

TEMPERATURE DEPENDENT Z→B TRANSITIONS IN POLY(dG-dC)

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ABSTRACT

The effect of temperature on the Z-form of poly(dG-dC) in a medium of low dielectric constant has been studied. A novel temperature-dependent reversible $Z \rightleftharpoons B$ transition is observed in 60% (v/v) alcohol. The mid-point of transition (T_m) is found to be lowered from 65° C to 40° C when an extra 1.5 mM NaCl was added to the solution having a sodium:phosphate ratio of 1:1. Analysis of the temperature dependent Z→B transitions both in the presence and absence of 1.5 mM NaCl reveals a positive change in enthalpy.

INTRODUCTION

THE discovery of left-handed Z-DNA in alternating purine-pyrimidine sequence¹ has developed a renewed interest in the understanding of polymorphism of DNA and its biological implications. It has been observed that poly(dG-dC) or cloned d(CG)_n sequence in supercoiled DNA can undergo B→Z transition under the specific influence of various cations^{2,3} or with change in solvent dielectric constant⁴. Chemical modifications like methylation of cytosine residues etc also favour a B→Z transition⁵. Several workers have suggested that reversible B↔Z transition may act as a regulatory control in biological processes like replication (Brahmachari, S. K., Parnaik, V. and Das, M. R., unpublished work), transcription⁶ etc. In spite of the vast amount of studies that have been carried out on the B→Z transition, the exact nature of its mechanism is not yet known.

Poly(dG-dC) in NaCl solution is known to undergo a reversible B↔Z transition² which is temperature independent ($\Delta H=0$). On the contrary, poly(dG-dC) in 0.3 mM MgCl₂-20% aqueous ethanol which gives a circular dichroic (CD) spectrum like that of B-DNA spectrum, undergoes a transition to Z*-form⁶ at 45° C. Even 4M aqueous LiCl induces a transition to Z-form⁷ at 67° C. All the studies reported so far are on B→Z transition which are all well characterized by several spectroscopic techniques⁸⁻¹⁰. Here for the first time we report the existence of a temperature-dependent ($\Delta H \neq 0$) reversible Z→B transition of poly(dG-dC) in a medium of low dielectric constant (aqueous alcohol).

A very recent report on the temperature-dependent ($\Delta H \neq 0$) R-L transition of poly(d(A-m⁵C).d(G-T))¹¹ has come to our notice when this manuscript was under preparation.

MATERIALS AND METHODS

Poly(dG-dC) (sodium salt) (Lot No. 217910) was

purchased from PL Biochemicals, Wisconsin, USA and was used without further purification. Sodium content of the poly(dG-dC) was estimated (flame photometrically) to be 3 mM in 1 mg/ml of the aqueous stock solution which is equivalent to a sodium to phosphate ratio of 1:1. The solution of A₂₅₈ (Absorbance at 258nm) ≈ 7 was diluted with absolute alcohol to get the desired alcohol concentration.

Circular dichroism (CD) measurements were carried out using a JASCO J20 automatic recording spectropolarimeter attached with MLS (GDR) constant temperature circulatory bath. Temperature of the cell holder was directly monitored. Constant temperature of the sample was obtained by prolonged equilibration. The end point of the equilibration was determined by checking the variation of θ at 290 nm with respect to time. A 2 mm cell was used for all the recordings. $[\theta]$ was calculated using the formula

$$[\theta] = \frac{\theta_{\text{deg}} \times 330}{l \times c}$$

$[\theta]$ = mean residual ellipticity

l = pathlength in decimeters

c = concentration in g/100 ml

Concentration of the polymer was calculated from A₂₅₈ by assuming $\epsilon = 7100$, where ϵ is the molar absorptivity¹².

Experiments were also repeated after layering liquid paraffin on top of the solution and sealing the cell cap with teflon tape. Spectra reported are the average of several scans. Experiments were carried out with freshly prepared stock solutions.

