Premelting Unwinding of the Deoxyribonucleic Acid Duplex by Aqueous Magnesium Perchlorate†

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ABSTRACT: The average rotation angle of the native PM-2 DNA duplex is reduced by up to 6% by the addition of the neutral denaturing salt magnesium perchlorate. Such duplex winding alterations may be monitored with high sensitivity by measuring the sedimentation coefficient ratio of closed to nicked DNA as a function of the amount of salt added, since the special topological properties of the closed duplex require that duplex winding changes be balanced by equal and opposite changes in the extent of supercoiling. Experiments performed at temperatures between 5 and 40° indicate that the effects of salt and temperature increases are additive, and that 1.0 N $Mg(ClO_4)_2$ is the equivalent of a 20° rise in temperature. The magnesium perchlorate induced unwinding appears to be largely noncooperative and proceeds at the rate of 0.42° per base pair per equivalent per liter. The duplex unwinding induced by $Mg(ClO_4)_2$ may also be complemented and extended by the intercalating dye ethidium bromide, as demonstrated by the results of the dye–sedimentation velocity titration at 5°. At high levels of $Mg(ClO_4)_2$ the helix–coil transition occurs at all temperatures, and the closed circular PM-2 DNA assumed a highly compact form characterized by a greatly increased sedimentation coefficient. The salt concentration necessary to bring about the transition varies from 2.45 M at 5° to 1.75 M at 40°. Nicked circular PM-2 DNA is precipitated by Mg^{2+} upon the occurrence of strand separation. The denatured, single-stranded species appear to be resolubilized, however, in the high perchlorate and temperature regions.

Deoxyribonucleic acid, which is the primary unit for the storage of genetic information, is stable as a native structure in which the bases are removed from direct contact with both the solvent and with other external molecules. The exposure of the nucleotides for the decoding of this information requires localized opening of the duplex at temperatures which are probably far removed from those required to bring about the cooperative transition to the coiled form. The understanding of the nature and mechanism of such structural changes requires determination of the limits of stability of the duplex under various and controlled environmental conditions.

It has recently become clear that the native DNA secondary structure is subject to a certain amount of winding flexibility at temperatures well below the thermal melting region. Thus, the average duplex rotation angle increases by approximately 0.07° when the solvent is changed from 2 M NaCl to 2 M CsCl (Wang, 1969). A slight unwinding of the helix occurs as the temperature is raised, whereas an increase in the ionic strength from 0.1 to 1.0 M in CsCl causes an increase in the duplex winding of 0.15° per nucleotide pair (Wang, 1969). Wide-angle X-ray scattering experiments have indicated that the turn angle is decreased by 8.5% for DNA in 0.05–0.15 M aqueous salt solution compared to the B form, whereas DNA in the nucleohistone complex has a secondary structure very similar to the B form (Bram, 1971).

The addition of certain neutral salts to aqueous solutions containing DNA also affects its structural stability, as indicated by a concommitant lowering of the midpoint of the thermal helix–coil transition (Hamaguchi and Geiduschek, 1962). The relative effectiveness of such salts as destabilizing agents generally follows the lyotropic or Hofmeister (1888) series (Hamaguchi and Geiduschek, 1962; von Hippel and Schleich, 1969a,b), although no molecular mechanism has yet been generally accepted for the specific mode of action. Robinson and Grant (1966) found that a good correlation exists between the effectiveness of electrolytes as denaturants and their ability to lower the activity coefficients of the bases. This and other lines of inquiry indicate that the net free energy of the transition helix (native) → coil is lowered by the addition of neutral salts.

It has been recently demonstrated (Bauer, 1972) that the addition of sodium perchlorate, a denaturing salt, to aqueous solution brings about a structural transition in DNA at temperatures well below those required to initiate the cooperative helix–coil transition. Native PM-2 DNA, a closed circular double-stranded molecule isolated from mature bacteriophage PM-2 (Espejo and Canelo, 1968, 1969), is unwound by up to 3.9% at 20° as a result of the addition of $NaClO_4$ to a concentration of 7.2 M as monitored by the progressive and characteristic sedimentation coefficient alterations in closed relative to nicked circular PM-2 DNA (Upholt *et al.*, 1971). Such a reduction in the average duplex winding angle is consistent with the observed optical rotatory dispersion (ord) changes attending the addition of denaturing salts such as LiCl (Tunis and Hearst, 1968) and $NaClO_4$ (Hamaguchi and Geiduschek, 1962) to DNA.

