The DNA Melting Transition in Aqueous Magnesium Salt Solutions†

Gary S. Ott,‡ Robert Ziegler,§ and William R. Bauer*

ABSTRACT: The melting transition of the magnesium salt of DNA has been systematically examined in the presence of various types of anions. The addition of ClO_4^- to a concentration of 3.0 *N* results in a biphasic optical transition, with the first phase exhibiting rapid reversibility and independence of the DNA concentration. This subtransition, which is interpreted as an intramolecular condensation to a collapsed form of DNA, is followed by a DNA concentration-dependent aggregation reaction. The aggregation can be reversed by increasing the ClO_4^- concentration to 6.0 *N* while elevating the temperature to post-transition levels. Alternatively, both the collapse and the aggregation can be prevented by melting in the presence of trichloroacetate, the most strongly chaotropic solvent for DNA which has been reported (K. Hamaguchi and E. P. Geiduschek (1962), *J. Am. Chem. Soc. 84,* 1329). The forces responsible for mediating both the collapse and the aggregation are superficially similar to those involved in maintaining duplex stability. The collapsed form, in particular, possibly possesses features in common with the condensed structures which can be produced in aqueous solution of certain polymers, such as polyethylene glycol (Lerman, L. S. (1971), *Proc. Natl. Acad. Sci. U.S.A. 68,* 1886).

It has long been clear that the structure of polynucleotides is at least partly dependent upon the nature of the accompanying counterion. The divalent ions, in particular, exert a significant influence upon the structure of tRNA (Lynch and Schimmel, 1974; Cole et al, 1972), rRNA and ribosomes (Ts'o et al., 1958; Edelman et al., 1960), chromatin (Huberman, 1973), and DNA (Eichhorn and Shin, 1968; Sandor and Ts'o, 1971). In the case of DNA, in particular, the divalent ions may be grouped into two categories according to their mode of interaction. Some, such as Ni^{2+}, Cu^{2+}, and Zn^{2+}, can bind to the bases as well as to the phosphate residues along the backbone chain. Others, typified by Mg^{2+}, apparently interact with the phosphates only.

The history of investigation of MgDNA interactions has been complicated by the precipitation of even partially and denatured MgDNA under certain salt, nucleic acid, temperature, and pH conditions (Lyons and Kotin, 1964, 1965; Sandor and Ts'o, 1971; Baba and Kagemoto, 1974). This has led to uncertainty concerning both the stoichiometry of ion binding and the value of the binding constant. The melting of DNA, in particular, is shifted to higher temperatures at some Mg^{2+} concentrations (Dove and Davidson, 1962), but is shifted to lower temperatures at yet other Mg^{2+} concentrations (Lyons and Kotin, 1964, 1965). In a previous investigation of the addition of Mg^{2+} to DNA in destabilizing solvents (Bauer, 1972), the MgDNA was apparently soluble at low ionic strength, insoluble at intermediate ionic strength, and then resolubilized at higher ionic strength. This effect was dependent upon temperature, the resolubilization being favored by an increase in temperature. The molecular nature of the binding of Mg^{2+} to DNA has been a subject of some dispute, probably due to the aforecited dependence of the results upon the detailed experimental arrangement. It now appears that the primary mode of binding yields a maximum of one Mg^{2+} ion per two phosphate residues. It has been proposed (Lyons and Kotin, 1965) that higher Mg^{2+} levels might result in "charge reversal", i.e., in a MgDNA which is effectively a polycation having one positive charge per nucleotide residue. There is, however, no direct evidence in the literature for such a mode of binding.

In the present investigation we examine the melting behavior of various species of MgDNA over an extended range of ionic strengths, and with a variety of ionic species as counterions for the added salt. In agreement with previous reports, we find that the addition of Mg^{2+} stabilizes DNA against melting at low salt levels. As the Mg^{2+} concentration is increased, an ionic strength region is encountered in which precipitation of the denatured MgDNA occurs during the course of melting. Next, the helix–coil transition profile assumes a complex form at intermediate salt concentrations in chaotropic solvents, typically becoming biphasic in the solvent systems examined. Finally, at high concentrations of Mg^{2+} accompanied by destabilizing anions, and especially at higher temperatures, MgDNA is soluble even as a denatured species.

Materials and Methods

Reagent grade $Mg(ClO_4)_2 \cdot 6H_2O$ was obtained from the G. Frederick Smith Chemical Company, Columbus, Ohio. All solutions were filtered through a Pyrex No. 36060 medium pore fritted filter before use, and concentrations were determined from the refractive index at 25° as previously described (Bauer, 1972). The activity coefficients of $Mg(ClO_4)_2$ solutions and of $MgCl_2$ solutions were obtained from Robinson and Stokes (1970). Formaldehyde was prepared from paraformaldehyde by heating to 90° at pH 12 for 15 min in a closed vessel. All other chemicals were reagent grade and were used without further purification.

Source of Nucleic Acids. DNAs from calf thymus and *Clostridium perfringens* were obtained from the Sigma

† From the Department of Microbiology, Health Sciences Center, State University of New York, Stony Brook, New York 11794. *Received August 8, 1974.* This work was supported by Grants No. GM-19190 and GM-21176 from the National Institutes of Health.

‡ University of Colorado, Department of Chemistry, Boulder, Colorado 80302.

§ Present address: Duke University Medical Center, Durham, North Carolina 27710.

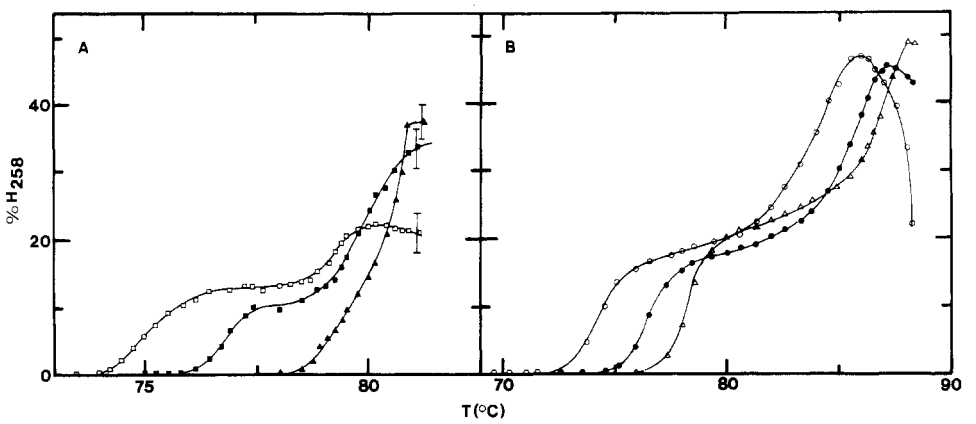

FIGURE 1: Thermal denaturation profiles of *Cl. perfringens* DNA in $MgCl_2$–0.005 *M* Tris–0.0005 *M* EDTA (pH 7.5). (A) The %*H* at 258 nm is plotted as a function of temperature for $MgCl_2$ concentrations of 0.01 *M* (▲), 0.05 *M* (■), and 0.10 *M* (□). The initial absorbances were 0.636, 0.600, and 0.588, respectively. (B) The $MgCl_2$ concentrations were 0.5 *M* (△), 1.0 *M* (●), and 1.5 M (○). The initial absorbances were 0.598, 0.651, and 0.596, respectively.

