Viscometric Studies on the Stability of DNA-Proflavine Complex-II

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DNA-Proflavine Complex, Viscometric Studies

The binding of proflavine to native DNA increased its stability against thermal denaturation as measured by viscometric method. Up to a moderate ionic strength of 0.058 M, the melting temperature of the complex increased almost linearly with the increase of dye concentrations and a saturation was reached when one proflavine molecule was added per four to five DNA-Phosphates ($D/P \cong 0.2$). The extent of stabilization (ΔT_m) produced by dye binding decreased gradually with the increase of ionic strength and no stabilization effect was observed at an ionic strength of about 0.3 M. The maximum melting temperature attained by Proflavine binding was almost independent of the ionic strength of the medium. The same maximum value was reached as obtained simply by increasing the sodium ion concentration.

Introduction

In a previous communication the thermal stability of DNA-Proflavine complex in 0.02 M BPES buffer was studied by viscometric technique [1]. The present report concerns a detailed viscometric studies on the thermal denaturation of DNA-Proflavine complexes (for different dye to nucleotide ratios, D/P) measured in solvents of six different molarities. From these results the relative stabilization produced by the bound cationic dyes have been discussed.

Materials and Methods

Salmon sperm DNA (Sigma Chemical Company, USA) was used in this study. Stock DNA solution prepared in BPES [1] buffer, pH 6.8 was stored at 0 °C. It was established that even at low ionic strength the pH of the buffer did not show any significant change within the range of temperature used in the measurement. DNA solutions in different molarities of the same buffer were prepared from the stock. The concentration of DNA solutions was estimated spectrophotometrically by assuming a molar extinction coefficient of 6600 $M^{-1}\,cm^{-1}$ for DNA at 258 nm with a PMCII spectrophotometer (Carl Zeiss, West Germany).

For the preparation of the dye-DNA complex, proflavine hemisulphate (Imperial Chemicals, England) was used without further purification. Molarity of the dye solution was estimated spectrophotometrically by assuming a molar extinction coefficient of $4.1 \cdot 10^4$ $M^{-1}\,cm^{-1}$ at 445 nm.

Preparations of the complex and viscosity measurements were done as descrbied in the previous communication [1]. The same method was followed to draw the melting profiles.

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Results and Discussions

Figs 1 – 3 show the variation of the ratio of specific viscosity at temperature T to that at 35 °C, $(\eta'_{sp})_T/(\eta'_{sp})_{35\,°C}$, for DNA-Proflavine complexes at different dye to nucleotide ratios (D/P) for three different molarities of the solvent. For comparison, the melting curves for native DNA, the details of which is the subject of a separate communication, have been included in the figures in dotted lines. There are very well-marked features between the three figures.

At 0.002 M Na^+ (Fig. 1) the melting curves showed that with increasing dye concentration in

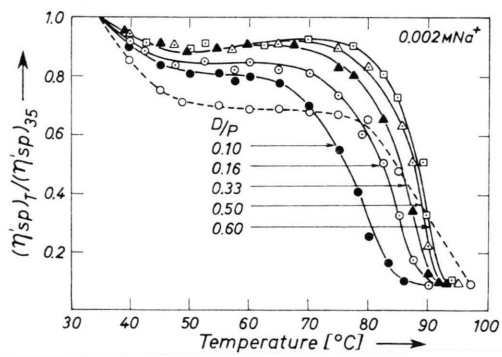

Fig. 1. Melting profiles of DNA-Proflavine complexes at different D/P values. The dotted curve is for native DNA ($D/P=0$). Final DNA concentration = 20.0 ν/ml in each complex. Na^+ = 0.002 M.

the complex, the initial decrease of specific viscosity observed with native DNA was reduced and the curves were shifted to higher temperatures resulting in a corresponding rise in melting temperature (T_m). The cooperativity of the transition increased with increasing level of dye binding. A value of 88.0 °C for T_m was attained at about $D/P = 0.6$. There was no further significant rise in T_m after this D/P value.

The increase of ionic strength to 0.005 M abolished the initial low-temperature decrease of the viscosity ratio (Fig. 2), and no intermediate plateau

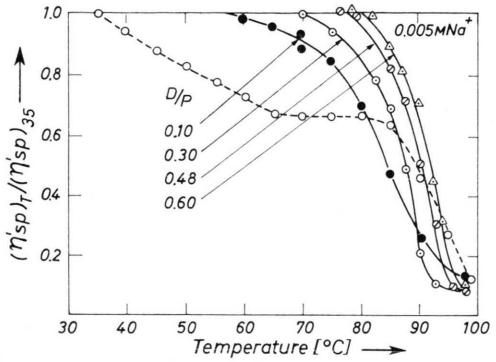

Fig. 2. Melting profiles of DNA-Proflavine complexes at different D/P values. Na^+ = 0.005 M. Other experimental conditions are the same as in Fig. 1.

appeared before the cooperative transition occurred. This was true for D/P varying between 0.1 to 0.6. The gradual shift of the melting curves towards higher temperatures continued upto the D/P value of about 0.6, as in the previous case. The cooperativity of the transition also increased with increasing D/P ratio. The maximum value of T_m reached at a D/P ratio of 0.6 was 91.0 °C.