The elegant proton magnetic resonance (pmr) experiments of Prestegard and Chan (1969) have shown that certain neutral salts of the lyotropic series bring about conformational changes in the pucker of the furanose ring and in the torsion angle between base and sugar in several uracil derivatives. These experiments indicated that magnesium perchlorate is much more effective in shifting the conformational equilibrium than is the corresponding sodium salt. The experiments reported in the present communication show that $Mg(ClO_4)_2$ is also much more potent than $NaClO_4$ in bringing about a reduction in the average duplex pitch in native PM-2 DNA. The effects of $Mg(ClO_4)_2$ upon the sedimentation co-

† From the Department of Chemistry, University of Colorado, Boulder, Colorado 80302. *Received March 16, 1972.* Supported by Grant GB-19190 from the National Science Foundation. This is Part II in a series on the structure of DNA in denaturing solvents; the first paper is Bauer (1972).

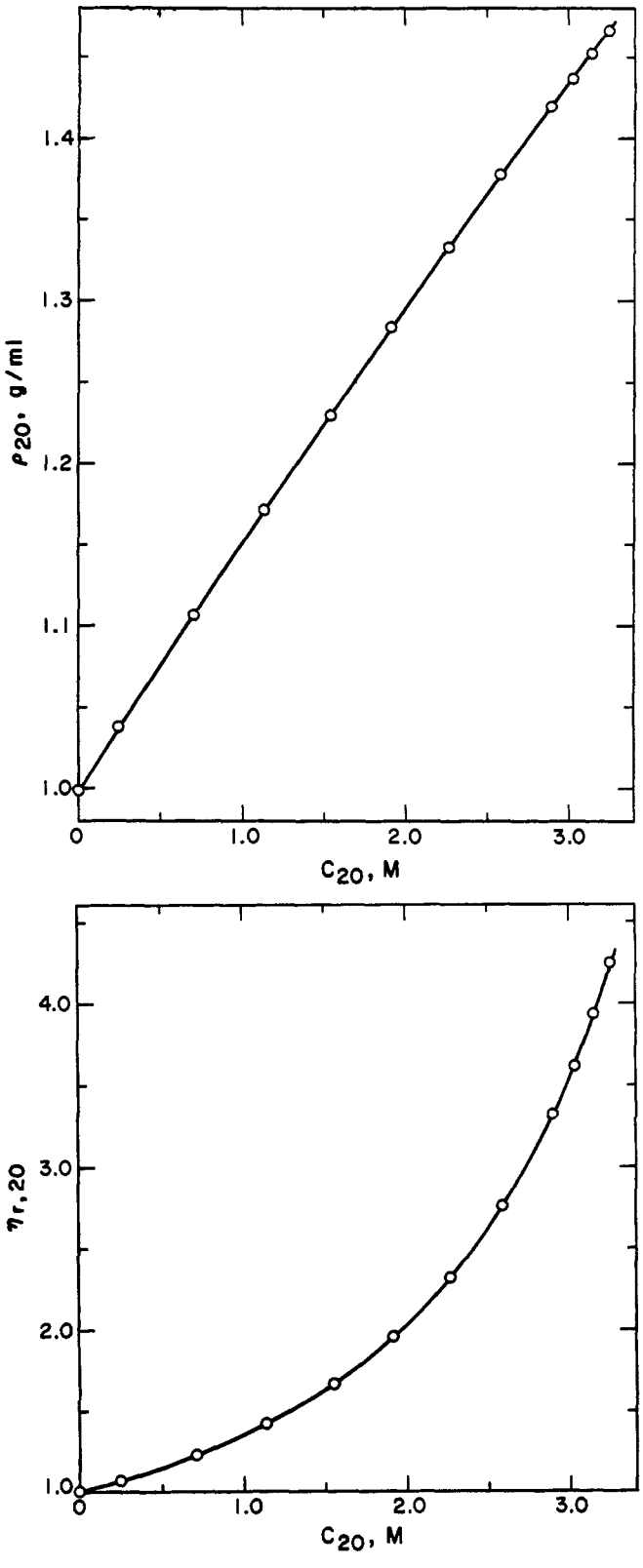

FIGURE 1: Solution parameters of aqueous $Mg(ClO_4)_2$ at 20°. (a, top) Density, ρ_{20}, plotted as a function of C_{20} for concentrations ranging up to saturation at this temperature. (b, bottom) Relative viscosity, $\eta_{r,20}$, as a function of concentration. The solid line represents a plot of fitted eq 3.

efficient of closed PM-2 DNA are dramatic and suggest that the duplex is unwound prior to the onset of the cooperative melting transition by nearly 6% at 20°. The results therefore serve to define the rotational limits of DNA duplex stability under these solution conditions. Sedimentation velocity–salt addition titrations performed at various temperatures indicate that the unwinding effects of increases in temperature and in salt concentration are codirectional.