Chemical Company. They were dissolved by extensive shaking at 4°, followed by phenol extraction and dialysis into 0.001 *M* EDTA and either 0.01 *M* Tris (pH 7.5) or 0.01 *M* $Na_2B_4O_7$ (pH 8.5) and stored at 4°. Both exhibited an absorbance ratio, A_{260}/A_{280}, of greater than 1.8. The DNA from bacteriophages T7 and $\lambda b_2 b_5 c$ were prepared by first isolating the viruses by equilibrium banding in CsCl, followed by phenol extraction (Davison and Freifelder, 1962). The T7 DNA had an absorbance ratio of 1.90 and sedimented as a single band in neutral CsCl. The $\lambda b_2 b_5 c$ DNA had an absorbance ratio of 1.86 and also sedimented as a single band. The T4 DNA was a gift from Dr. Lawrence Gold. The alternating copolymer poly[d(A-T)] was obtained from General Biochemicals, Chagen Falls, Ohio.

Determination of Melting Profiles. All melting experiments were conducted with a Gilford Model 2000 automatic recording spectrophotometer equipped with automatic zero, auxillary offsets, and thermosensor. The rate of temperature rise was controlled with a temperature programmer obtained from Northeast Scientific Corp., used at a heating rate of ⅓°/min. Excessive evaporation of the aqueous solutions during the melting experiments was prevented by the addition of a low density, nonmiscible mineral oil. Results are expressed in terms of the percent hyperchromicity, $\%H_\lambda$, which is related to the absorbance, A_λ, by $\%H_\lambda = [A_\lambda(T) - A_\lambda(25°)]/A_\lambda(25°)$. The transition breadth, δ, is taken to be the temperature interval between 10 and 90% of the maximum %*H*. The melting temperature, T_m, is the temperature at which the %*H* is 50% of the maximum obtained. The precipitation temperature, T_p, is the temperature at which a precipitate begins to form in certain MgDNA systems. This temperature is generally taken to be that at which erratic behavior of the absorbance recorder just becomes evident.

Spectra. The absorbance spectra were measured with a thermostated Cary 15 spectrophotometer. Temperature was measured by placing a thermometer at the circulating water exit from the chamber. Thirty minutes were allowed to elapse before each spectrum was measured to allow for thermal equilibration.

Irreversible melting profiles were determined by suspending the sample in a Lauda temperature bath. Five minutes after the bath had come to equilibrium the temperature was recorded from a thermometer which had been cross-calibrated with the Gilford thermistor by means of a thermocouple and voltmeter. Approximately 0.3 ml of the sample was transferred into a clean dry cold cuvet held in an ice-water slush at 0°. The sample was maintained at 0° for 5 min and then allowed to come up to 25°, at which time the absorbance at 260 nm was measured with the Gilford 2000.

Results

The Helix-Coil Transition in $MgCl_2$. The melting behavior of the magnesium salt of DNA depends markedly upon the magnesium ion activity, upon the DNA concentration, and upon the nature of any excess anions. We first examine MgDNA in $MgCl_2$, confirming and extending earlier investigations (Dove and Davidson, 1962; Lyons and Kotin, 1964, 1965). The denaturation profiles obtained for *Cl. perfringens* DNA at various $MgCl_2$ concentrations are shown in Figure 1A. The transition at 0.01 *M* $MgCl_2$ is monophasic and exhibits a T_m of 80° and a $\%H_{max}$ of 37%, before precipitation prevents further measurement. The value of the $\%H_{max}$ is comparable to the 40.5% reported for a DNA of similar base composition in 0.01 *M* NaCl by Felsenfeld and Hirschman (1965), indicating that precipitation does not obscure a major portion of the transition. The transition width, δ, is 3.5°.

Increase in the $MgCl_2$ concentration to 0.05 *M* results in the appearance of a markedly different, biphasic transition profile. The first of the two subtransitions gives rise to 10% hyperchromicity, with a melting temperature, T_{m1}, of 73.6°. A lag of several degrees is observed before a second subtransition brings the total $\%H_{max}$ to 33%, accompanied by the onset of visual turbidity. This value of $\%H_{max}$ is significantly less than that found in 0.01 *M* $MgCl_2$, suggesting that precipitation has preceded the completion of denaturation. The optical transition occurs over a range, δ, of 8.3°, much of that in the lag at the plateau between subtransitions; and δ_1, the width of the first subtransition, is only 2°. The pattern of denaturation in 0.10 *M* $MgCl_2$ is similar: $\%H_{max}$ is further diminished to only 22%, T_{m1} is reduced to 70.2°, and the transition occurs over a range of 10°, while δ_1 is increased to 3.7°. The %*H* found in the first subtransition, 13%, is slightly greater than that observed in 0.05 *M* $MgCl_2$.

Increasing the $MgCl_2$ concentration to the level of 0.1 *M* thus brings about two principal effects. First, the melting temperature is lowered, in conjunction with precipitation of DNA at progressively reduced levels of denaturation. Second, the profile divides into two subtransitions, accompa-

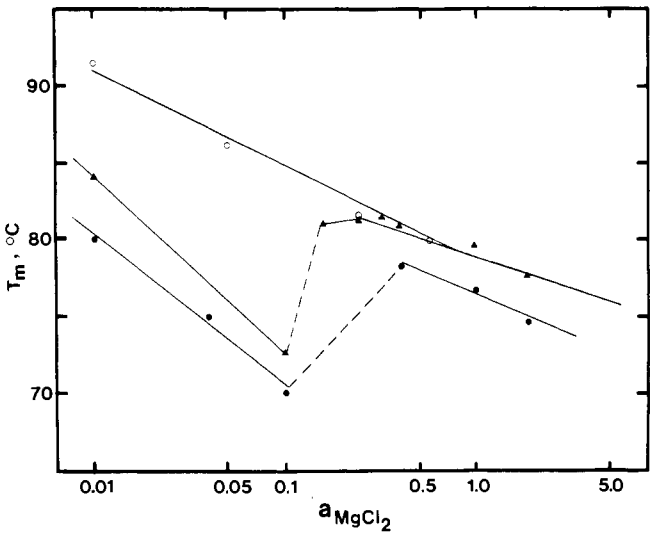

FIGURE 2: The DNA melting or precipitation temperature, T_{ml} or T_p, plotted as a function of the $MgCl_2$ activity: *Cl. perfringens* DNA–0.005 *M* Tris (pH 7.5) (●); calf thymus DNA–0.005 *M* Tris (pH 7.5) (▲); calf thymus DNA–0.005 *M* Tris (pH 7.5) with ionic strength held constant at 2.0 *M* by NaCl addition (○).

