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DNA-POLYCATION INTERACTIONS: UREA DEPENDANCE

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ABSTRACT

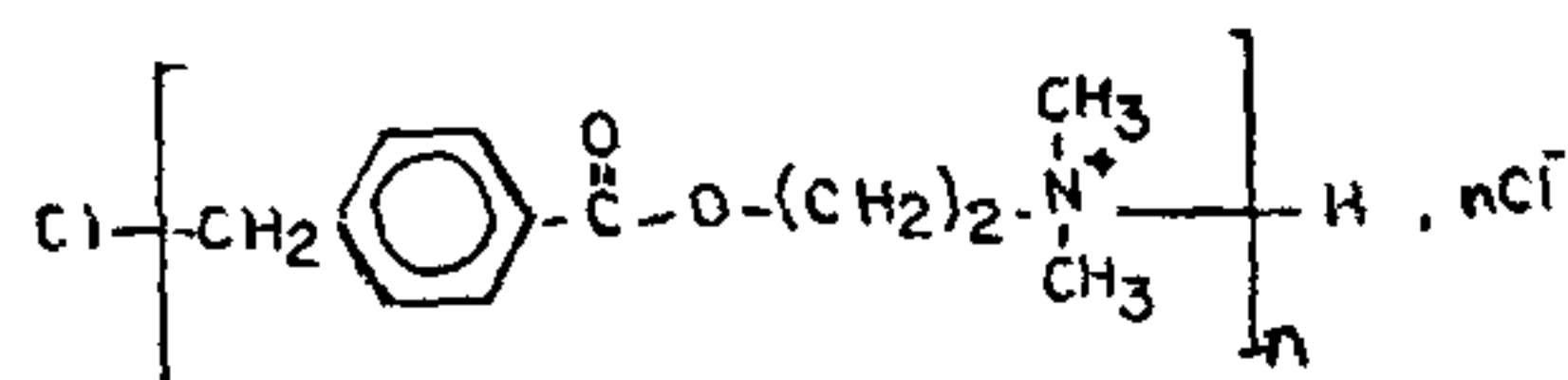
Thermal denaturation of DNA in the presence of novel polycations has been studied at varying concentrations of sodium chloride and urea. T_m values of DNA-polycation complexes decreased linearly with increasing urea concentrations. It is proposed that urea is responsible for destroying intermolecular hydrogen bonds and hydrophobic interactions which may be involved in the maintenance of tertiary structure of DNA-polycation complex. Both electrostatic and hydrophobic effects probably influence the stability of the DNA-polycation complex.

INTRODUCTION

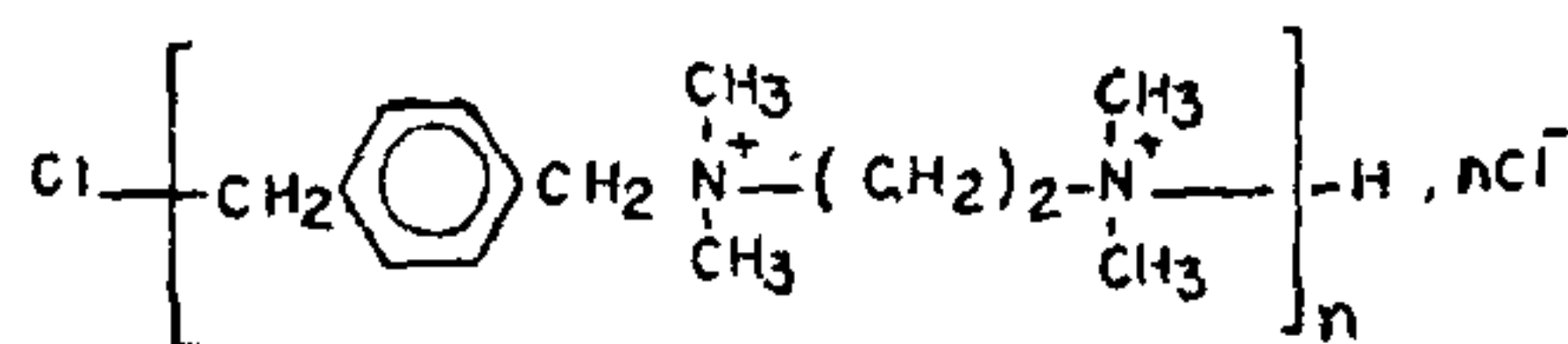
It is well known that various types of polycations interact strongly with nucleic acids which are polyanions¹⁻¹⁰. The effects of urea on the secondary structure of nucleic acids have been extensively investigated^{11,12}. The effects of high concentration of urea on the macromolecular structure and interaction are well known. These arise from competitive hydrogen bonding and disruption of hydrophobic interactions¹³. Such effects frequently show co-operativity and do not arise below critical concentrations. However with small molecules whose residues may be incorporated into macromolecules, the effects may be detected at low concentrations of urea or urea derivatives and follow linear or monophasic functions to higher concentrations¹⁴. Thermal denaturation studies have been utilised to probe the interactions of basic proteins and other polycations with DNA¹⁵. In this paper we report our investigations on the melting behaviour of polycation-DNA complex in different concentrations of urea.

