Studies on the Melting of DNA

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The thermal denaturation of the native DNA in solvents of varying salt concentrations was studied by viscometric and spectrophotometric methods. It was observed that within the molarity range of 0.02 M to 0.3 M, the melting temperatures obtained by the two independent methods agreed well, but that at lower ionic strength the agreement was not satisfactory. Both the viscometric and the spectrophotometric measurements showed an increase of the melting temperature with increasing counterion concentration and a levelling off effect in the neighbourhood of 0.3 M.

Introduction

The intrinsic viscosity and the sedimentation constant of the native and the completely denatured DNA, as affected by the counterion concentration, have been investigated by several workers [1–6]. The appearance of the DNA molecules in these two states under the electron microscope has been correlated with these physical parameters [3, 7, 8]. However, very little work has been done on the effect of counter ion concentration on the hydrodynamic properties of DNA during the different stages of its transition from the completely native to the fully denatured state [9–11]. The present report concerns a comparative study of the viscometric and the spectrophotometric measurements on the melting of native DNA at different ionic strengths under identical conditions.

Materials and Methods

Salmon sperm DNA (Sigma Chemicals, USA) was used in this study. DNA solutions were made in BPES [12] buffer at different molarities from the same stock. The concentrations of the DNA solutions were deduced from their absorbances at 258 nm. The value of the molar extinction coefficient was taken as 6600 $cm^{-1} M^{-1}$ at this wavelength. Spectrophotometric measurements were done with a PMQ II spectrophotometer (Carl Zeiss, West Germany) at room temperature. The method of viscosity measurements of the DNA solutions denatured to varying extent have been described in a previous communication [12]. The heating and the subsequent rapid chilling of the samples at the two low ionic strengths (0.005 M, 0.002 M) were carried out simultaneously. It was observed that the pH was maintained at high temperatures even at the lowest buffer concentration. [13].

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Results and Discussion

Figs 1 and 2 show the thermal denaturation pro-

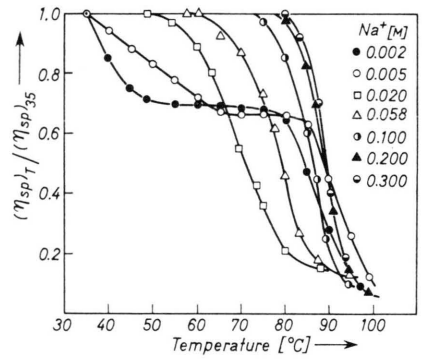

Fig. 1. Viscometric melting profiles of native DNA at different molarities of the solvent. DNA concentration approximately 20 γ/ml in each case.

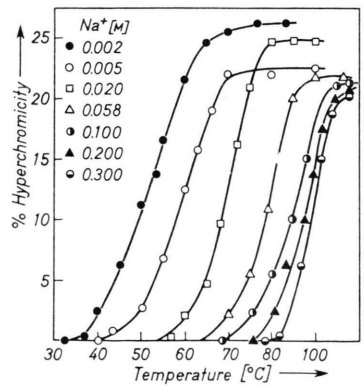

Fig. 2. Spectrophotometric melting profiles of native DNA at different molarities of the solvent identical to those in Fig. 1.