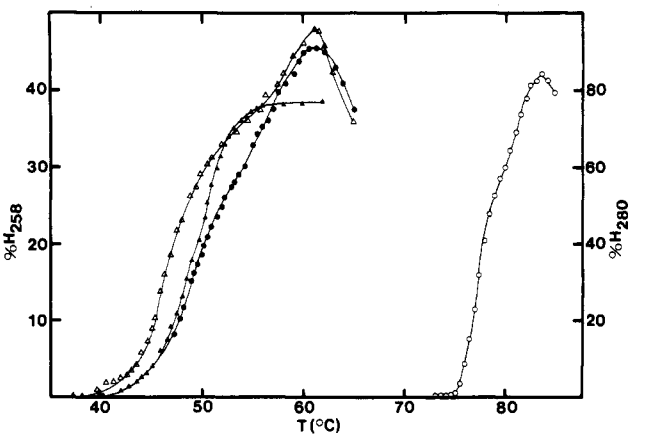

FIGURE 3: The %*H* at 258 nm for calf thymus DNA as a function of temperature for various Mg salts in 0.005 *M* Tris–0.0005 *M* EDTA (pH 7.5): 1.5 *M* $MgCl_2$ (○), 1.5 *M* $Mg(ClO_4)_2$ (△), 0.8 *M* $Mg(CCl_3CCO_2)_2$ (▲); and for 2.0 *M* $Mg(SCN)_2$–0.005 *M* $Na_2B_4O_7$ (pH 8.5), 280 nm (●). The initial absorbance values were 0.707, 0.701, 0.407, and 0.960, respectively.

nied by the appearance of an intermediate plateau. Denaturation profiles for *Cl. perfringens* DNA in more concentrated $MgCl_2$ solutions lack the erratic behavior found at the termination of melting in less concentrated $MgCl_2$ solutions. This is shown in Figure 1B for $MgCl_2$ concentrations of 0.5, 1.0, and 1.5 *M*. The hyperchromic region is now followed by a monotonic, nonerratic decrease in absorbance. Accompanying this change to a soluble form (no precipitate settles out, but the solution is visibly turbid) is a return of T_{ml} to higher temperatures and an increase in $\%H_{max}$ to 45%. The first subtransition width, $\delta_1 = 3°$, is comparable to that observed in less concentrated salt solutions; but the overall transition width δ is extended to 12°, due to an increase in the width of the second transition, δ_2, from 6 to 9°. A moderate increase in the Mg^{2+} concentration thus abolishes precipitate formation upon denaturation in favor of a soluble, probably aggregated species.

An increase in the $MgCl_2$ concentration above the solubilizing level of about 0.2 *M* results in a small decrease of T_{ml}. The relationship between T_{ml} or T_p and the chemical potential of $MgCl_2$ is shown for both calf thymus DNA and *Cl. perfringens* DNA in Figure 2. It should be noted that a higher $MgCl_2$ concentration is required to induce biphasicity in calf thymus, compared to *Cl. perfringens* DNA. Above the concentration of $MgCl_2$ necessary to avoid precipitation, T_{ml} is seen to be linearly related to log (a_{MgCl_2}), i.e., to the chemical potential. If excess NaCl is added to maintain a constant ionic strength (μ = 2.0 *M*), this linear relationship extends back to lower concentrations of Mg^{2+} and precipitate formation does not occur. Such linear behavior matches that theoretically predicted by Crothers (1971) for the dependence of T_m on the activity of a ligand which binds to the helix and coil forms of DNA with different numbers of binding sites. The initially large diminution of T_m as $MgCl_2$ is added in the absence of supporting electrolyte is probably assisted by the precipitate formation and accompanying loss of DNA from solution. In the region between 0.1 *M* and 0.5 *M* $MgCl_2$, precipitate formation no longer occurs; hence the melting temperature exhibits a corresponding transient increase with added salt. The subsequent negative slope is most likely associated with salt-induced changes in the activity coefficients of the bases, as discussed below.

Table I: Transition (δ) and Subtransition (δ_1,δ_2) Widths, and $\%H_{max}$, for the Melting of Calf Thymus DNA in Various Magnesium Salts (see Figure 3).

Solvent	δ_1 (°C)	δ_2 (°C)	δ (°C)	$\%H_{max}$
1.5 *M* $MgCl_2$	3.3	2.4	5.7	42
2.0 *M* $Mg(SCN)_2$	8.0	4.0	12.0	89[a]
1.5 *M* $Mg(ClO_4)_2$	7.6	7.6	15.2	48
0.8 *M* $Mg(CCl_3CO_2)_2$			8.0	38

[a] Measured at 280 nm.

Denaturation of DNA in Chaotropic Solvents. The experiments described above demonstrate that the presence of high salt concentrations broadens the temperature range over which the collapsed form of DNA is soluble. We next present the results of similar experiments employing a variety of destabilizing or chaotropic (von Hippel and Schleich, 1969a,b) anions. Denaturation profiles for calf thymus DNA in the presence of MgX_2, where X = Cl^-, SCN^-, ClO_4^-, or $CCl_3CO_2^-$ are shown in Figure 3. Salt concentrations were chosen so as to bring about denaturation at similar temperatures except for $MgCl_2$, for which sufficiently high salt concentrations are unattainable. The profiles obtained in $MgCl_2$, $Mg(SCN)_2$, and $Mg(ClO_4)_2$ are apparently biphasic and the hyperchromic rise is again followed by a monotonic decrease in absorbance. The solutions were turbid at the conclusion of melting, but no actual precipitation occurred. The profile found in $Mg(CCl_3CO_2)_2$ resembles the familiar cooperative, monophasic melting curve observed upon denaturation to single strands in solvent systems containing no divalent cations. No post-transitional decrease in absorbance is observed, nor was there any turbidity or precipitation. The transition widths and maximum hyperchromicities are listed in Table I.