METHODS AND MATERIALS

DNA was purchased from Sigma Chemical Company (USA) as its sodium salt (sodium deoxynucleotide of calf thymus DNA highly polymerised). Sodium deoxynucleate (1 mg/ml) was added to SSC buffer (sodium chloride 0.01 M and sodium citrate 0.001 M pH 7) over a few drops of carbon tetrachloride. The



POLYCATION I $n = 35 \pm 5$



POLYCATION II $n = 10$

exact amount of DNA was estimated spectrophotometrically assuming $\epsilon = 6.6 \times 10^3 \text{ cm}^{-1} \text{ M}^{-1}$ at 260 nm. The A_{260}/A_{230} ratio of this solution was 2.3. The following polycations were synthesised according to the procedure of Rembaum and coworkers^{16,17}.

Stock solutions were prepared in the following concentrations: DNA (1 mg/ml) in sodium chloride 0.01 M and sodium citrate 0.001 M pH 7, polycation (0.01 M), sodium chloride (1 M) and urea (10 M). The reaction mixture contained the following order: DNA (7.5×10^{-5} in DNA phosphate), sodium chloride, EDTA (0.001 M), polycation and urea. The total volume of the mixture was made up to 4 ml by diluting with an appropriate volume of SSC buffer. The polycation and DNA ratio is represented by r . Before determining melting temperature (T_m) the A_{260} absorbance of the

mixture was measured using a Gilford spectrophotometer model 240. Thermal denaturation studies were carried out in microcuvettes using a Gilford spectrophotometer equipped with thermoprogrammer model 2527. The samples were heated from room temperature to 100°C at a rate of 1°C/min. No correction for thermal expansion was made to the absorbance readings in routine experiments. The derivative melting profile was obtained by

$$\frac{d\epsilon_{260}(t)}{dt} = \frac{\epsilon_{260}(t+1) - \epsilon_{260}(t-1)}{(t+1) - (t-1)}$$

RESULTS AND DISCUSSION

The effect of urea (0.5 to 7M) on T_m of DNA-polycation I ($r=0.461$) in sodium chloride (0.1 M and 0.15 M) depicted in figure 1 shows that there is gradual decrease in T_m with increase in urea concentration. Variation of T_m of DNA-polycation I ($r=0.231$) in sodium chloride (0.05 M, 0.15 M) with variation of urea concentration is depicted in figure 2. The effect of