Experimental Section

Materials. Reagent grade $Mg(ClO_4)_2 \cdot 6H_2O$ was obtained from the G. Fredrick Smith Chemical Co., Columbus, Ohio. The aqueous solutions were slightly opalescent and were clarified by filtration through a 0.22 μ Millipore filter before use. The resulting solutions, which possess no significant absorbance at 260 nm, were not purified further. Ethidium bromide was obtained from the California Corp. for Biochemical Research, Los Angeles, Calif. PM-2 viral DNA was prepared as described previously (Bauer, 1972). Tris-HCl buffer, used throughout at a concentration of 0.01 M, was prepared from Trizma Base, reagent grade, obtained from the Sigma Chemical Co., St. Louis, Mo. The pH of concentrated $Mg(ClO_4)_2$ solutions was measured with a Sargent general purpose combination electrode after 30-fold dilution with glass-distilled, deionized water.

Analytical Ultracentrifugation. A Beckman Model E analytical ultracentrifuge equipped with electronic speed control, photoelectric scanner and multiplex accessory was used. All experiments were conducted with 12-mm charcoal-filled Epon, type I band-forming centerpieces (Bruner and Vinograd, 1965). Approximately 0.5 μg of DNA was used per band in each experiment, an amount well below the level at which effects of concentration-dependent sedimentation become important. Rotor temperature was controlled with the RTIC unit modified to permit variable heating wattage, as as described by Upholt *et al.* (1971). The rotor temperature was continuously monitored during the experiments with a Monsanto recorder, Model 530 A. The use of black anodized rotors was found to be unnecessary even at 5° provided that the heating wattage is suitably reduced. Rotor speed was monitored with a Hewlett-Packard Eput meter, Model 522 B. Sedimentation coefficients and sedimentation coefficient ratios were calculated with a Nova 1200 computer as described previously (Bauer, 1972).

Magnesium Perchlorate Solution Parameters. Densities and viscosities were measured at 20° in a water bath thermostated to ±0.05°. Densities were determined by weighing aliquots of approximately 25 ml in calibrated Fisher pycnometers. Viscosities were measured with a Cannon capillary viscometer. Flow times ranged from 296 sec with distilled, deionized water to 854 sec with 3.28 M $Mg(ClO_4)_2$. Refractive indices were measured at 25° with a Bausch and Lomb refractometer thermostated with a Haake circulating bath. Stock solutions were prepared by weight (molal scale) and were converted to volume concentrations at 20° (molar scale) with the aid of the measured densities. Figure 1a presents the variations of density with concentration for $Mg(ClO_4)_2$ at 20°, and Figure 1b shows the variation of relative viscosity with concentration under the same conditions. The best least-squares equations relating density (ρ_{20}) and refractive index (n_{25}), concentration in molarity (C_{20}) and refractive index and relative viscosity ($\eta_{r,20}$) and concentration are given by eq 1, 2, and 3, respectively.

$$\rho_{20} = 16.4297 - 31.0253n_{25} + 14.5926n_{25}^2 \quad (1)$$

$$C_{20} = -1181.12 + 2740.07n_{25} - 2155.39n_{25}^2 + 573.556n_{25}^3 \quad (2)$$

$$\eta_{r,20} = 0.998896 + 0.31684C_{20} - 0.11158C_{20}^2 + 0.206658C_{20}^3 - 0.0788435C_{20}^4 + 0.0140015C_{20}^5 \quad (3)$$

Correction of Sedimentation Coefficients to Standard Conditions. The Svedberg correction procedure was used, in which $s^*_{20,w} = s\eta_{r,T}(1 - \bar{v}\rho)_{20,w}/(1 - \bar{v}\rho)_T$. Since the values of η_r and ρ were available at 20° only, the first-order corrections were made using $\rho_{T,c} = \rho_{20,c} + \rho_{T,w} - \rho_{20,w}$; and, for the relative viscosity, $(\eta_r)_{c,T} = (\eta_r)_{c,20} \cdot (\eta_r)_{w,T}$. The partial specific volume of the magnesium salt of DNA was estimated using the additive volume method of Hearst (1962), assuming that one equivalent of Mg^{2+} neutralizes each equilvalent of DNA phosphate. The crystal molar volume per equivalent of Mg differs from that of the corresponding lithium salt by 0.06 cm³/equiv in the case of the bromide and by −0.32 cm³/equiv for the chloride. Using the average value of −0.13 cm³/equiv and following Hearst, it is estimated that $\bar{v}$ = 0.552 ml/g for MgDNA.