The magnitude of δ_1 is seen to be about twice as great in the chaotropic solvents as in $MgCl_2$, a result which parallels that of Hamaguchi and Geiduschek (1962) for the corresponding alkali metal salts. δ_2 increases in the series $Cl^- < SCN^- < ClO_4^-$, a trend which is similar to the Hofmeister series. The most potent anion in this series examined,

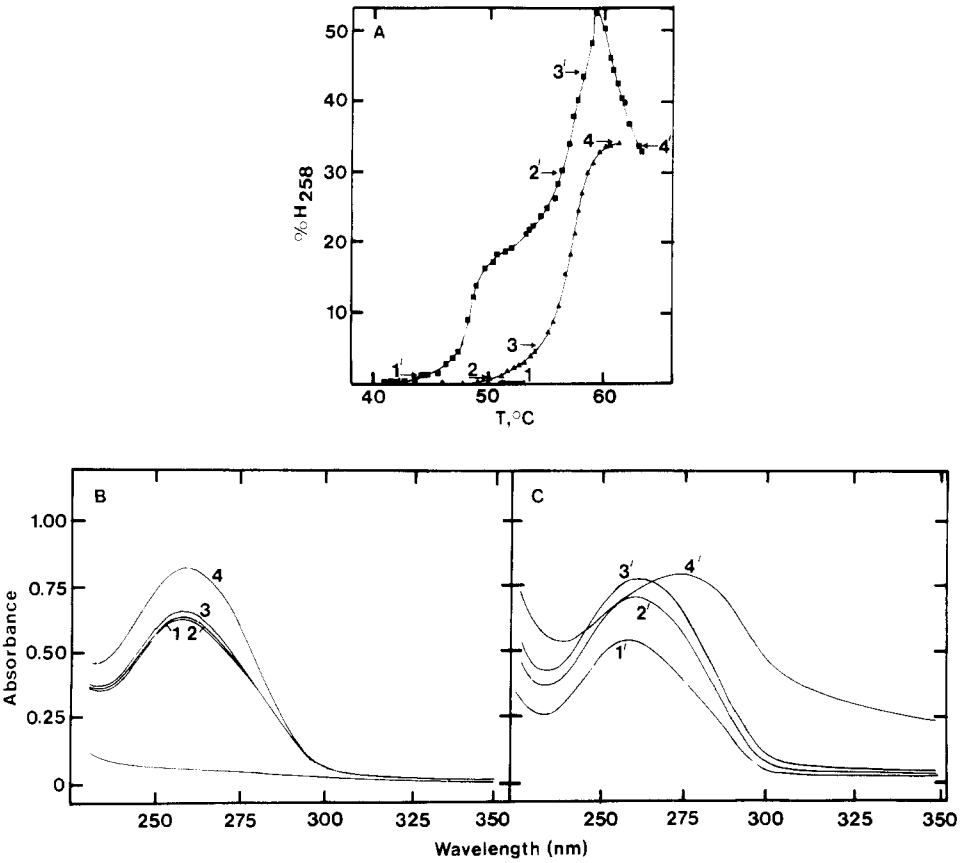

FIGURE 4: Native and hyperchromic spectra of T7 DNA in perchlorate solutions. (A) Melting profiles in 1.5 *M* $Mg(ClO_4)_2$–0.001 *M* $Na_2B_4O_7$ (pH 8.5), initial absorbance 0.482 (■); and in 5.4 *M* $NaClO_4$–0.01 *M* $Na_2B_4O_7$ (pH 8.5), initial absorbance 0.533 (▲). (B) Spectra determined at the temperatures and hyperchromicities indicated by the arrows in (A) for the $NaClO_4$ series. (C) Spectra determined at the temperatures and hyperchromicities indicated by the arrows in (A) for the $Mg(ClO_4)_2$ series. The reference cuvets contained distilled water in (B) and 1.5 *M* $Mg(ClO_4)_2$–0.01 *M* $Na_2B_4O_7$ in (C).

$Cl_3CCO_2^-$, eliminates the second subtransition altogether and abolishes the post-transition drop in absorbance. Visual inspection of the cuvets at the conclusion of melting showed no evidence of turbidity. The $Cl_3CCO_2^-$ anion thus destabilizes duplex DNA and reduces the number of optically distinct species during and after the melting. In particular, aggregation by Mg^{2+} is abolished.

The Spectrum of MgDNA in Chaotropic Solvents. The spectra of the DNA species present at different stages of the denaturation procedure were examined in 1.5 *M* $Mg(ClO_4)_2$, a solvent which is transparent at 258 nm. In order to determine useful temperatures at which spectra should be measured, the melting profiles for T7 DNA in 1.5 *M* $Mg(ClO_4)_2$ and in 5.4 *M* $NaClO_4$ were determined at 258 nm (Figure 4A). At the temperatures indicated by the arrows on each curve, the absorbance spectra were determined from 230 to 350 nm. These spectra are displayed in Figure 4B and C.

The melting profile obtained in 5.4 *M* $NaClO_4$ is monophasic, as previously reported by Hamaguchi and Geidushek (1962). A maximum hyperchromicity of 33% is obtained. Determination of the spectrum for the fully denatured material (Figure 4B) reveals no significant spectral distortions. The wavelength of maximum absorbance remains at approximately 260 nm, and the hyperchromic contribution exhibits essentially the same functional dependence on wavelength as that found by Felsenfeld and Hirschman (1965) for *Escherichia coli* DNA in 0.01 *M* NaCl.

Table II: Analysis of the Spectrum of T7 DNA in 1.5 *M* $Mg(ClO_4)$ at Various Stages of the Denaturation Process, Curves 1′–4′ of Figure 4C.

Spectrum	λ_{max} (nm)	A_{258}	A_{280}	A_{320}	A_{258}/A_{280}
1′	257.9	0.540	0.305	0.025	1.78
2′	259.0	0.710	0.440	0.039	1.62
3′	260.0	0.786	0.520	0.055	1.51
4′	273.5	0.745	0.780	0.315	0.96

The denaturation profile obtained for T7 DNA in 1.5 *M* $Mg(ClO_4)_2$ (Figure 4A) is similar to that found for calf thymus DNA in the same solvent. There are two subtransitions very widely separated by the broad inflection region, and the hyperchromic transition is followed by the typical decrease in absorbance at higher temperatures. Spectra (Figure 4C) obtained in the first subtransition, intermediate region, and the second subtransition exhibit a slight red shift and develop a small absorbance in the spectral region above 300 nm. A spectrum obtained in the region of decreasing absorbance, No. 4′ of Figure 4C, is radically shifted with substantial absorbance above 300 nm. Spectral characteristics are tabulated in Table II.