RESULTS

CD spectrum of B-form of Poly(dG-dC) in water at 27° C is shown in figure 1. In the presence of 60% (v/v) alcohol at 27° C poly(dG-dC) exhibits a Z-DNA spectrum with a strong negative band at ~290 nm (figure 1). On raising the temperature to 71° C a characteristic

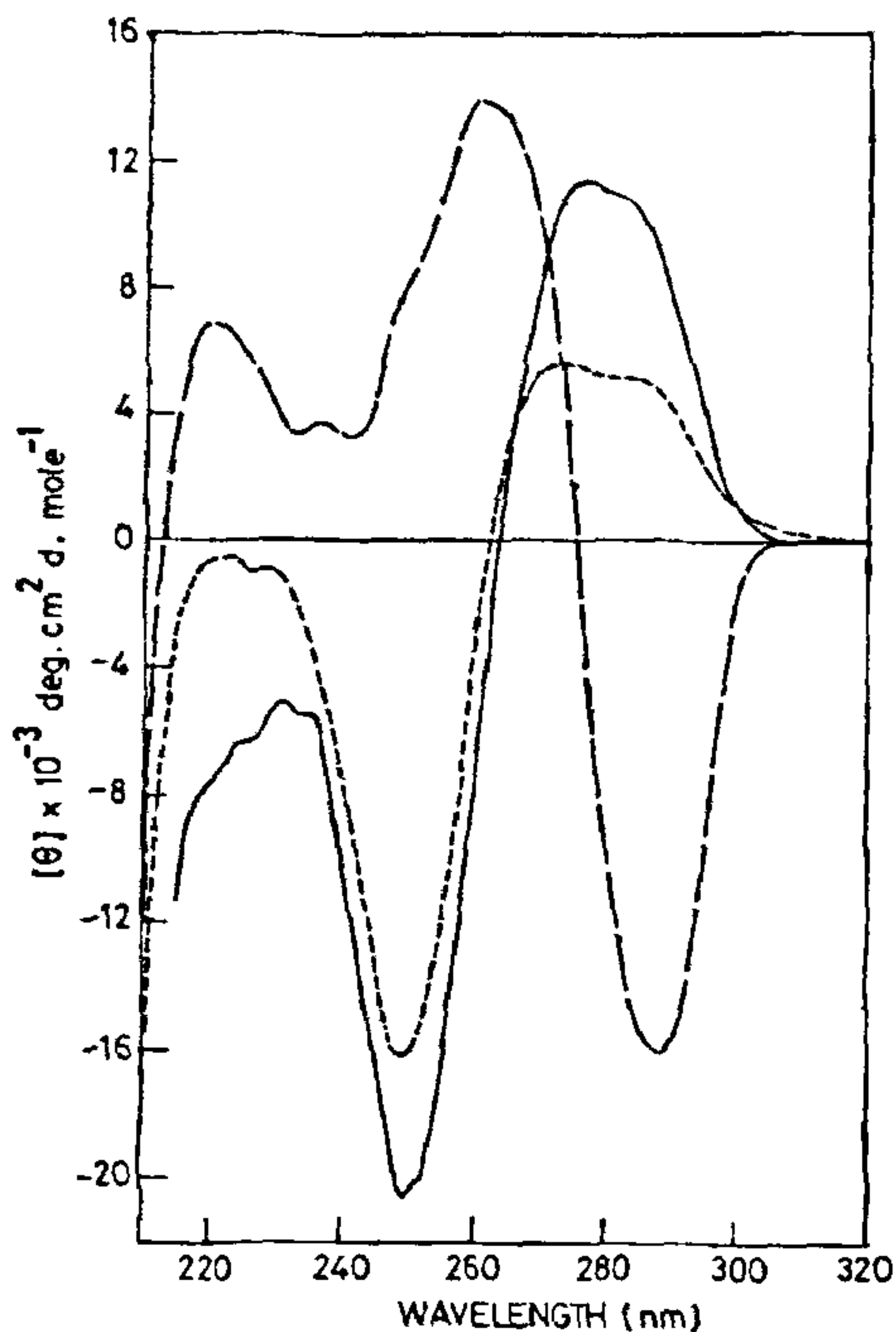


Figure 1. CD spectra of poly(*dG-dC*): in water at 27° C (---); in 60% alcohol at 27° C (— —) and at 71° C (—).

