

# Articles

## Anomalous Absorbance-Temperature Profile of Calf Thymus DNA in Presence of Spermine

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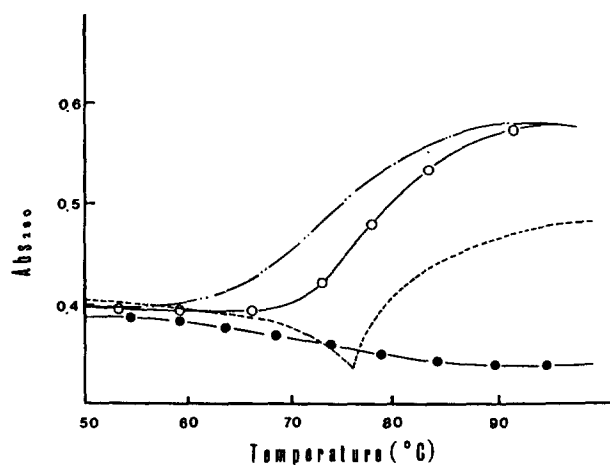
An anomalous absorbance-temperature profile of calf thymus DNA, having a hypochromic trough just before the rise of the  $T_m$ -region phase, occurs at the spermine concentration where the DNA collapses into a compact structure. The trough phase can be eliminated by the addition of ethidium bromide and also by a hydrophobic environment.

### Introduction

The stabilization of collapsed forms of DNA by polyamines *in vivo* has attracted wide attention, because most DNA, for example, in chromosomes, bacterial nucleoids and viruses, is present *in vivo* in a compact form through interaction with cationic species such as histones or polyamines<sup>1-9</sup>, and polyamines occur in nature as major cationic constituents of virus and bacteriophage heads. Although this conformational collapse of DNA has been successfully described by Manning's counterion condensation theory<sup>10</sup>, as applied by Wilson and Broomfield<sup>2</sup>, there still remain other factors to be elucidated. It seems that the collapse of DNA can occur spontaneously primarily through entropy-driven nonspecific hydrophobic interactions, whenever a critical fraction of the negative charges of the DNA phosphate has been neutralized and thereby the electrostatic repulsion has been sufficiently reduced. In this case, the collapsed state of the DNA may be favored by temperature increase *via* increased stability of the hydrophobic interactions. The present study was undertaken to obtain further insight into the mechanism of the spermine-induced conformational collapse of calf thymus DNA and its temperature dependency. Here we present the anomalous absorbance-temperature profile, of the DNA, which occurs at the spermine concentration where the DNA collapses into a compact structure.

### Materials and Methods

Calf thymus DNA (Type 1) and spermine were purchased from Sigma Chemical Co. and used without further purification. Calf thymus DNA was used throughout this work unless indicated otherwise. Ethidium bromide (Sigma) was recrystallized once from methanol prior to use. The heat-denatured DNA was prepared by heating the DNA ( $A_{260}=1.4$ ) dissolved in 8 mM citrate buffer, pH 7, in a boiling water bath for 20 min, and then cooling rapidly to 0°C in an ice-water. The DNA concentrations are expressed in terms of nucleotide phosphate by using the extinction coefficient of  $\epsilon_{260}=6,600 \text{ M}^{-1}\text{cm}^{-1}$ . The DNA concentrations used in each experiment was  $6.1 \times 10^{-5} \text{ M}$ . The concentration of ethidium bromide (EtBr) was determined spectrophotometrically by using the

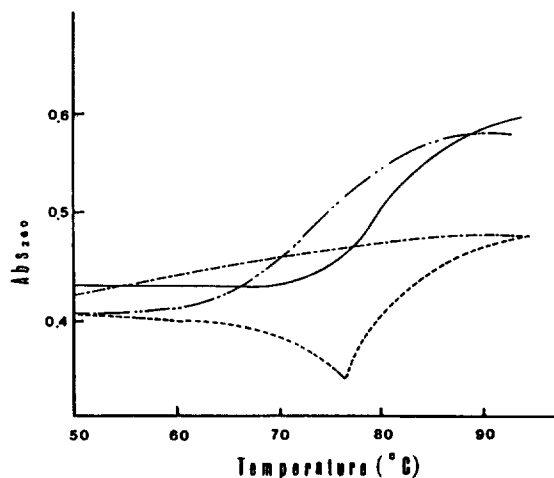


**Figure 1.** Effect of spermine on the absorbance-temperature profiles of DNA. Spermine concentrations: ---, minus spermine; —○—,  $5 \times 10^{-5} \text{ M}$ ; ·····,  $3 \times 10^{-4} \text{ M}$ ; —●—,  $3 \times 10^{-3} \text{ M}$ .

extinction coefficient of  $\epsilon_{480}=5,680 \text{ M}^{-1}\text{cm}^{-1}$ . To make spermine-DNA complex, spermine solution was added slowly through the wall of the tube of the DNA solution while swirling the solution gently. The spermine and EtBr ligands were usually mixed with DNA for 10 min. Absorbance-temperature profiles were obtained as previously reported<sup>11</sup>.