Fishman et al. (1967) have reported a similar spectral shift for *E. coli* DNA denatured in $MgCl_2$ solutions. Their interpretation of the shift is based upon a postulated interaction between the purine and pyrimidine nitrogens and

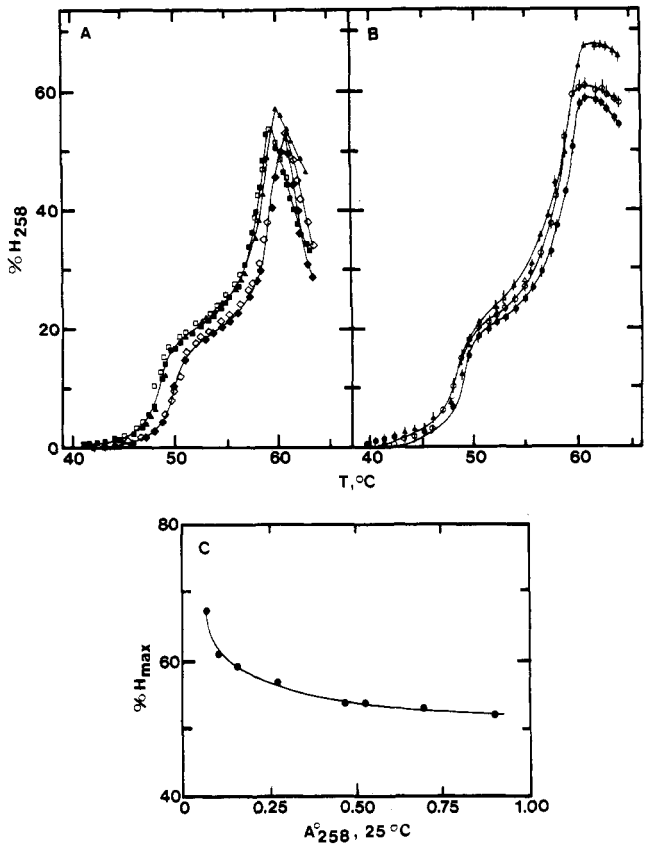

FIGURE 5: (A) Melting profiles of T7 DNA in 1.5 *M* $Mg(ClO_4)_2$–0.01 *M* $Na_2B_4O_7$ (pH 8.5). The initial absorbance values at 25° were 0.896 (♦), 0.692 (◇), 0.522 (■), 0.463 (□), and 0.266 (▲). (B) Same as (A) with initial absorbance values of 0.149 (●), 0.097 (○), and 0.067 (△). The vertical bars indicate the estimated precision at a full scale recorder setting of 0.01 absorbance unit. (C) The maximum value of %*H* for the experiments described in (A) and (B) plotted as a function of initial absorbance.

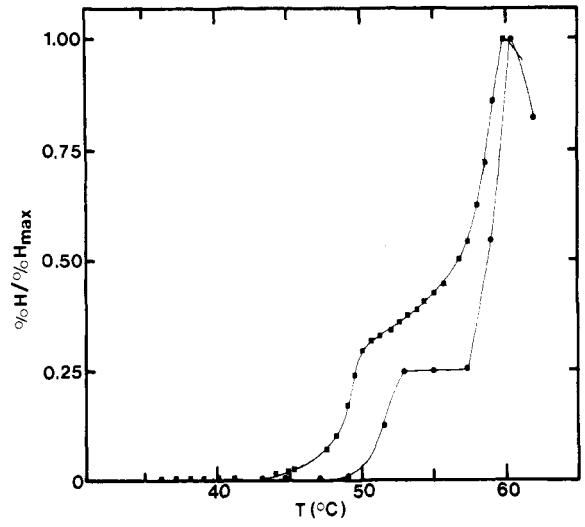

FIGURE 6: The fraction of the maximum %*H* plotted vs. temperature for T7 DNA in 1.5 *M* $Mg(ClO_4)_2$–0.01 *M* $Na_2B_4O_7$ (pH 8.5): d-assay (■) and i-assay (●).

Mg^{2+}. Experimental evidence to support such an interpretation is, however, lacking. Electron microscopic examination of extensively denatured material reveals the formation of large aggregates (Ott, Bastia, and Bauer, in preparation) and suggests that light scattering is responsible for the spectral shift. A comparison of the observed dispersion curve external to the absorbance band with the theoretically expected turbidity due to light scattering, as discussed below, lend support to this interpretation.

As is evident from inspection of Figure 4C, monitoring the helix–coil transition at a single wavelength is potentially misleading in the region of the melting curve between points 3′ and 4′, Figure 4A. If the principal cause of the observed spectral shifts is light scattering resulting from aggregate formation, it is to be expected that these anomalies will disappear as the polynucleotide concentration is diminished. Figure 5A and B displays the melting profiles for the T7 DNA denatured in 1.5 *M* $Mg(ClO_4)_2$ with initial absorbances ranging from 0.067 to 0.896. Several features are significant. The shape of the profile up to the beginning of the second subtransition is apparently unaffected by changes in DNA concentration; that is, the early denaturation appears to be an intramolecular phenomenon. The maximum hyperchromicity, and necessarily the height of the second subtransition, are inversely related to DNA concentration (Figure 5C). Finally, the post-transitional drop in hyperchromicity becomes less pronounced as the DNA concentration is reduced. At an initial absorbance of 0.067, this drop is essentially eliminated. The latter two observations are consistent with the interpretation that a phase transition limits the $\%H_{max}$ and is responsible for the decrease following $\%H_{max}$. Under sufficiently dilute conditions that transition does not occur.

Reversibility Experiments. The nature of the various DNA species throughout the transition was further investigated by examining the type I reversibility (Geiduschek, 1962). Figure 6 compares the melting behavior of T7 DNA in 1.5 *M* $Mg(ClO_4)_2$ under the conditions of both the d (dissociation of base pairs) assay, where absorbance is measured at *T*, and the i assay, which measures irreversibility or hyperchromicity which is not rapidly lost upon quenching. In this latter experiment the cuvet is removed at *T* and plunged into an ice bath, then the absorbance is measured at 25°. The two are plotted as the fraction of the maximum percent hyperchromicity in each case. The preinflection melting is seen to be completely reversible, as is usual for the early melting stages in a number of other solvents. At the inflection point between subtransitions the reversibility is lost, and about 25% of the maximum hyperchromicity remains after quenching. No more extensive irreversibility is seen until the denaturation is well into the second subtransition, whereupon a second sharp upsurge in irreversible hyperchromicity is observed. These results are consistent with the intramolecular condensation seen at the inflection point by electron microscopy (Ott, Bastia, and Bauer, in preparation). We term the result of such an intramolecular condensation a *collapsed form.*

The lack of rapid reversibility of the species in the second subtransition poses the question of whether it is reversible at all, or whether covalent bonds might be formed. The native form of DNA in 3.0 *M* $Mg(ClO_4)_2$ is sufficiently unstable so that T_m lies below 0°. From the spectra in Figure 4 it can be seen that either the absorbance ratio, 258–280 nm, or the absorbance at 320 nm would provide a good spectral index for the presence of aggregated material. Table III summarizes the alterations in these spectral landmarks for calf thymus DNA in 3.0 *M* $Mg(ClO_4)_2$ at temperatures above T_m. The data from Table III indicate that in high salt at temperatures far above T_m the aggregates disaggregate, giving rise to species which spectrally resemble single stranded, denatured DNA. This is similar to the