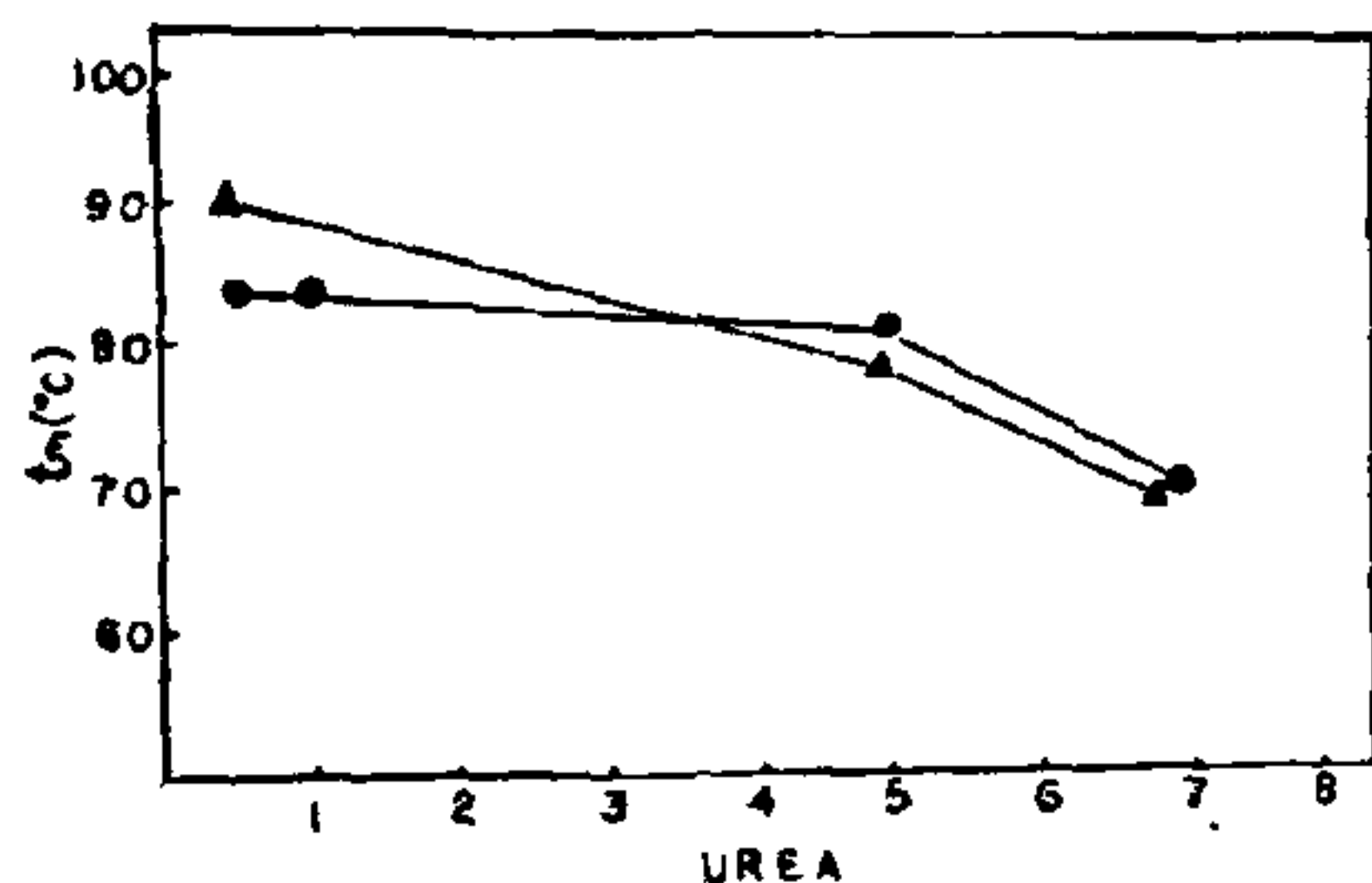


Figure 1. Variation of T_m of DNA-polycation I ($r=0.461$) with variation of urea concentration. Closed circle represents sodium chloride (0.15 M) and closed triangle for sodium chloride (0.01 M)

