DAEHAN HWAHAK HWOEJEE
(*Journal of the Korean Chemical Society*)
Vol. 26, No. 4, 1982
Printed in the Republic of Korea

단 신

DNA의 吸光度-溫度 樣相에 미치는 스페르민의 영향*

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(1982. 2. 4 접수)

Effect of Spermine on the Absorbance-Temperature Profile of DNA

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(Received Feb, 4, 1982)

Conformational transitions of genes would be key molecular events in differentiation and de-differentiation of cells. Especially cooperative conformational transitions of genes in the events are considered to be an underlying basic mechanism of gene activation and regulation. On the other hand, numerous studies have shown that polyamines interact with nucleic acids and that polyamines have a variety of stimulatory effects on syntheses of DNA, RNA, and proteins[1~3]. Recently the possible involvement of polyamines in cell transformation and the variation of the concentration level of polyamines in cancerous cells have attracted wide attention[4]. Thus, the investigation of the effect of polyamines on structural-functional properties of genes would be useful for the understanding of the conformational transition of genes in relation with its functions. In the present work, in an attempt to improve our understanding on the characteristics of the absorbance-temperature profile of calf thymus DNA, influenced by spermine, the effect of spermine on the molar enthalpy of transition (ΔH_{app}) and cooperative length (n) of the transition, in addition to the value of transition midpoint (T_m), was estimated under the assumption of two-state model of transition[5].

E. coli. DNA was prepared according to the procedures of Marmur[6], and calf thymus DNA (Type I) was purchased from Sigma Chemical Co. DNA solutions were prepared in phosphate buffer, pH 7.0, composed of 0.006 *M* phosphate and 0.001 *M* EDTA, and the initial concentration was adjusted to have the absorbance of 0.3 cm^{-1} at the wavelength of 260 nm at 20 °C. Spermine tetrahydrochloride was from Sigma. The absorbance-temperature profile was scanned at 260 nm with Pye Unicam 1800. The heating of the cell of the DNA solution was performed by circulating water with Haake Constant Temperature Bath Circulator connected to the cell holder of the spectrophotometer. The rate of heating of the Circulator was 3 °C per min. The temperature corresponding to an absorbance was checked all through the scanning. By testing the temperature-difference between the

* This work was supported by Korea Science and Engineering Foundation Grant.

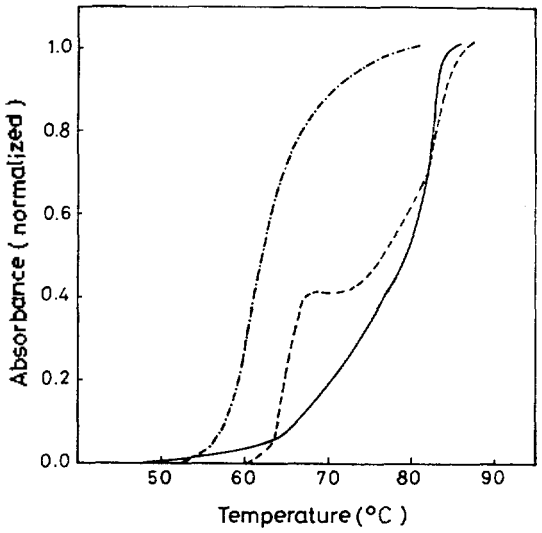

Fig. 1. Absorbance-temperature profiles of DNA species in the absence and presence of 0.03 *M* spermine 4HCl. —·—·: Calf thymus DNA, minus spermine. - - -: Calf thymus DMA, plus spermine. ——: *E. coli* DNA, minus spermine.

Table 1. Transition midpoint (T_m); molar enthalpy of transition (ΔH) at T_m; ratio of cooperative lengths (n) of the species of DNA solutions.

DNA solutions	T_m, °C	ΔH kcal/mole	Ratios for n
Calf thymus, minus spermine 4HCl	63	43.6	1.0
Calf thymus plus spermine 4HCl	64 84	97.2 120.4	2.2
E. coli, minus spermine 4HCl	78	51.9	1.2

temperature of the water in the Circulator and the temperature of the solution in the cell during the interval of the heating, the temperature of the former was corrected to that of the latter, but correction for the thermal expansion of water was not done.

The Absorbance-Temperature Profiles of the DNA Solution in the Absence and Presence of Spermine. In the presence of spermine at the concentration of 0.03 *M*, the profile is shown to be characterized by polyphasic insead of monophasic nature, *i.e.*, rather discrete stepwise than continuous transition phases, and increased sharpness of the transition profile. The characteristic increase in the cooperative length (n) and the molar enthalpy of transition, in each phase at T_m, whose values are associated with the sharpness of the transition in cooperative transition, was calculated for the two-state model. In this case, if the fractional *i.e.*, normalized, increase in absorbance is shown as θ, then the apparent equilibrium constant K_{app} can be equated as[7]: $K_{app}=K^n=\frac{\theta}{1-\theta}$, where K is the intrinsic equilibrium constant, and at a transition midpoint,

$$\left(\frac{d \ln K_{app}}{dT}\right)_{T_m}=\left[\frac{d}{dT}\left(\ln\frac{\theta}{1-\theta}\right)\right]_{T_m}$$
$$=\frac{\Delta H_{app}}{RT^2}=\frac{n\cdot\Delta H}{RT^2}$$

where ΔH is the intrinsic molar enthalpy of transition. The value of the apparent molar enthalpy of transition, ΔH_{app}, was obtained from the slope of the van't Hoff plot: In K_{app} *vs.* $\frac{1}{T}$. Taking the values of the intrinsic mo lar enthalpy of transition, ΔH, for the transitions in the presence and absence of spermine to be the same, the ratios of cooperative length (n) of the transition profiles in the presence and absence of spermine were calculated. The values of T_m, ΔH_{app}, and n for the phases of the profiles are shown in *Table* 1. In the table, we can see the increase in T_m, ΔH_{app} and n in the presenc of spermine. Thus, the preference of spermine for binding to helical structure of DNA[8] and its influence on conformational stability and cooperativity of the conformational transition of the DNA can be confirmed. It is tempting to speculate some relationship between the discrete conformational transition profile of the DNA induced by spermine and cancerous cell transformation

associated with abnormal level of polyamine concentration.

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