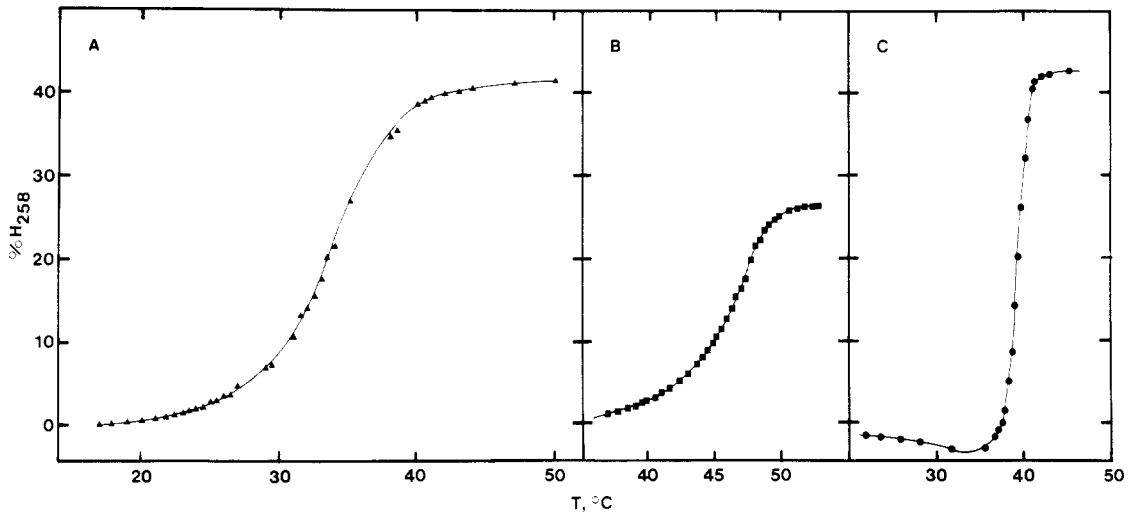

FIGURE 7: (A) Melting of $\lambda b_2 b_5 c$ DNA in 1.5 *M* $Mg(ClO_4)_2$–0.01 *M* $Na_2B_4O_7$ (pH 8.5)–8% CH_2O. (B) The melting of poly[d(A-T)] in 1.14 *M* $Mg(ClO_4)_2$–0.01 *M* Tris (pH 7.5). (C) The melting of T4 DNA in 1.5 *M* $Mg(ClO_4)_2$–0.01 *M* Tris (pH 7.5).

Table III: Spectral Characteristics of Calf Thymus DNA in 3.0 *M* $Mg(ClO_4)_2$–0.01 *M* $Na_2B_4O_7$,(pH 8.5).

Temp (°C)	A_{232}	A_{258}	A_{280}	A_{320}	A_{258}/A_{280}
4	0.578	0.578	0.523	0.140	1.11
15	0.554	0.554	0.503	0.146	1.10
25	0.268	0.551	0.317	0	1.74
55	0.252	0.548	0.284	0	1.94

melting behavior in $Mg(Cl_3CCO_2)_2$ and indicates that under severe denaturing conditions the soluble aggregates are no longer preferred.

Several other systems exist in which the aggregates are not stable. When denaturation is accomplished in the presence of formaldehyde the exocyclic amino nitrogens of the DNA bases are hydroxymethylated (Feldman, 1973). No broad inflection region or phase change is observed when denaturation is accomplished in 1.5 *M* $Mg(ClO_4)_2$ and 8% CH_2O (Figure 7A). In has previously been shown that an increase in Mg^{2+} binding normally seen upon denaturation is abolished with hydroxymethylated DNA (Zubay and Doty, 1958).

Two polynucleotides have been found which exhibit no aggregation in $Mg(ClO_4)_2$. The melting profile for poly[d(A-T)] is shown in Figure 7B, and that of the DNA from wild type phage T4 in Figure 7C. This is perhaps related to the glucosylation of cytosine in this phage DNA, since the melting profile for a poorly characterized DNA from a T4 mutant deficient in glucosylation was found to be biphasic (data not shown). The factors which prevent aggregation in the above systems are currently being investigated.

Discussion

Destabilization of Native DNA by Mg^{2+}. Other investigations have indicated that the binding constant of Mg^{2+} is nearly the same to the helix as to the coil form of DNA, and that saturation occurs with duplex DNA at a Mg^{2+}/base pair ratio of 1.0 (Sander and Ts'o, 1971). The explanation for the observed reduction in T_m at high Mg^{2+}/nucleotide ratios might, therefore, lie in the opening up of additional binding sites on denatured DNA. We examine this possibility with the aid of the theory described by Crothers (1971). The expected variation of the melting temperature in the presence of a ligand which binds differently to the helix and to the coil, $\Delta T_m = T_m' - T_m$, is

$$\Delta T_m = (RT_mT_m'/\Delta H_m)[B_c \ln (1 + K_c'a) - B_h \ln (1 + K_h'a)] \quad (1)$$

where T_m' and T_m are the melting temperatures with and without the ligand, which is present at activity a and which binds to helix (h) and to coil (c) with effective binding constants K' (see below) and maximum binding ratios B ions per nucleotide pair. The enthalpy of melting, ΔH_m, is taken as −8 kcal/mol. At high Mg^{2+} concentrations $K'a \gg 1$ and, approximately

$$\Delta T_m = (RT_mT_m'/\Delta H_m)[(B_c \ln K_c' - B_h \ln K_h') + (B_c - B_h) \ln a]$$

The variation of T_m with ln a is then approximately

$$dT_m/d \ln a = (RT_m^2/\Delta H_m)(B_c - B_h) \quad (2)$$

and, using the value of $dT_m/d \ln a$ of −5° over the region above 0.2 *M* in Figure 2, we estimate that $B_c - B_h = 0.064$. A small (6%) increase in the number of Mg^{2+} binding sites upon denaturation is, therefore, adequate to explain the slope of this curve. Physically, such an arrangement might correspond to the binding of a few Mg^{2+} ions at phosphate sites which are already surrounded by Mg^{2+}–phosphate pairs. The greater flexibility of the coil compared to the helix might allow such interactions to take place.

This result may be used to predict the magnitude of ΔT_m itself provided that the effective binding constants K' are known. The value of the intrinsic binding constant, K, has been estimated to be $6 \times 10^3\ M^{-1}$ in dilute phosphate buffer (Sander and Ts'o, 1971). The effective binding constant, K', is related to K by

$$K' = Ke^{WB} \quad (3)$$

where W is a constant which accounts for the electrostatic interaction between bound ligands. For a DNA of the base composition of calf thymus DNA T_m is 94.7° in 2 *M* salt (Schildkraut and Lifson, 1965), and eq 1 may be cast into numerical form

$$\Delta T_m = -5.29(\log K' - \log a) \quad (4a)$$

The constant W is equal to 4.05 for a model in which the binding sites are separated by 3.4 Å along the backbone chain (Clement et al., 1973) and, combining with eq 4a

$$\Delta T_m = -10.6 + 5.3 \log a \qquad (4b)$$

Equation 4b predicts no net destabilization at a $Mg(ClO_4)_2$ activity of 0.01 M; a decrease in T_m of 5.3° at 0.1 M; and a decrease in T_m of 10.6° at 1.0 M. These semi-empirical predictions are close to the experimental values (Figure 2).