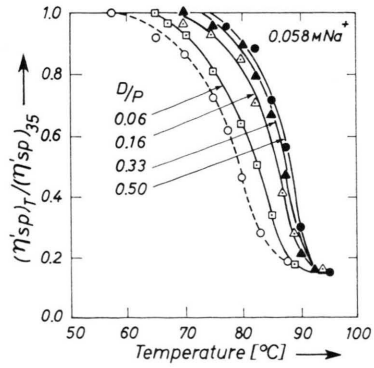

Fig. 3. Melting profiles of DNA-Proflavine complexes. Na^+ = 0.058 M. Other experimental conditions are the same as in Fig. 1.

At 0.058 M Na^+ (Fig. 3), the shift of the melting curves towards higher temperature for the same D/P ratio was relatively low compared to that at the lower ionic strengths. The melting profiles appeared to be as sharp as that of native DNA. Very little broadening of the profiles could be detected even at low D/P values. The curve for $D/P = 0.50$ was almost coincident with that for $D/P = 0.33$. The highest T_m-value of the complex observed at $D/P = 0.5$ was 88.0 °C.

At still higher ionic strengths, the pattern of the profiles remained unchanged but the extent of stabilization (ΔT_m) was less. The maximum T_m-values of the complex ($D/P = 0.5$) at the ionic strengths of 0.1 M and 0.2 M were 89.0 °C and 90.0 °C respectively. No stabilization effect due to dye binding was observed at an ionic strength of 0.3 M. Melting of both the DNA and that of the complex ($D/P = 0.5$) occured at a temperature of 89.5 °C.

The melting temperatures (T_m) of DNA-Proflavine complexes at different ionic strengths were deduced from the melting curves. These are plotted in Fig. 4. The points on the ordinate represent the

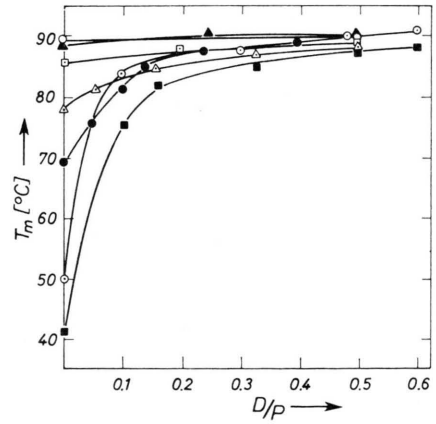

Fig. 4. Melting temperatures (T_m) plotted against D/P at different ionic strengths: -■-, 0.002 M; -⊙-, 0.005 M; -●-, 0.02 M; -△-, 0.058 M; -□-, 0.10 M; -▲-, 0.20 M; -○-, 0.3 M. Data for 0.02 M has been taken from a previous publication (ref. 1).

melting temperatures of native DNA ($D/P = 0$) at different molarities of the solvent. The figure showed that for increasing concentrations of sodium ion the thermal stability of native DNA increased due to the electrostatic shielding and a saturation was approached in the neighbourhood of 0.3 M [2, 3]. The maximum T_m-value attained at this ionic strength was 89.5 °C.

For Na^+ concentration in the range of 0.002 M to 0.058 M the melting temperatures of the complexes increased almost linearly with the increase of D/P up to a ratio of about 0.10. The increase then slowed down and attained almost a saturation value in the neighbourhood of 0.2. Afterwards, no significant rise in the T_m occurred with increasing dye concentration in the complex. It is known that at about $D/P = 0.2$, the strong binding also reaches its completion. It, therefore, follows that mainly the strong binding of the proflavine cations was responsible for the helical stability of DNA-Proflavine complex [1, 4–9]. The weakly bound dye molecules were mostly dissociated at premelting temperatures and contributed little to the stability of the complex. This effect was very negligible at higher ionic strengths. The rate of increase in melting temperature was also greater at the lower ionic strengths. For $D/P < 0.2$ greater number of dye molecules are strongly bound with DNA with the lowering of ionic strength that accounted satisfactorily for the observed effect [10].

It is seen from Fig. 4 that Proflavine binding increases the melting temperature upto a limiting value. The maximum T_m-value obtained for the DNA-Proflavine complexes at different ionic strengths was in the neighbourhood of 90.0 °C, same as obtained with native DNA at a molarity of 0.3 M. From the present study it is therefore, concluded that a DNA molecule of a definite base composition has an upper limit of its thermal stability which may be attained either by incresaing the counter ion concentration of the medium or by increasing the degree of proflavine binding.

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