Results

Dependence of Sedimentation Coefficients on $Mg(ClO_4)_2$ Concentration and Temperature. The sedimentation coefficients of both closed and nicked circular PM-2 DNAs vary markedly with the neutral salt concentration at all temperatures studied. Figure 2 presents the results of measurements of sedimentation coefficient, s, for both components as a function of C_{20}, the magnesium perchlorate concentration in moles per liter as determined at 20°, for experiments conducted at 5, 20, 30, and 40°. The sedimentation coefficients increase uniformly with temperature, as is to be expected from the temperature dependence of the solution viscosity and density. The monotonic reduction in s_{II}[1] which is clearly evident in each case is likewise to be expected as the solution viscosity and density are increased by the addition of salt. The initial decrease in s_{II} continues in each case until a particular salt concentration is attained, at which point the nicked molecule undergoes a cooperative helix–coil transition and the resulting single strands are precipitated. This latter phenomenon has been previously observed upon the addition of $MgCl_2$ to heat-denatured DNA (Lyons and Kotin, 1964). The value of the critical salt concentration clearly depends upon the temperature, decreasing from 2.4 M at 5° to 1.7 M at 40°.

The sedimentation coefficient of the closed molecule, s_I, varies in a more complex manner over the same salt concentration range. The initial monotonic decrease, similar to that of II, is interrupted by first an increase, then a subsequent decrease in the slope of the curve. This region of the velocity curves is then terminated at each temperature by a transient joining of the s *vs.* C_{20} curves of both I and II. The shapes of both curves remain approximately unchanged as the temperature is raised, except that the initial portions appear to be progressively truncated.

The region of salt concentrations to the right of the junction point is characterized by a rapid increase in s_I to a maximum, followed by a terminal decrease. This behavior is most likely due to the formation of a highly compact closed molecule as a result of a massive loss of ordered secondary structure at high $Mg(ClO_4)_2$ concentrations. The final decrease in s_I with increasing C_{20} arises from the rapid increase in solution viscosity with salt concentration in this region (Figure 1b). Two additional slow components were observed at the highest salt concentrations employed at temperatures between 20 and 40°. These components appeared only at saturation at 20° (Figure 2b) and at progressively lower salt concentrations at 30 and 40° (Figure 2c,d). The identity of these components, which are possibly resolubilized single-stranded linear and circular species, is currently under investigation.

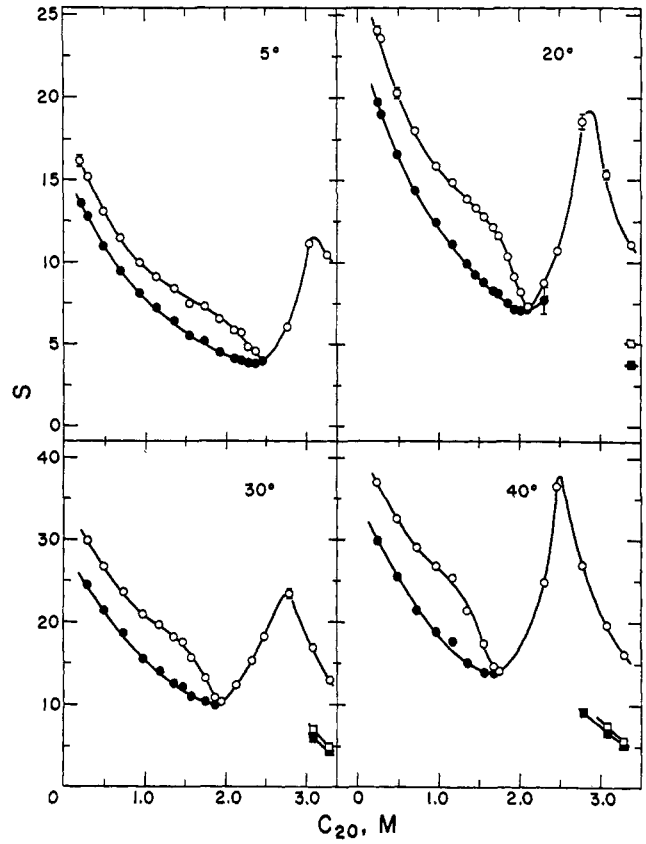

FIGURE 2: The sedimentation coefficient of closed (●) and nicked (○) circular PM-2 DNAs as a function of $Mg(ClO_4)_2$ concentration at 5, 20, 30, and 40°. Points not showing error bars are characterized by standard deviations of less than ±0.05 S. The slower components which appear at high salt concentrations and temperatures might represent single standard circular (□) and linear (■) molecules (see text).