Optical Properties of DNA Aggregates. The formation of large aggregates of DNA might be expected to alter the spectral properties by at least two mechanisms: screening of the interior by the exterior of the aggregates might produce a diminution in the average extinction; and the increased particle size will result in light scattering with accompanying turbidity. A combination of these two mechanisms appears to be sufficient to explain the spectral changes documented in Figure 4C. It should be noted that the hypochromicity and red shift which we observe resemble similar changes induced in the spectrum by poly(ethylene oxide) (Evdokimov et al., 1972).

By analogy with the treatment of Felsenfeld and Hirschman (1965) we define the normalized hyperchromic spectrum, $h(\lambda)$, as

$$h(\lambda) = [A(\lambda) - A_0(\lambda)]/A_0(258\text{ nm}) \qquad (5)$$

Figure 8A presents the variation in $h(\lambda)$ with wavelength for T7 DNA in both 5.4 M $NaClO_4$ and in 1.5 M $Mg(ClO_4)_2$. The data for this analysis were taken from hyperchromic spectra shown in Figure 4B and C. The values of $h(\lambda)$ for the $Mg(ClO_4)_2$ curve were increased by the ratio of the maximum absorbance at 258 nm attained during denaturation to that remaining at the temperature of the spectrum, a factor of 1.13. This correction accounts, to a first approximation, for the screening of some aggregated material from the incident light. Also presented in Figure 8A is the normalized difference between the hyperchromic spectra in the two solvents, $\Delta h(\lambda)$. The hyperchromicity across the absorbance band exhibits an anomalous dispersion in $Mg(ClO_4)_2$, an effect consistent with a scattering contribution to the apparent extinction.

Figure 8B shows the variation in the logarithm of $\Delta h(\lambda)$ with the logarithm of the wavelength in the region outside the DNA absorbance band. A least-squares analysis of the linear region of this curve yields a slope of -4.18 ± 0.12 (SD). This value is essentially that to be expected from a Rayleigh scatterer and suggests that no new interaction of Mg^{2+} with DNA, such as with the bases, is required to explain the observed hyperchromic spectra.

Formation of Condensed and Aggregated DNA. The production of condensed forms of DNA has been reported in other solvent systems, including complexes with histone (Fasman et al., 1970; Shih and Rosman, 1971) and with polylysine (Shapiro et al., 1969) and in the presence of certain polymers such as poly(ethylene oxide) (Lerman, 1971; Jordan et al., 1972; Evdokimov et al., 1972). It is noteworthy that in the case of poly(ethylene oxide) the reported condensation is intramolecular at low DNA concentrations (Lerman, 1971) but leads to the formation of aggregates at higher DNA concentrations (Jordan et al., 1972). The effect is critically dependent upon the salt concentration, in that case NaCl.

The formation of collapsed and aggregated forms of MgDNA may be rationalized on a molecular basis by considering the effects of charge repulsion and of nucleotide solubility. It would seem straightforward that collapse or aggregation is strongly disfavored in the absence of substantial charge neutralization along the DNA backbone chain, and it is reasonable to assume that this is the primary effect of the Mg^{2+} counterion. It has been established that the salt concentration is crucial in the poly(ethylene oxide) induced collapse of DNA (Lerman, 1971), and Mg^{2+} is

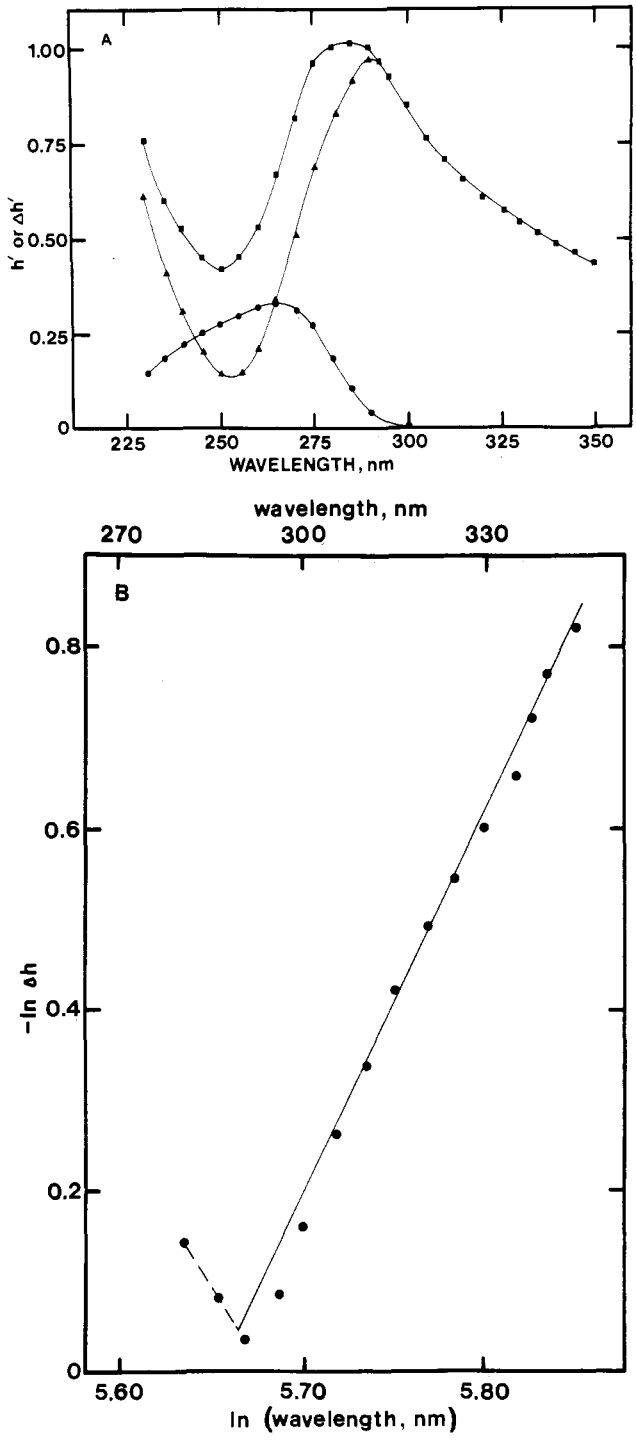

FIGURE 8: (A) The hyperchromic spectrum (see text) plotted as a function of wavelength for T7 DNA in 0.01 M $Na_2B_4O_7$ (pH 8.5) and 5.4 M $NaClO_4$ (●) or 1.5 M $Mg(ClO_4)_2$ (■). Also plotted is Δh, the turbidity contribution to the hyperchromic spectrum (▲). The initial absorbances were 0.620 in $NaClO_4$ and 0.545 in $Mg(ClO_4)_2$ (0.484 when adjusted for apparent loss of absorbance due to aggregation). (B) The quantity $-\ln \Delta h$ plotted vs. $\ln \lambda$ for the data shown in (A).

known to be much more effective than is Na^+ in reducing repulsions between phosphate groups (Clement et al., 1973). The most stable form assumed by the essentially neutral MgDNA is expected to depend, then, upon the free energy of interaction of the bases with each other as opposed to interaction of the bases with solvent.