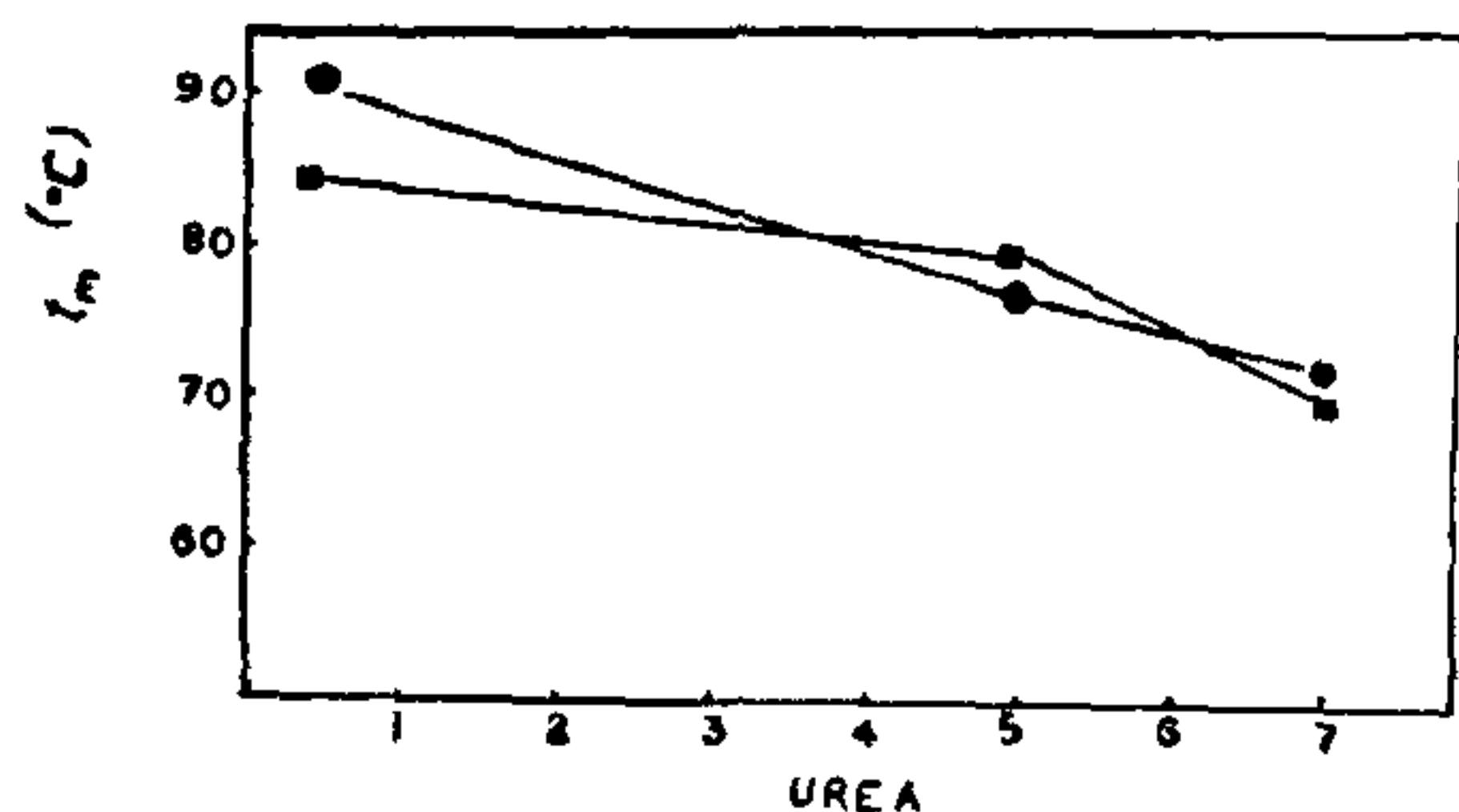


Figure 2. Variation of T_m of DNA-polycation II ($r=0.231$) with variation of urea concentration. Closed circle represents sodium chloride (0.15 M) and closed square for sodium chloride (0.05 M)