Correction to $s^*_{20,w}$. The procedure for correction to standard conditions of measured sedimentation coefficients for DNA depends upon the nature of the sedimentation solvent and upon the magnitude of the three-component interactions. For NaDNA in aqueous NaCl, for example, the classical Svedberg correction procedure gives consistent results with the use of the value 0.556 ml/g (Hearst, 1962) for the partial specific volume. For CsDNA in CsCl (Bruner and Vinograd, 1965) or MgDNA in $MgSO_4$, (Vijayendram and Vold, 1971), plots of $s \cdot \eta$ *vs.* ρ are linear and the Bruner–Vinograd (1965) correction applies. A similar analysis of the data presented in Figure 2 at 20° revealed that this latter procedure does not result in a straight line for the variation of $(s_{II} \cdot \eta)_{20}$ *vs.* ρ_{20} for MgDNA in $Mg(ClO_4)_2$, although the deviations are small until the region of the cooperative helix–coil transition is approached.

Figure 3 shows the results of the application of the Svedberg correction procedure to both s_I and s_{II} in aqueous $Mg(ClO_4)_2$ at temperatures ranging between 5 and 40°. The correction results in consistent values of $s^*_{20,w}$, the apparent

[1] Abbreviations used are: EB, ethidium bromide; I, closed circular PM-2 DNA; II, nicked circular PM-2 DNA.

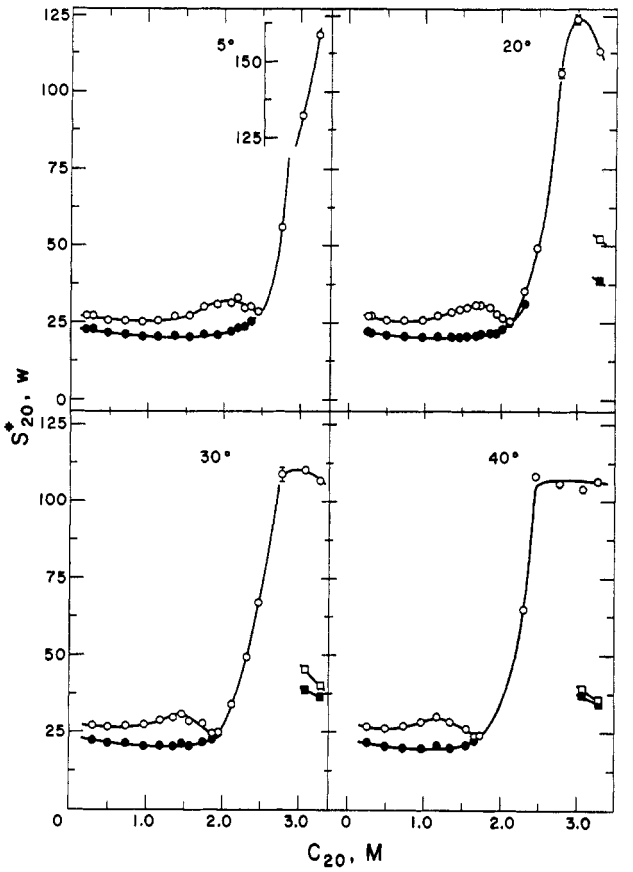

FIGURE 3: Calculated values of $s^*_{20,w}$, the apparent standard sedimentation coefficient, plotted as a function of $Mg(ClO_4)_2$ concentration, C_{20}, for closed (●) and nicked (○) circular PM-2 DNAs at 5, 20, 30, and 40°. The Svedberg correction was used to obtain $s^*_{20,w}$, as described in the text. Other symbols are defined in the legend to Figure 2.