### Results and Discussion

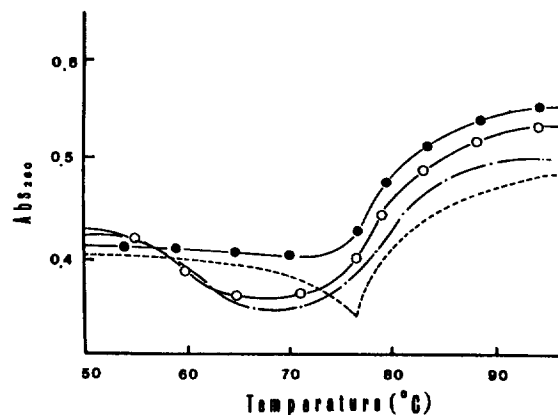
The absorbance-temperature profile of the DNA is shown in Figure 1. As the concentration of the added spermine is increased to  $3 \times 10^{-4} \text{ M}$ , the melting profile is shifted toward the right and the  $T_m$  is increased. These effects have been observed<sup>11-13</sup> when the added small ligands bind to the double helical DNA rather than the single strand DNA. In Figure 1, we notice a trough occurring just before the rise of the  $T_m$ -region profile curve. This anomalous absorbance-temperature profile at the spermine concentration of  $3 \times 10^{-4} \text{ M}$  can be obtained only with the native DNA but not with the denatured DNA as shown in Figure 2. We also previously found that the native DNA is collapsed rapidly into a compact form at this concentration of spermine as determined



**Figure 2.** Anomalous absorbance-temperature profile of DNA. The concentration of spermine added is  $3 \times 10^{-4}$  M. —, minus spermine; - - -, denatured DNA plus spermine; ·····, native DNA plus spermine; — · —, native DNA plus spermine and  $2.8 \times 10^{-6}$  M EtBr.

by viscometric titration<sup>14</sup>. These results indicate that there is an anomalous behavior in the absorbance-temperature profile of the native DNA at the spermine concentration where the native DNA collapses into a condensed state.

The trough of the anomalous absorbance-temperature profile is indicative of hypochromicity due to an increased conformational collapse of DNA induced by temperature increase. Widom and Baldwin<sup>3</sup> previously reported that the condensed state of DNA, induced by  $\text{Co}^{3+}(\text{NH}_3)_6$ , is more stable at higher temperatures. Chatteraj *et al.*<sup>6</sup> provided microscopic evidence that collapsed DNA structures formed by spermidine at high and low temperatures are similar. We, hereby, speculate that nonspecific hydrophobic interactions may play an important role in stabilization of the tertiary structure of the collapsed state of DNA, when a critical fraction of the DNA phosphate charge is neutralized by any cationic species. Since the nonspecific hydrophobic interactions which stabilize the tertiary structure of DNA is entropy-driven, temperature increase may favor the hydrophobic interactions, thus leading to the stabilization of the collapsed conformation of DNA. Although there is no detailed information available with respect to the tertiary structure of DNA, structure of kinky<sup>15,16</sup> and circumferentially wound toroids, which can be a good compromise for the extended rigid DNA duplex to accommodate minimized bending, have been described<sup>17,18</sup>. In formation of such a condensed structure, packing density of the quasi-parallel DNA segments should be increased by entropy-driven hydrophobic interactions of the DNA segments. Since entropy-driven process should be favored by temperature increase, maximization of packing density, and accordingly compaction of DNA structure would be favored by temperature increase. The hypochromicity trough in the anomalous absorbance-temperature profile of DNA, which occurs at the spermine concentration where the DNA molecules get collapsed, may be indicative of the compact structure of DNA, favored by the temperature increase. In order to look into this possibility and some characteristics of the phase transition of the DNA structure corresponding to the trough



**Figure 3.** Effect of EtBr on the anomalous absorbance-temperature profile of DNA in the presence of  $3.0 \times 10^{-4}$  M spermine. EtBr concentration (M): - - -, minus EtBr; — · —,  $8.9 \times 10^{-7}$  M; —○—,  $1.9 \times 10^{-6}$  M; —●—,  $2.8 \times 10^{-6}$  M.

formation, we examined the differential effect of ethidium bromide, as a probe, on the two phases; of the trough formation (phase I), and of the  $T_m$ -region (phase II) of the anomalous absorbance-temperature profile.

In Figure 3, we can see that as the concentration of EtBr added is increased, the depth of the trough is decreased, while the width becomes broader and the trough phase is shifted to the left, and thereby the phase transition midpoint ( $T_c$ ) of the trough formation. Relative cooperative lengths ( $n$ ) vs. the concentrations of EtBr for the transitions of phase I and phase II calculated as shown below on the assumption that the structural transition monitored by the absorbance change at the wavelength of 260 nm takes place in two-state transition.