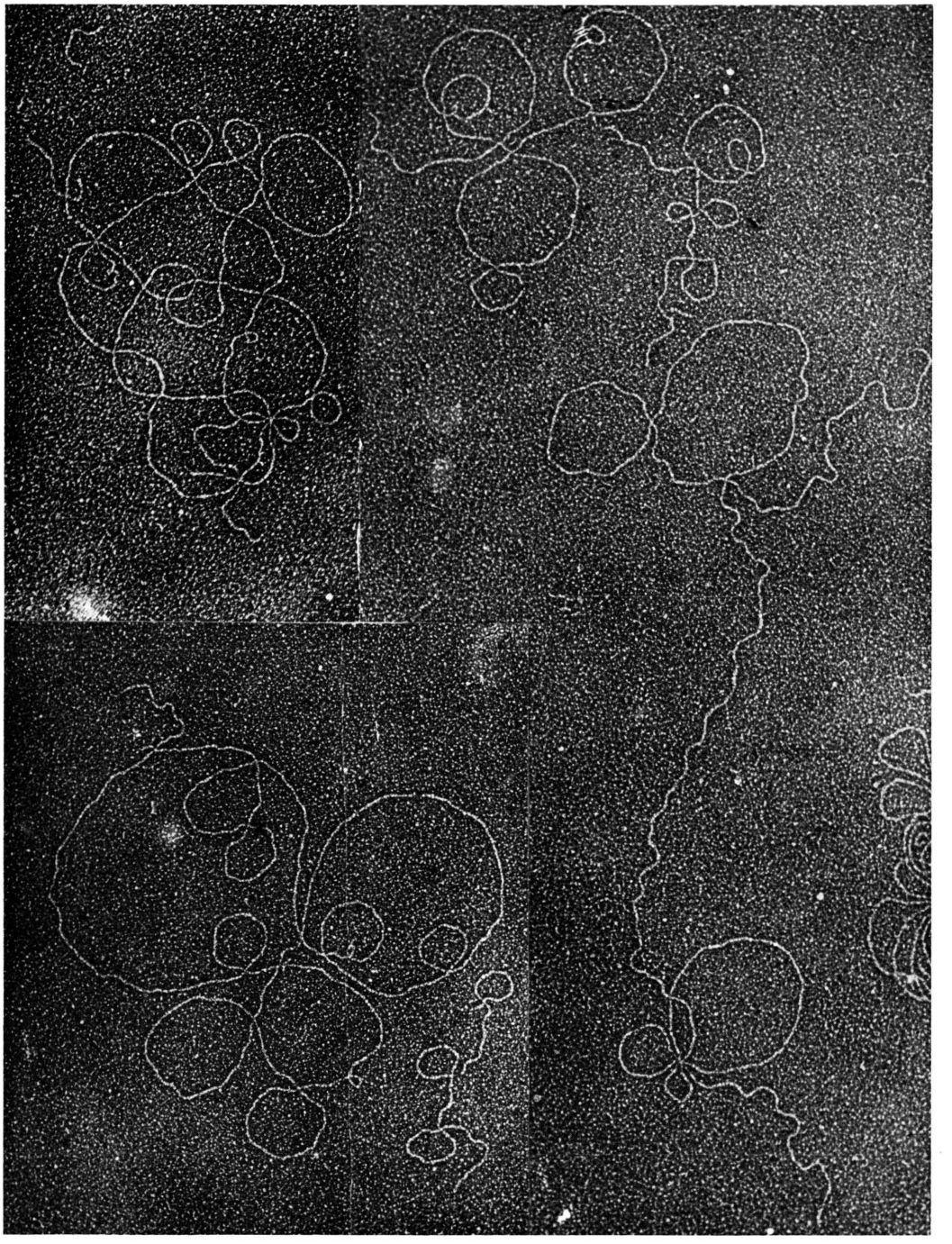

Fig. 1. Native full length T-7 DNA molecules prepared by the protein monolayer technique with triple distilled water hypophase. The molecules show circular loops and smooth contour. X 40,500.