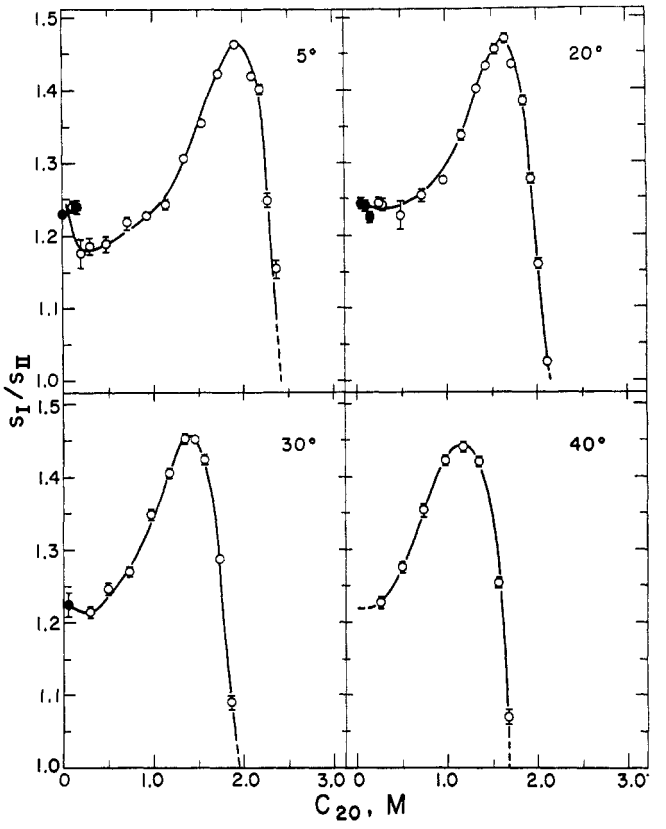

FIGURE 4: The ratio $s_I:s_{II}$ for PM-2 DNA plotted as a function of $Mg(ClO_4)_2$ concentration, C_{20}, for sedimentation data obtained at 5, 20, 30, and 40°. (○) Solvent was aqueous 1.0 M $MgCl_2$ at 5°. (●) D_2O added to 50% by weight of total water for density stability.

standard sedimentation coefficient, for the nicked molecule over salt concentrations ranging up to the region of the critical point and between 5 and 40°. At a value of C_{20} = 0.97 M, for example, the calculated values of $s^*_{20,w,II}$ are 20.25, 20.65, 20.15, and 19.95 S at 5, 20, 30, and 40°, respectively. It is appreciated that several assumptions were made in the course of these calculations (see Experimental Section), but the results are nonetheless useful for comparing the effects of the denaturing solvent upon the frictional coefficients of the various sedimenting species.

Figure 3 shows that the salt concentration required to relax the closed molecule decreases steadily with temperature, while the concentration interval between the first maximum in s_I and the relaxation point remains approximately constant (*cf.* Figure 1). As discussed in greater detail below, the effects of temperature and salt addition are complementary.

The value of $s^*_{20,w}$ calculated for the rapidly sedimenting form, to the right of the critical salt concentration, is much less certain due to the unknown effects of duplex structural alterations upon the effective partial specific volume. The value of approximately 110 S is obtained at both 30 and 40°, indicating that the structure of the fast form is similar under these two conditions. At 20°, the calculated value of $s^*_{20,w}$ rises to 125 S at 3.0 M before dropping to 112 S at 3.3 M; at 5° the high value of 170 S is obtained at saturation in $Mg(ClO_4)_2$ concentration. These results indicate that the structural transition to the fast form is dependent upon both salt concentration and temperature. The transition is essentially complete (as measured by sedimentation) at temperatures greater than 20° and for salt concentrations greater than 3 M, with the effects of salt and temperature being again complementary. At 5°, or at 20° and 3.0 M, an intermediate, possibly stiffer structure is stable. No indication of the precipitation the closed molecule was found under any of the conditions.

Sedimentation Coefficient Ratios in $Mg(ClO_4)$ from 5 to 40°. The effects of aqueous $Mg(ClO_4)_2$ upon the structure of PM-2 DNA are most clearly revealed in Figure 4, in which the ratio $s_I:s_{II}$ is plotted as a function of C_{20} for experiments performed at 5, 20, 30, and 40°. The curves move progressively to lower salt concentrations as the temperature is increased, without large distortions in shape. The curves are similar in appearance to the region to the left of the critical point in the plot of $s_{20,w}$ versus EB binding ratio for PM-2 DNA as obtained by Upholt *et al.* (1971). The complex shapes of these curves and the corresponding appearance of PM-2 DNA I in electron micrograph have been explained as resulting from the successive transition through three different tertiary structural forms (Upholt *et al.*, 1971). These include a native, highly branched superhelix; an increasingly more relaxed but still nearly rigid rod as $|\sigma|$ decreases; and a floppy, essentially random coil in the region of lowest $|\sigma|$.

Ethidium Bromide–Velocity Titration. Figure 5 shows the effects upon s of the addition of ethidium bromide to aqueous 1.92 M $Mg(ClO_4)_2$ at 5°. The curve reveals that only a small region of initial unwinding remains under these conditions and, consequently, that the effects of $Mg(ClO_4)_2$ and EB are codirectional. The appearance of the curve in Figure 5 is