In cooperative transition<sup>18</sup>, the sharpness of the transition generally increase with the "cooperative length",  $n$ . Among other things, this leads to a characteristic increase in the molar enthalpy of transition  $\Delta H_{app}$ . This enthalpy at the transition midpoint ( $T_c$ ) in each phase was calculated for a two-state model. In this case, the apparent rate constant is:

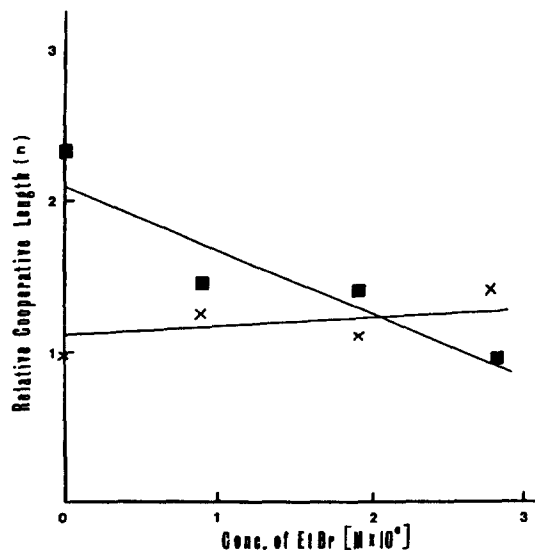
$$K_{app} = K^n = \frac{\theta}{1-\theta}, \text{ where } \theta = \frac{[I]}{[I] + [II]},$$

and [I], [II]=concentration of species. If the normalized increase in absorbance during the transition can be equated with the quantity,  $1-\theta$ , then at a transition midpoint:

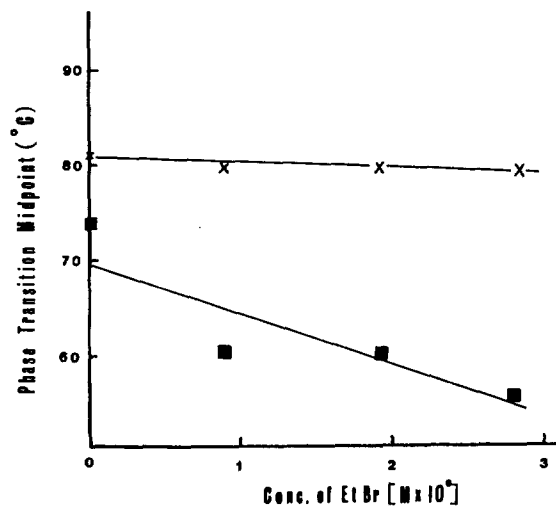
$$\begin{aligned} \left[ \frac{d \ln K_{app}}{dT} \right]_{T_c} &= \left[ \frac{d}{dT} \left( \ln \frac{\theta}{1-\theta} \right) \right]_{T_c} \\ &= \frac{\Delta H_{app}}{RT_c^2} = \frac{n \cdot \Delta H_u}{RT_c^2} \end{aligned}$$

where  $\Delta H_u$  is the molar enthalpy of transition for the elementary transition process. Thus, from a van't Hoff plot, *i.e.*  $\ln K_{app}$  against  $1/T$ , and taking the values of  $\Delta H_u$  to be the same, the ratios for cooperative lengths  $n$  were calculated. The  $n$  data are plotted in Figure 4.

The relative cooperative length of the transition in phase I is decreased, while that of the phase II is increased, as the concentration of EtBr is increased. Differential effects of EtBr on phase I and II can be seen again in the change



**Figure 4.** Effect of ethidium bromide on the relative cooperative length ( $n$ ). —■—, phase transition to the downward (trough) peak; —x—, phase transition in the  $T_m$  region.



**Figure 5.** Effect of ethidium bromide on phase transition midpoint ( $T_c$ , —■—;  $T_m$ , —x—).

of phase transition midpoint vs. the concentration of EtBr, as shown in Figure 5. The transition midpoint ( $T_c$ ) for the phase I is decreased, as the EtBr concentration is increased. In contrast, the transition midpoint ( $T_m$ ) of phase II is kept almost constant as the concentration of EtBr is increased, whereas the  $T_m$  of free DNA is raised as stated above. Mechanism for this difference in the EtBr effect on spermine-complexed and free DNA is not clear at this point. However, based on these data of differential effects of EtBr on the

two phases, we believe it very likely that the three dimensional structural levels corresponding to the two phases respectively are dissimilar and that phase I is more sensitive to ethidium bromide than phase II. Studies on the extent of binding of spermine as a function of EtBr concentration should be needed before further progress can be made with this aspect of problem.

From our studies we draw the following tentative conclusion: if phase II is primarily affiliated to the thermal transition of the secondary structure, the phase I may be a reflect of the transition of tertiary structure resulting from the thermal stabilization of the monomolecular condensed spermine-DNA complex. The present study showing the occurrence of anomalous absorbance-temperature profile of calf thymus DNA at the spermine concentration where the conformational collapse of DNA begins will be useful for the understanding of the mechanism of the declination of melting profile of some DNA solutions prior to the rise of the profile.

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