B-DNA like CD spectrum was obtained (figure 1). On slow cooling to room temperature once again a *Z*-DNA spectrum was obtained thereby indicating that the conformational change was not due to the loss of alcohol by evaporation.

When the concentration of Na⁺ was increased by an additional 1.5 mM (Sodium: phosphate ≈ 2.5) a complete transition from *Z*-form to *B*-form was achieved at 47° C (figure 2). Further addition of salt up to 50 mM, at 27° C showed an exponential decrease of the 290 nm negative CD band.

Figure 3 shows that the temperature induced *Z* → *B* transition both in the absence and in the presence of NaCl, is highly co-operative and complete transition takes place within a temperature range of 10° C. The transition is completely reversible on lowering the temperature. The midpoints of these transitions (*T*_{1/2}) are 64.5° ± 0.5° C and 39.5° ± 0.5° C respectively (figure 3). Analysis of this temperature dependent equi-

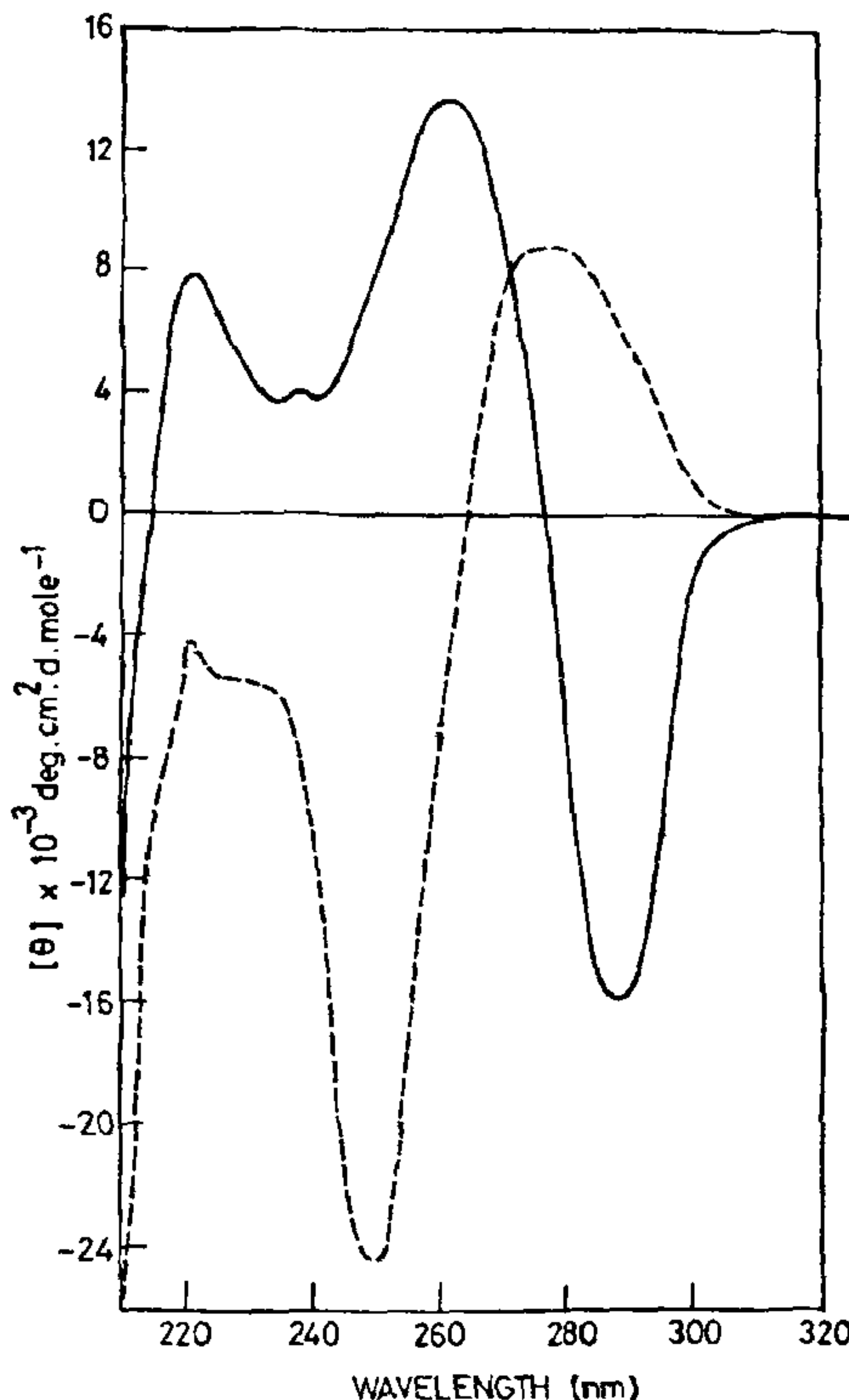


Figure 2. CD spectra of poly(*dG-dC*) in 60% ethanol containing an additional 1.5 mM NaCl at 27° C (—), at 47° C (---).

brium constant for *Z* ⇌ *B* transition using Van't Hoff equation yields an enthalpy change of 0.95 MJ/mol and 0.54 MJ/mole in the absence and presence of the additional NaCl respectively. The change in entropy is 2.8 kJ/mole/° K for the former and 1.7 kJ/mole/° K for the latter.

We have observed that poly(*dG-dC*) in 30% (v/v) alcohol which exhibits a *B*-DNA like spectrum with or without 1.5 mM NaCl does not undergo a *B* → *Z* transition as a function of temperature.

DISCUSSION

For the first time we show that in a medium of low dielectric constant (aqueous alcohol) there exists a temperature-dependent *Z* → *B* transition which is