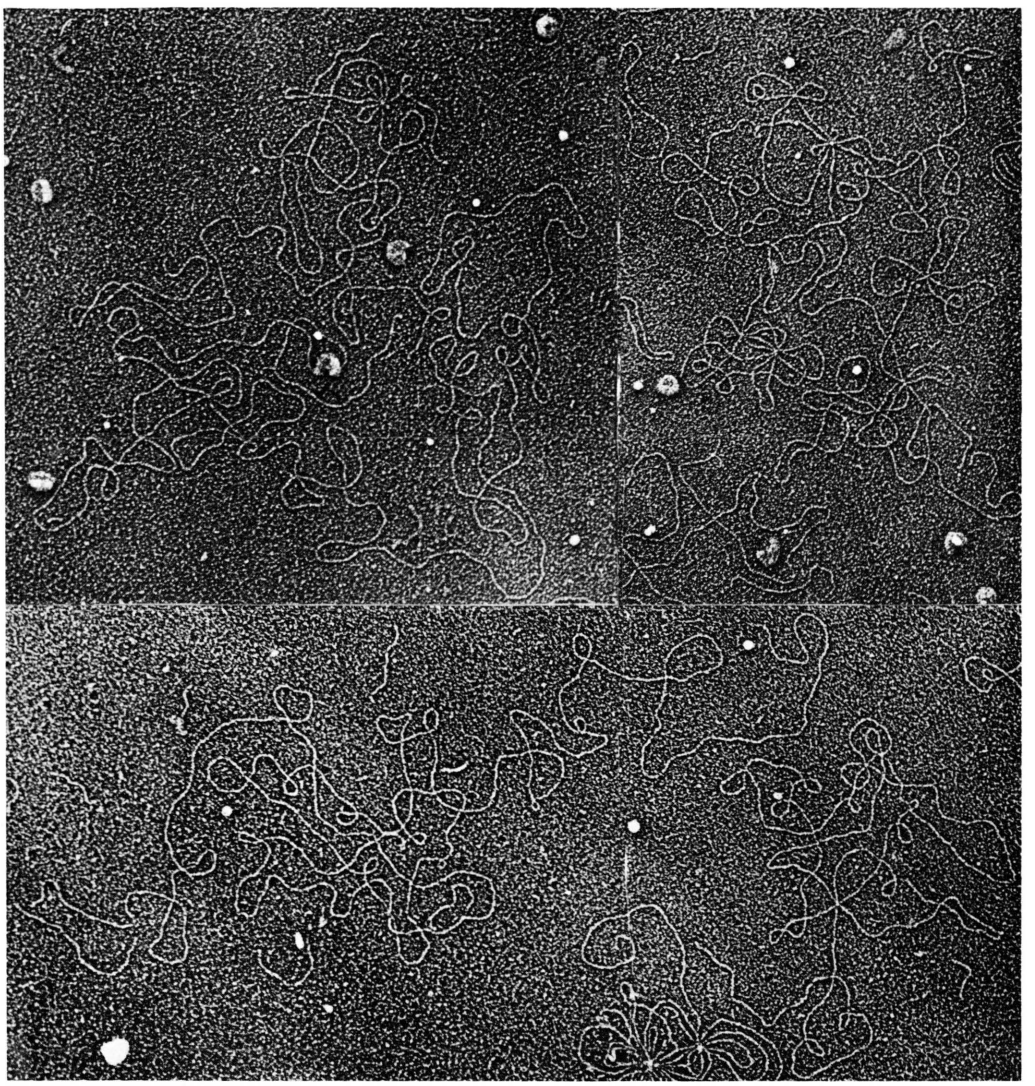

Fig. 2. T-7 DNA molecules bound with PF at P/D=1 and prepared by the protein monolayer technique with triple-distilled water hypophase containing PF at the same concentration as in the complex. The molecules have lost the circularity of the loops but greater smoothness of contour and appear to be more closely packed than the native, dye-free DNA in Fig. 1. X 40,500.

files of the native DNA, obtained by viscometric and spectrophotometric measurements under identical molarities of the solvent in the range of 0.002 M to 0.3 M Na^+. In the first case there was a gradual decrease in the ratio of specific viscosities at temperature T to that at 35 °C, $(\eta_{sp})_T/(\eta_{sp})_{35}$, and in the second case a gradual increase in hyperchromicity when the temperature was raised above a certain limit. In both the cases, within the molarity range of 0.02 M to 0.3 M, the curves became sharper as the ionic strength was increased, showing a greater cooperativity. There was little difference between the curves at 0.2 M and 0.3 M, indicating a levelling off effect in the neighbourhood of this molarity.