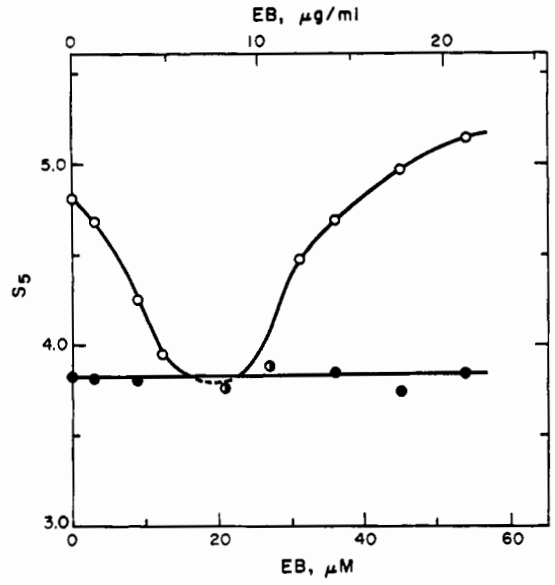

FIGURE 5: The sedimentation velocity–EB addition curve of PM-2 DNAs in aqueous 1.92 M $Mg(ClO_4)_2$ at 5°. The sedimentation coefficient is completely uncorrected. (○) Closed DNA; (●) nicked DNA; (◑) overlapping, nonresolved mixture of I and II.

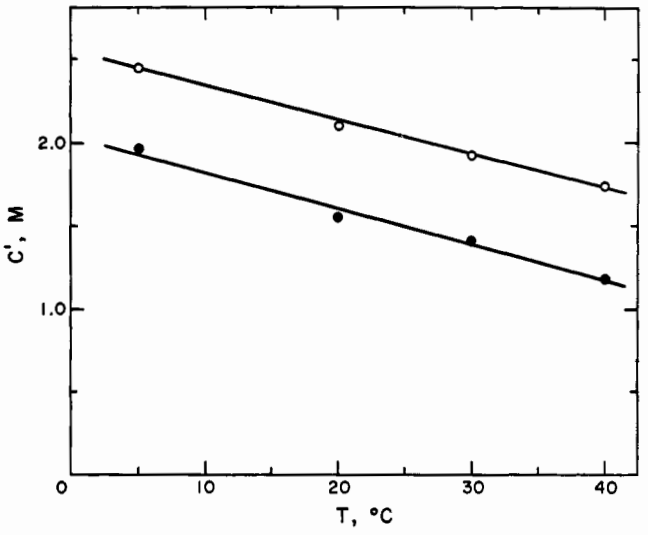

FIGURE 6: The concentration of $Mg(ClO_4)_2$, C', required to achieve a given amount of sedimentation coefficient alteration in closed PM-2 DNA as a function of temperature. (○) C' necessary to reach the junction between the curves of Figure 3. (●) C' necessary to reach the maximum in the curves of Figure 4.

that to be expected from a DNA of greatly reduced superhelix density relative to native PM-2 DNA. The minimum in the velocity curve corresponds to the removal of all superhelical turns and the subsequent rise indicates the formation of a positive superhelix.

Discussion

Effects of Salt and Temperature on the Duplex Rotation Angle. The complementarity between increases in temperature and in $Mg(ClO_4)_2$ concentration in unwinding the DNA duplex is illustrated by Figure 6, in which the equivalent concentration of perchlorate necessary to reach both the junctions between the curves of Figure 3 and the maxima in Figure 4 are plotted as a function of temperature. The two curves are seen to be parallel and to be nearly linear, a behavior which is consistent with a minimal extent of cooperative duplex unwinding by either means. The negative slope of the curves in Figure 7 is 0.05 equiv/°C; *i.e.*, the addition of $Mg(ClO_4)_2$ to a concentration of 0.05 N is comparable in duplex unwinding to a 1° increase in the temperature. The corresponding slope in aqueous $NaClO_4$, 0.06 equiv/°C (Bauer, 1972), is the same within experimental error. Thus, the addition of 1.0 N perchlorate is structurally equivalent to a temperature rise of 20° in both aqueous perchlorate binary solvents. The addition of CsCl to aqueous SV 40 DNA of low superhelix density, on the other hand, is equivalent to a temperature *decrease* in the region between 0.4 and 2.0 M (Upholt *et al.*, 1971). The same authors reported a negative shift in the superhelix density of SV 40 DNA as the concentration of NaCl is increased between 1.0 and 5.0 M demonstrating a negative correlation between the effects of salt and temperature in this case as well.

The extent of duplex unwinding by magnesium perchlorate may be expressed in terms of changes in the average duplex rotation angle (Wang *et al.*, 1967; Wang, 1969). At 20°, the addition of 4.2 N perchlorate is required to remove the initially present superhelical winding of 5.3% or 1.9 deg/base pair. If the premelting unwinding is assumed to be noncooperative, the specific unwinding is $\Delta\theta/\Delta N = -0.42°$/base pair per equiv per l. As noted above, the sign of $\Delta\theta/\Delta N$ is positive for NaCl and CsCl solutions. In aqueous $NaClO_4$, a value of $\Delta\theta/\Delta N = -0.20°$/base pair per equiv per l. was obtained (Bauer, 1972). Thus $Mg(ClO_4)_2$ is about twice as effective on an equivalent basis as $NaClO_4$ in bringing about the premelting unwinding of DNA.