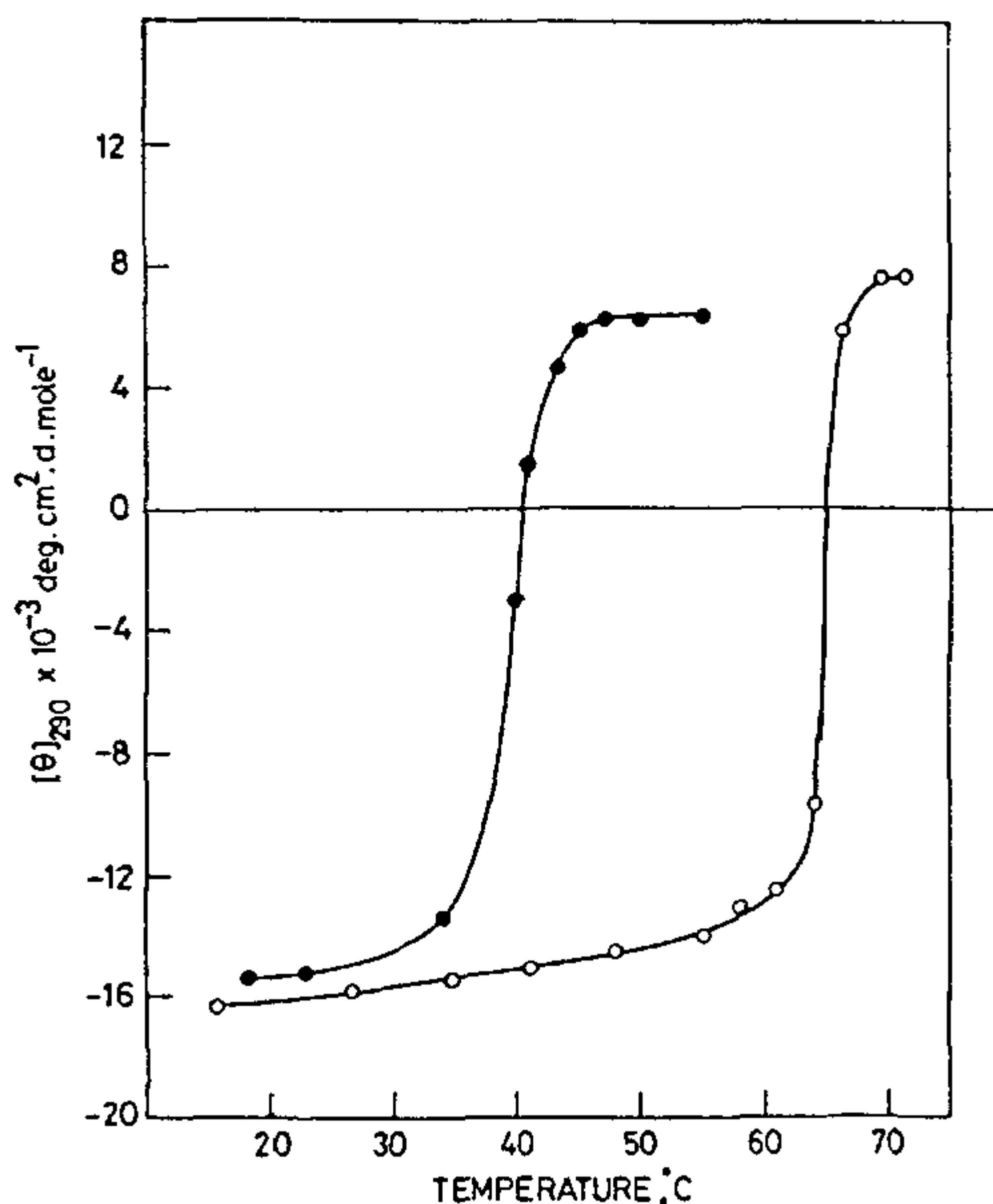


Figure 3. Temperature dependence of Z-form of poly(*dG-dC*) in 60% alcohol: without NaCl (—o—), with an additional 1.5 mM NaCl (—●—).

highly co-operative. Moreover millimolar quantity (1.5 mM) of NaCl present in the medium brought down the transition temperature significantly. The $Z \rightarrow B$ transition observed by us is not isoenthalpic. This is in contrast to the reported temperature-independent $B \rightarrow Z$ transition² where $\Delta H = 0$.

It is believed that $B \rightarrow Z$ transition is brought about by the lowering of the water activity by reagents like salt and alcohol. Increase in temperature is also known to change water activity. However, contrary to the observation that the increase in temperature induces a $B \rightarrow Z^*$ transition in poly(*dG-dC*) in the presence of 0.3 mM Mg^{++} and 20% alcohol, our data show that a temperature dependent reverse transition (*i.e.* $Z \rightarrow B$) takes place in the presence of 1.5 mM NaCl in 60% alcohol. This suggests that a decrease in water activity may not be the major driving force for the salt/alcohol induced $B \rightarrow Z$ transition.

