to the specificity of binding of the polypeptide with DNA or to the extent of aggregation of the complex.

The method described in this paper combines the very useful tools of thermal denaturation analysis with CD spectroscopy. The method will be of use for studying the binding of both histones and synthetic polypeptides to DNA in order to examine the conformational consequences, stability and specificity of binding between these macromolecules.

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