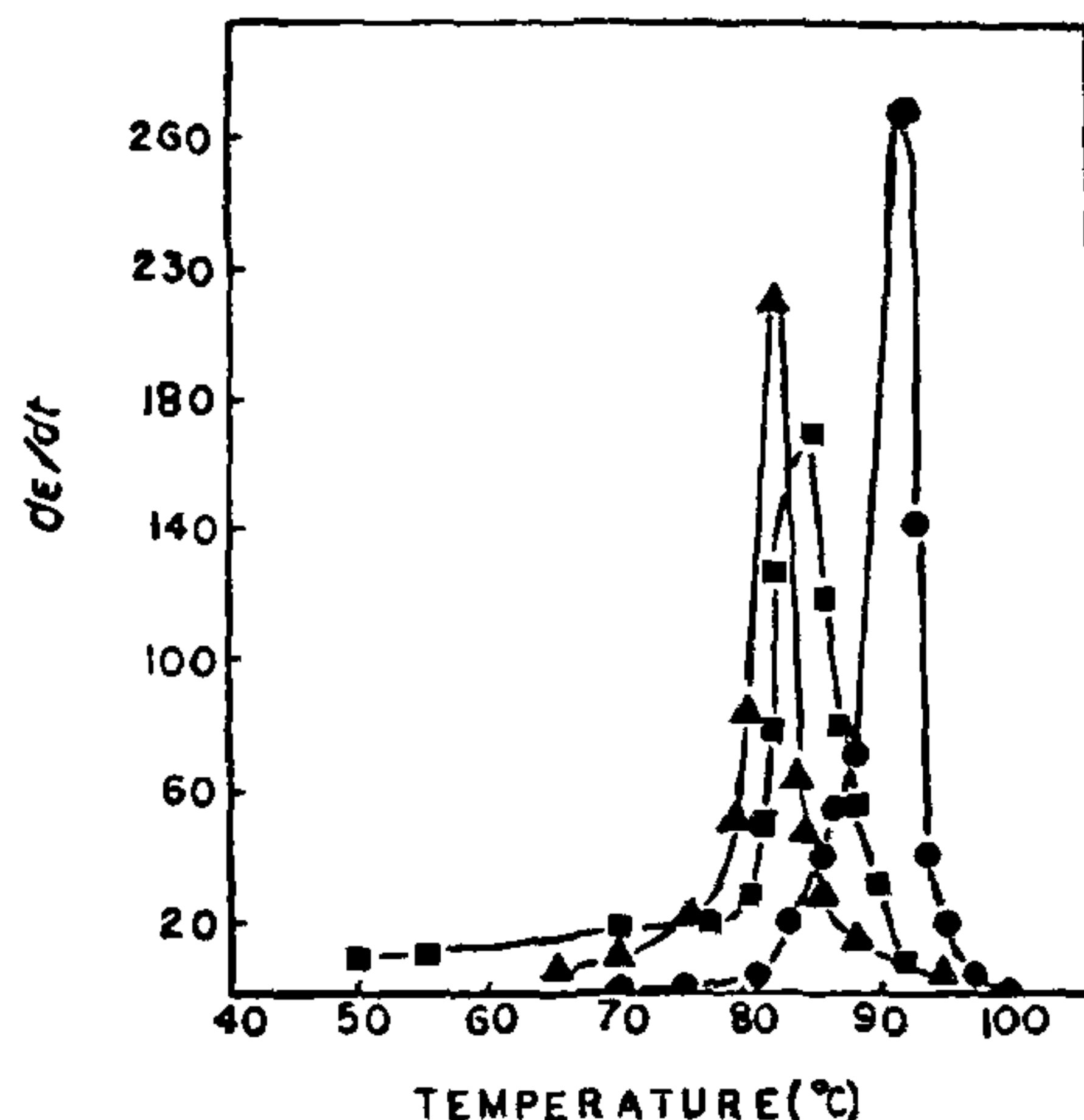


Figure 3. The derivative melting profile of DNA-polycation II ($r=0.461$) in 0.15 M NaCl in the presence of various concentration of urea. Closed triangle represents 7 M urea, closed square for 5 M urea and closed circle for 0.5 M urea.

variation T_m of DNA-polycation-II ($r=0.461$) in 0.15 M NaCl in presence of different concentration of urea is shown in figure 3. The melting band at 70°C represents the melting of the free base pairs of DNA not bound by polycation. The base pairs of DNA bound by polycation ($r=0.461$) melt at 91°C in 0.5 M sodium chloride. The observed increase in the value indicates the stabilization of the helical component of DNA in the complex against thermal denaturation. Similar to polycation number of simple cationic compounds, spermine, spermidine, diamines, polylysine and polyornithine etc have been shown to stabilise native DNA against thermal denaturation presumably by preferential binding to the native DNA helix¹⁸. The base pairs of DNA bound by polycation II ($r=0.461$) melt at 80°C, 85°C and 97°C in 0.15 M sodium chloride in 7 M, 5 M and 0.5 M urea respectively (figure 3). The effect of hydrophobic interactions of DNA-polycation complex is also observed in the melting temperature studies. Urea (7 M) caused the DNA-polycation II ($r=0.231$) in sodium chloride (0.15 M) and DNA-polycation II ($r=0.461$) in sodium chloride (0.1 M and 0.15 M) to be completely restored to the T_m of DNA alone ($T_m=70^\circ\text{C}$). Urea functions by destroying intermolecular hydrogen bonds and hydrophobic interactions which may be involved in the maintenance of tertiary structure of DNA-polycation complex. Thus our results suggest that DNA-polycation complex involves hydrophobic interactions. Electro-