It is known that alkali metal ions, when present in water, modify the water structure significantly¹³. The ability of these ions in making the water structure falls off in the order $Li > Cs > Rb > K > Na$. Our observation, that 1.5 mM NaCl decreased the transition temperature (T_i) considerably, is compatible with the water structure making property of Na^+ . Thus, we would expect that the alkali metal ions succeeding Na

as above should lower the T_i further. As a preliminary indication we have observed that 1.5 mM CsCl present in 60% (v/v) alcohol reverts the Z-form to B-form at 27° C itself (manuscript in preparation). Interestingly, the mid-point of the salt induced $B \rightarrow Z$ transition in poly(*dG-dC*) also falls off from Cs^+ to Na^+ . Li^+ does not at all induce a $B \rightarrow Z$ transition at room temperature¹⁴.

It may be noted that the $Z \rightarrow B$ transition takes place with an increase in temperature by a few degrees. Sasisekharan and Brahmachari¹⁵ have reported that very mild conditions like mere equilibration at 40% RH humidity, can induce a $B \rightarrow Z$ transition in poly(*dG-dC*) fibre. They have argued that the handedness of the duplex in both the forms of DNA should be the same since this transition takes place in the solid state. Our observation that a small change in temperature induces $Z \rightarrow B$ transition lends support to their conclusion that the handedness of the duplex in both B-form and Z-form should be the same *i.e.* left handed.

