Viscometric Studies on the Stability of DNA-Proflavine Complex

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Viscometric technique has been used to estimate the relative contributions of strong and weak binding modes towards thermal stabilization of the Proflavine-DNA complex. Variation of the ratio of the specific viscosity of the dye-bound DNA to that of the DNA solution ($\eta^\text{sp}/\eta^\text{sp}$) with different dye to DNA-phosphate ratios ($D/P$) shows that the saturation is attained at $D/P$ value of about 0.2. This effect is more pronounced at lower ionic strengths. Heat-induced helix-coil transition curves at different $D/P$ values at a fixed ionic strength of the buffer reveal a gradual shift towards higher temperature with the increase of $D/P$ and levelling off at $D/P$ of about 0.22. It is suggested that only the strong binding mode causes thermal stabilization of the DNA double helix and the double helix having all the possible intercalating sites saturated by the dye molecules attains the most stable configuration.

The study of the interactions between DNA and the cationic acridine dyes is of great interest because some of them possess mutagenic and carcinogenic properties. Two distinguishable binding processes have so far been observed. In the "strong" binding mode, occurring at low values of the dye to DNA phosphate ratio ($D/P$), the dye molecules are intercalated between the base pairs, which is followed by the local untwisting and the lengthening of the double helix. This phenomenon has been investigated by spectrophotometric, electron microscopic, autoradiographic, and X-Ray diffraction studies as well as by the hydrodynamic methods. In the "weak" binding mode occurring at high $D/P$ values, the dye molecules are stacked on the surface of the DNA molecule. The stability of the proflavine bound DNA (PF – DNA) was studied by optical methods. It was shown that the bound DNA was more stable and that the helix-coil transition temperature was raised. The present paper reports the results of viscometric studies on the binding of different amounts of proflavine with DNA and its variation with the ionic strength of the solvent. The relative contributions of the strong and the weak binding modes, on the stability of the double helix have also been investigated.

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England) were used in this study. Stock solutions of DNA and dyes were prepared in BPES (0.006 M Na₂HPO₄, 0.002 M NaH₂PO₄, 0.001 M EDTA and requisite amount of NaCl controlling the ionic strength) buffer, pH 6.9. The concentrations of DNA and dye solutions were estimated spectrophotometrically by assuming a molar extinction coefficient of 6600 M⁻¹ cm⁻¹ for DNA at 258 m/μ and 4.1 x 10⁴ M⁻¹ cm⁻¹ for proflavine at 440 m/μ. Complexes were prepared by mixing equal volumes of DNA solutions of a fixed molarity (P) and dye solutions of varying molarities (D). The resulting mixture has been characterised by the corresponding (D/P) value.

The viscosities were measured by a Cannon-Manning semi-micro viscometer, mounted rigidly in a thermostatic bath maintained at (35.0 ± 0.1) °C. Specific viscosity (ηₛₚ) of the native DNA dissolved in BPES was obtained from the relation:

\[ ηₛₚ = \frac{(t - t₀)}{t₀} \]

where \( t \) and \( t₀ \) were the efflux times of the DNA solution and the buffer respectively. The specific viscosities of the dye-DNA solutions (\( ηₛₚ \)) were next obtained with the same DNA concentrations (P) but varying amounts of dye (D). BPES buffer was used as a reference in this case also, since there was very little difference between the efflux time (\( t₀ \)) of the buffer and the dye solutions in buffer at the concentrations used in this study.

The variation of the ratio of two specific viscosities, (\( ηₛₚ / ηₛₚ \)) has been plotted in Fig. 1 as a function of (D/P) for three different ionic strengths (0.001 M, 0.01 M, 0.195 M Na⁺). Each curve was the mean of three independent measurements. The three curves for different ionic strengths showed a steep increase of the ratio of (\( ηₛₚ / ηₛₚ \)) with the increase of (D/P), up to a limiting value of about 0.2. After this, the increase slowed down and attained a saturation value. This state of affairs continued up to the maximum measured (D/P) value of approximately 0.85. For (D/P) > 1.0 the precipitation occurred and the viscometric studies could not be continued. At any particular (D/P), (\( ηₛₚ / ηₛₚ \)) was higher for lower ionic strengths. These results also showed that two different processes of binding were operative, the first one nearly reached its completion at about (D/P) = 0.2 and that afterwards the second process continued. The contribution of the second process in changing the specific viscosity was negligible.

The changes in the specific viscosities of dye-bound DNA with temperatures (\( ηₛₚ \)) for different values of (D/P) were next investigated. Samples were taken in sealed ampuoles, heated at different constant temperatures for 15 min and then rapidly chilled in ice. These were then slowly brought to the room temperature and the specific viscosities were measured at 35 °C. The variation of (\( ηₛₚ \))/\( ηₛₚ \) of the dye-bound DNA solutions with temperature at a fixed ionic strength (0.02 M BPES) has been shown in Fig. 2 for six different (D/P) ratios (0.05, 0.10, 0.14, 0.24, 0.40 and 0.50). These results showed that the transition curve shifted to higher temperatures as the (D/P) is increased. In other words, the PF – DNA complex became more stable at higher (D/P) and a saturation was reached near about (D/P) = 0.24 (compare the transition curves for (D/P) = 0.24 and 0.4).

The midpoints of each transition curve (\( Tₘ \)) were deduced from Fig. 2 and these have been shown in Table I and also have been plotted against (D/P) in Fig. 3.

Fig. 1. The specific viscosity of dye-bound DNA solution relative to that of DNA solution alone (\( ηₛₚ / ηₛₚ \)) at the same ionic strength plotted against (D/P). Final DNA concentration in each complex (P/2) = 1 x 10⁻⁴ M.

Fig. 2. Heat-induced helix-coil transition curves of dye-bound DNA solution in 0.02 M BPES at different (D/P) values.
The melting temperature for the native DNA in 0.02 M BPES was 69 °C and has been indicated as the point A in Fig. 3. Compared to the native DNA, the melting temperature for the dye-bound DNA was higher and increased with the increase of D/P. It reached a maximum value of 87.5 °C at the point B at about D/P = 0.22 after which it levelled off. Upto the maximum measured value of D/P = 0.5 represented by the point C, no further change in Tm occurred.

From the results presented here the following conclusions can be made. (i) The intercalative binding leads to the increase of specific viscosity which is most pronounced at the lowest ionic strength and a saturation in this binding mode is approached in the neighbourhood of D/P = 0.2. (ii) The gradual increase of melting temperature with the increase of dye concentration continues up to the D/P value of about 0.22.

(iii) It appears that only the first binding mode causes thermal stabilization of the double helix and the most stable structure is one at which all the intercalating sites have been saturated.

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