The effects of increasing temperatures upon θ may be estimated by combining the slope calculated from Figure 7 with the above results, and $\Delta\theta/\Delta T = (\Delta\theta/\Delta N)(-\Delta N/\Delta T) = 0.01°$/base pair per °C. This value is in the same direction and about twice as large as the corresponding quantity measured in NaCl or CsCl (Upholt *et al.*, 1971; Wang, 1969). The difference in magnitude is probably not significant at the level of approximation used in the present calculations.

Is the Duplex Unwinding Localized or Generalized? No definitive answer to this question is yet possible. Generalized unwinding is expected if the salt-induced instability is independent of base composition and sequence. The formation of a locally unwound "blister" region, the remainder of the duplex remaining unchanged, would result if particular sequences are especially destabilized. These mechanisms are not, of course, mutually exclusive.

The EB titration results at 5° (Figure 6) suggest that no significant denatured region is present at this temperature under conditions in which the average duplex rotation angle has been decreased by nearly 5%. It is doubtful that the formation of a positive superhelix would proceed symmetrically with the loss of negative supercoils in the presence of a denatured region which could also wind to partially compensate the EB duplex unwinding. The results at 5° might, however, represent a special case. EB titrations at higher temperatures (W. Bauer, in preparation) provide suggestive evidence for the formation of such blister regions as the temperature is raised in the presence of sufficient $Mg(ClO_4)_2$. The complex appearance of the thermal melting transitions of a variety of DNAs in aqueous $Mg(ClO_4)_2$ (R. Ziegler and W. Bauer, in preparation) also suggests that certain base sequences are especially destabilized under the appropriate conditions of salt and temperature.

Molecular Mechanism of the Perchlorate-Induced Unwinding. Several theories have been presented to account for the general, destabilizing effects of neutral salts upon the structures of macromolecules (*e.g.*, Schleich and von Hippel, 1969a,b; Tanford, 1970). Two different sites of action are generally involved: direct interaction between electrolyte and polymer, and indirect effects through salt-induced breakdown of the structure of water. All such theories have been directed towards an explanation of the reductions in T_m which are brought about by the neutral salts.

The present results refer to alterations in the structure of the helix in the probable absence of transfer of the bases from the interior of the helix to a state in which they are surrounded by solvent. A reduction in the average duplex rotation angle may, however, be readily associated with a reduction in T_m, since the coil represents (ideally) a form in which $\langle\theta\rangle = 0$. It is worth noting that the temperature–salt proportionality calculated here, 20°/equiv per l., is similar to that estimated for the effects of temperature and $NaClO_4$ on the near infrared spectrum of HOD (Worley and Klotz, 1966). The similarity of these two numbers is, however, possibly peculiar to solutions of perchlorate salts (Schleich and von Hippel, 1970). The structure of water is clearly disrupted by the addition of perchlorate salts. Whether or not such effects are responsible for the duplex structure changes under the same conditions is still an open question.

It is perhaps more revealing to compare the results reported here to those of Prestegard and Chan (1969), who investigated the effects of electrolytes on the pmr spectra of several uracil derivatives. Aqueous $NaClO_4$ and $Mg(ClO_4)_2$ induce a shift in the average sugar conformation in the direction 2′-endo → 3′-endo, thereby permitting an extention of the sugar–base torsion angle toward more negative values. The latter salt is much more effective in this respect, just as it is in the effects reported here. Such shifts are reminiscent of those which occur in the B → A conformational transition of DNA (Arnott, 1971), in which the duplex is unwound by 9% (in addition, of course, to alterations in the base pair–axis inclination and other changes). The deoxyribose moieties in DNA are relatively accessible to solvent, and it is possible that the unwinding effects of these electrolytes are likewise to be accounted for in terms of a shift in the sugar ring pucker from the 2′-endo, characteristic of the B structure, toward the 3′-endo conformation. This explanation would not require direct interactions of either solvent or electrolyte with the relatively inaccessible bases. The possibility is not excluded, however, that the electrolytes also act as polarization competitors and thereby reduce the dipole-induced dipole contributions to helix stability (Bugg *et al.*, 1971). This latter effect might be facilitated by previous unwinding via the sugar-pucker mechanism. Such a combined mechanism might account for the general applicability of the Hofmeister series but for the lack of perfect correlation between the ability of the various neutral salts to destabilize water and native DNA. The former mechanism (effect on sugar pucker) might depend upon water structure changes, while the latter (base polarizability changes) might vary with the effective ionic charge density.

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