The viscometric and the spectrophotometric results for molarities between 0.02 M to 0.3 M will be described first. The melting temperatures deduced from these curves are shown in Table I. It will be

Table I. Melting temperatures (T_m) of native DNA at different molarities obtained from viscometric and spectrophotometric profiles.

Molarity of the solvent [M]	T_m [°C] (Viscometric	T_m [°C] (Spectrophotometric)
0.002	—	52.0
0.005	—	59.5
0.020	69.0	70.0
0.058	78.0	79.0
0.100	85.5	85.0
0.200	88.5	88.0
0.300	89.0	89.0

seen that the melting temperatures obtained by the two independent methods were almost identical. Thus the lowering of viscosity due to the overall decrease in the hydrodynamic volume of the macromolecule, corresponds with the increase in hyperchromicity due to the destruction of regular base stacking. This result, therefore, shows that at high ionic strengths the viscosity measurement can be used to determine the melting temperatures just as the conventional method of the measurement of hyperchromicity.

However, there were very marked differences between the viscometric and the spectrophotometric melting profiles at the molarities of 0.002 M and 0.005 M Na^+. The spectrophotometric profiles had a single ascending region from which the melting temperatures of 52 °C (at 0.002 M Na^+) and 59.5 °C (at 0.005 M Na^+) could be deduced. The temperatures for complete strand separation were 65 °C and 70 °C respectively.

The viscometric profiles in these two solvents showed two descending regions separated by a plateau. At the lowest ionic strength the viscosity ratio initially decreased at a faster rate than that of the latter but the plateaus in the two curves appeared at temperatures of 50 °C and 63 °C, the midpoints of these plateaus being approximately at 64 °C and 73 °C respectively. The viscosity ratios in the two plateaus, within the limit of the experimental error, were almost identical. If the intermediate plateaus in these profiles are regarded as the end of the helix-coil transition, one can deduce the melting temperatures at the two ionic strengths which are much lower than those deduced from the spectrophotometric melting curves. Comparing with the spectrophotometric data given above, it appears that the temperatures at the begining and at the middle of the viscometric plateaus can be used approximately as the substitute for the melting temperature and the temperature for complete strand separation.

In the high ionic strength solvents, the appearance of the single-stranded loops in the native structure, with rising temperature, make the double helix more compact [14–18]. This causes the gradual reduction in the specific viscosity in the molarity range of 0.02 M to 0.3 M which was very marked on the completion of the helix-coil transition. However, at 0.002 M and 0.005 M, the separated single strands retain considerable high viscosity [2]. Thus, at low ionic strengths, one is faced with the opposite tendency of the increase in viscosity due to the extended single-stranded zones in the native structure. This not only reduces the gradual fall in viscosity but also diminishes the total reduction in specific viscosity even on complete strand separation compared to that observed in a higher ionic strength solvents. This might also explain why the plateau in 0.002 M solvent appears earlier than in 0.005 M solvent and is very close to the latter.

At the end of the plateaus, a gradual fall in viscosity occurred which continued parallel at the two ionic strengths up to about 100 °C. It was also observed that in the case of the first transition, the original viscosity value was partially restored on annealing the heated samples at room temperature, while the second transition was completely irreversible in nature. This possibly represents the degra-

dation of separated strands due to the prolonged heating at low ionic strengths.

The appearance of the intermediate plateau is thus a specific feature of viscometric measurements on DNA melting only at low ionic strength. At higher ionic strengths this effect is absent. It has been observed that this effect is not caused by any change of pH with temperature at low buffer concentration. The increased cooperativity of the spectrophotometric melting curves with the increase of ionic strength has been interpreted by many workers in terms of different heterogeneity parameters [19–21]. The apparent decreased cooperativity of viscometric profiles with the lowering of ionic strength includes, besides these factors, the disproportionate decrease of specific viscosity due to the increased extension of the single-stranded regions.

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