The solubility of several of the bases, and of dA, in neutral salt solutions has been examined by Robinson and Grant (1966). From this investigation it was concluded that the order of the various anions in increasing base solubility is the same as the order in destabilizing the DNA duplex. Thus the Cl^- ion was generally found to decrease the base solubility with increasing concentration, whereas the addition of ClO_4^-, of SCN^-, or of $CCl_3CO_2^-$ brought about solubility increases in the order $ClO_4^- \simeq SCN^- \ll CCl_3CO_2^-$. For a given DNA, the most stable form will be determined by the temperature and by the salt type and concentration. In the presence of excess Mg^{2+}, the transition helix → coil is favored under conditions in which the solubility of the bases is maximal (addition of $CCl_3CO_2^-$) or in which base–base interactions are discouraged (reaction with H_2CO or presence of glucose residues on cytosine). Alternatively, the transition helix → aggregate is favored by raising the temperature in the presence of relatively low concentrations of $MgCl_2$. In this case the solubility of the bases is much reduced and the formation of a precipitate is the result.

The addition of ClO_4^- ion alters the base solubility to a level approximately midway between that pertaining in Cl^- and in CCl_3CO_2- solutions. The data presented above suggest that the reaction helix → aggregate is still favored in the vicinity of the melting region, although much less so than in the presence of low concentration of Cl^-. The similar appearance of the melting transition in the presence of high concentrations of $MgCl_2$ is more difficult to explain, due to the lack of pertinent data on the relevant base solubilities. It is to be noted, however, that the addition of $CaCl_2$ has no effect upon the solubility of thymine up to 1.0 *M*, whereas the solubility of this base is increased at higher salt levels (Robinson and Grant, 1966). In this region of base solubilities intermolecular aggregation, as evidenced by light scattering, occurs. The first optical subtransition is independent of DNA concentration and is to some extent rapidly reversible, leading to the possibility that some sort of ordered intramolecular collapsed structure is formed. The final stages of helix disruption are accompanied by an aggregation which is strongly dependent upon DNA concentration. These two features are highly reminiscent of the behavior of DNA in poly(ethylene oxide), and it is tempting to speculate that similar structural forms are involved. The driving force in this latter case has been attributed to excluded volume interactions (Jordan et al., 1972). Finally, at higher ClO_4^- concentrations and temperatures the reverse transition aggregate → coil is accomplished. The occurrence of this back reaction should make possible the detailed investigation of the various intermediates involved in the collapse and aggregation formation.

References

Baba, Y., and Kagemoto, A. (1974), *Biopolymers 13,* 339.

Bauer, W. (1972), *Biochemistry 11,* 2915.

Clement, R. M., Sturm, J., and Daune, M. P. (1973), *Biopolymers 12,* 405.

Cole, P. E., Yang, S. K., and Crothers, D. M. (1972), *Biochemistry 11,* 4358.

Crothers, D. M. (1971), *Biopolymers 10,* 2147.

Davison, P. F., and Freifelder, D. (1962), *J. Mol. Biol. 5,* 643.

Dix, D. E., and Straus, D. B. (1972), *Arch. Biochem. Biophys. 152,* 299.

Dove, W. F., and Davidson, N. (1962), *J. Mol. Biol. 5,* 467.

Edelman, I. S., Ts'o, P. O. P., and Vinograd, J. (1960), *Biochim. Biophys. Acta 43,* 393.

Eichhorn, G. L., and Shin, Y. A. (1968), *J. Am. Chem. Soc. 90,* 7323.

Evdokimov, Yu. M., Platonov, A. L., Tikohnenko, A. S., and Varshavsky, Ya. M. (1972), *FEBS Lett. 23,* 180.

Fasman, C. D., Schaffhausen, B., Goldsmith, L., and Adler, A. (1970), *Biochemistry 9,* 2814.

Feldman, M. Ya. (1973), *Prog. Nucleic Acid Res. Mol. Biol. 13,* 1–50.

Felsenfeld, G., and Hirschman, S. Z. (1965), *J. Mol. Biol. 13,* 407.

Fishman, M. M., Isaac, J., Schwartz, S., and Stein, S. (1967), *Biochem. Biophys. Res. Commun. 29,* 378.

Geiduschek, E. P. (1962), *J. Mol. Biol. 4,* 467.

Hamaguchi, K., and Geiduschek, E. P. (1962), *J. Am. Chem. Soc. 84,* 1329.

Huberman, J. (1973), *Annu. Rev. Biochem. 42,* 355.

Jordan, C. F., Lerman, L. S., and Venable, J. H., Jr. (1972), *Nature (London), New Biol. 236,* 67.

Kerker, M. (1969), The Scattering of Light and Other Electromagnetic Radiation, New York, N.Y., Academic Press.

Lerman, L. S. (1971), *Proc. Natl. Acad. Sci. U.S.A. 68,* 1886.

Lynch, D. C., and Schimmel, P. R. (1974), *Biochemistry 13,* 1841.

Lyons, J. W., and Kotin, L. (1964), *J. Am. Chem. Soc. 86,* 3634.

Lyons, J. W., and Kotin, L. (1965), *J. Am. Chem. Soc. 87,* 1781.

Robinson, D. R., and Grant, M. E. (1966), *J. Biol. Chem. 241,* 4030.

Robinson, R. A., and Stokes, R. H. (1970), Electrolyte Solutions, 2nd ed, Revised, London, Butterworths.

Sandor, C., and Ts'o, P. O. P. (1971), *J. Mol. Biol. 55,* 1.

Schildkraut, C., and Lifson, S. (1965), *Biopolymers 3,* 195.

Shapiro, J. T., Leng, M., and Felsenfeld, G. (1969), *Biochemistry 8,* 3219.

Shih, T. Y., and Rosman, G. D. (1971), *Biochemistry 10,* 1675.

Skerjanc, J., and Strauss, U. P. (1968), *J. Am. Chem. Soc. 90,* 3081.

Ts'o, P. O. P., Bonner, J., and Vinograd, J. (1958), *Biochim. Biophys. Acta 30,* 570.

von Hippel, P. H., and Schleich, J. (1969a), in Structure and Stability of Biological Macromolecules, Timasheff, S. N., and Fasman, G. D., Ed., New York, N.Y., Marcel Dekker, p 417.

von Hippel, P. H., and Schleich, J. (1969b), *Acc. Chem. Res. 2,* 257.

Zimmer, Ch., Luck, G., and Triebel, H. (1974), *Biopolymers 13,* 425.

Zubay, G., and Doty, P. (1958), *Biochim. Biophys. Acta 29,* 47.