Recently it has been suggested by Larsen and Weintraub¹⁶ that alteration in secondary structure of DNA caused by an embryonic Na^+ gradient may be responsible for the differential S1-nuclease sensitivity observed for the same DNA sequences. Thus, the shift in transition temperature with increasing salt concentration at the millimolar level could open up the possibility of a $Z \rightleftharpoons B$ equilibrium that is controlled by a salt gradient at ambient temperature. However, further work in this regard is necessary and has been initiated in order to arrive at the actual implication of the present observation.

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SYNTHESIS AND ANTIMICROBIAL EVALUATION OF SOME NEW *N*-SUBSTITUTED BENZYLIDENE-6,8-SUBSTITUTED-2-ETHYL-QUINAZOLIN-4-OXY-ACETIC ACID HYDRAZIDES

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INTRODUCTION

4(3H) Quinazoline-4-ones, have been reported to possess various biological activities¹⁻³ and exist as 4-ol-[II] tautomers in the presence of anhydrous potassium carbonate and dry acetone⁴. Although compounds synthesised from tautomeric form have not been reported so far to be biologically active, it was considered of interest to synthesise such compounds as having both the tautomeric 6,8-substituted-2-ethyl-quinazolin-4-oxy-acetic acid moiety as well as $-N=C <$ group, the presence of which in the compounds, as is well known^{5,6}, makes them highly biologically active. In this communication, the synthesis and the antibacterial and antifungal evaluation of the title compounds are described.

Reaction of propionic anhydride with 3,5-substituted anthranilic acids yielded 6,8-substituted-2-ethyl benzoxazin-4-ones [I] which were reacted with formamide to yield 6,8-substituted-2-ethyl-quinazolin-4-one [II]. The latter in the presence of anhyd. K_2CO_3 , dry acetone and chloroethyl-acetate, yielded 6,8-substituted-2-ethyl-quinazolin-4-oxy-ethyl-acetate [III], which on reaction with hydrazine hydrate in alcohol gave the corresponding hydrazide [IV]. *N*-benzylidene-derivatives from IV were obtained on condensation with different aromatic aldehydes and substituted acetophenones. The structure of the compounds was established on the basis of their elemental analysis and spectral data.

EXPERIMENTAL

The melting points were determined using sulphuric acid bath in open capillary tubes and are uncorrected. IR spectra in KBr were recorded on a Perkin Elmer spectrophotometer (ν max in cm^{-1}) and PMR spectra in DMSO d_6 on a Varian A-90D instrument using TMS as internal standard (chemical shift in δ ppm). The purity of each compound was checked on TLC.

6,8-Substituted-2-ethyl-benzoxazin-4-ones [I]

A mixture of substituted anthranilic acid (0.1 mol) and propionic anhydride (0.2 mol) was refluxed for 4 hr. The reaction mixture was poured into ice cold water and the solid that separated was recrystallised from hot water and methanol. Yield 85-90% IA ($R = R' = H$) mp 120°, IB ($R = Br, R' = H$) mp 180°, IC ($R' = R = Br$) mp 125°. Their IR spectra showed bands around 2950 cm^{-1} , 1660 cm^{-1} characteristic of $(-C-H)$ and $(-C=O)$ groups respectively.

6,8-Substituted-2-ethyl-quinazolin-4-ones [II]

The compound I (0.1 mol) and formamide (0.15 mol) were heated at 170° for 4 hr. On cooling with ice, a solid, separated which was recrystallised from hot water. Yield 70-75%. Following compounds were thus prepared:— IIA ($R = R' = H$) mp 212°, IIB ($R = Br, R' = H$) mp 180°, IIC ($R = R' = Br$) mp 270°. I.R. spectra showed bands around 3300, 2900 and 1670 cm^{-1} characteristic of (NH), (CH) and (CO) groups respectively.