tatic interactions influence the stability of DNA-polycation complex². Previous reports of thermal denaturation of DNA-polycation in the presence of 5 M urea restored T_m of free DNA region¹⁹. The addition of 5 M urea to complex resulted in elimination of the extrinsic band without affecting the CD. Therefore hydrophobic interactions influence the stability of DNA-polycation complex. Herskovites and Bowen¹⁴ have found close correlation between the solubility parameters, relative hydrophobicity and effectiveness of urea and amides on DNA as well as relative importance of van der Waal's forces. Frishman *et al*²⁰ have shown that urea functions as structure breakers even extremely low concentrations which arise from their competitive ability for hydrogen bonding. Aslanyan *et al*²¹ have studied the effect of urea on calf thymus DNA and phase T₂ by CD and thermal denaturation. The values of DNA decreased linearly with increasing urea concentration. They concluded that urea destabilises A-T and G-C base pairs to different extent. Effect of urea on the conformation of DNA double helix was studied by Aslanyan and Babayan²². They concluded that as urea has not been shown to bind directly to DNA the observed effects are probably due to the disruption of the hydration shell of the DNA by urea. The helical secondary structure of DNA was destabilised by an increasing concentration of urea²³. Interaction of DNA-polycation II ($r=0.461$) in 0.1M NaCl/citrate in the presence of urea was carried out by Mulimani *et al*.⁶ and it resulted in the conversion of essentially biphasic curve at 0.4-1M urea with no diminution of T_m .

The phosphate groups impart greatest negative charge density in the minor groove²⁴. Therefore the interaction of polycation is likely to be in this region. Our results suggest that DNA-polycation interactions involve hydrophobic forces in addition to electrostatic interactions. Further studies are in progress to understand the mechanism of urea on DNA-polycation complex in different concentrations of sodium chloride.

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