Structural Transitions of Deoxyribonucleic Acid in Aqueous Electrolyte Solutions. II. The Role of Hydration†

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ABSTRACT: The data and approach reported in paper I (Hanlon et al., 1975, preceding paper) have been used to calculate the fractional changes in secondary structure of calf thymus deoxyribonucleic acid which occur in aqueous solutions as a function of the concentration of NaCl, KCl, LiCl, CsCl, and NH_4Cl. There is a continuous loss in the "B" character of the nucleic acid with concomitant production of the C and, in some instances, an A form, as well, as the salt concentration increases. Sedimentation velocity studies suggest that there is an accompanying change in the hydrodynamic characteristics of the DNA molecules, as well. Utilizing the existing hydration data in the literature (Hearst and Vinograd, 1961a,b; Hearst, 1965; Tunis and Hearst, 1968a; Cohen and Eisenberg, 1968; Falk et al., 1962, 1963a,b), we have found that a gradual loss of "B" character and a decrease in the frictional coefficient of DNA occur as the net hydration of DNA is reduced from the fully hydrated form (60–80 mol of H_2O/mol of nucleotide) to values of ca. 12–14 mol of H_2O/mol of nucleotide. Below that value, a more precipitous decrease in these properties occurs. Extrapolation of the linear relationship observed between the fractional B content and the net hydration in the latter regions yield values of ca. 18 mol of H_2O/mol of nucleotide at 100% B and ca. 4 mol of H_2O/mol of nucleotide at 0% B (i.e., 100% C or C + A) for the alkali metal salts of DNA. The ammonium salt retains somewhat more H_2O in the C and A forms (ca. 7). These results together with the hydration site assignments of Falk et al. (1962, 1963a,b) are interpreted in terms of a hydration model for DNA in aqueous solution in which an intact primary hydration shell of ca. 18 mol of H_2O/mol of nucleotide is required for the maintenance of the "B" conformation. Removal of all but those water molecules solvating the phosphate groups results in the conversion to the C forms, predominantly, with a small amount of A structure formed as well in some salts. The accompanying changes in the sedimentation coefficients suggest that the DNA molecule assumes a more compact and/or flexible form under these conditions in which it is mainly in the C and A structures. The combination of these two events which ensue upon dehydration create a polymeric structure which can be more easily packaged in biological systems.

In the preceding paper (Hanlon et al., 1975) of this series, the change in the circular dichroism (CD) spectra of DNA in increasing concentrations of five electrolytes, NaCl, KCl, LiCl, CsCl, and NH_4Cl, was interpreted in terms of transconformational reactions: As the concentration of all of these electrolytes increased from dilute levels (0.01–0.04 *m*) to saturation, the conformation of DNA underwent a major transformation from a form resembling the Watson–Crick B structure,[1] designated as the "B" form, to the C structure with a small proportion of the bases converting to an A form at very high concentrations of LiCl, CsCl, and NH_4Cl. In this process, the effectiveness of the several salts varied, with NaCl being the least effective, and NH_4Cl the most effective on the basis of concentration. The order of the effectiveness could not be correlated with any simple ionic property in aqueous solution nor with the affinity of the cation of the salt for DNA. The differences in efficiency at low concentrations of these ions were attributed to differences in the manner in which they accommodated to the helical DNA structures as they became site bound to the negatively charged phosphate groups along the backbone. Clearly, however, this cannot be the only factor involved in promoting the B → (C + A) transition, as the DNA sites should be saturated at concentrations ranging from 0.1 to 0.5 *m*, whereas the transition in secondary structure continues as the concentration of a given electrolyte increases above this level. It is thus obvious that a property of DNA, other than its direct interaction with the ions of the medium, is being affected in these solutions in such a manner as to facilitate these transconformational reactions.

An obvious property of DNA which would be expected to have some influence on secondary structure and which changes dramatically as the concentration of salt is varied is the preferential solvation of this macromolecule. Hearst and coworkers (Hearst and Vinograd, 1961a,b; Hearst, 1965; Tunis and Hearst 1968a) as well as Cohen and Eisenberg (1968) have demonstrated that as the water activity, a_w, decreases with increasing concentrations of salts of Na^+, Li^+, and Cs^+ in aqueous solutions of DNA, the preferential solvation or net hydration of the macromolecule, Γ (in mol of H_2O/mol of nucleotide), decreases. This effect is more or less independent of the nature of the cation and dependent only on the value of a_w for a given DNA. There is a small dependence on base composition, with AT pairs being hydrated to the extent of 2 additional mol of water compared to GC pairs (Tunis and Hearst, 1968b).

It seemed plausible, therefore, that the observed transformation in the secondary structure of DNA could be correlated with its state of hydration in these solutions. The results of initial calculations reported previously (Wolf et al.,

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[1] As suggested by Bram (1971) and Ivanov et al. (1973), the conformation of DNA present at low concentrations of salts differs from the Watson-Crick B form in having a smaller rotational angle between successive base pairs in the helix. Presumably this is true of the ionically unperturbed conformation of DNA, which we have designated as the "B" form.

1972) confirmed that this was indeed the case. A rough correlation has also been pointed out by Zimmer and Luck (1973) who observed that the lowering of the positive CD band in the spectrum of DNA in aqueous electrolyte solutions followed the same course as the hydration of DNA in these solutions reported by Chattoraj and Bull (1971). In this present paper, we have used the conformational data reported in paper I (Hanlon et al., 1975) together with the net hydration data available in the literature, and the hydration site assignments of Falk et al. (1962, 1963a,b) to demonstrate and interpret the effects of hydration on secondary structure of DNA. Sedimentation velocity studies have been employed to follow an accompanying change in the hydrodynamic properties of the DNA coil.

The two sets of hydration data available for DNA (Hearst and coworkers, and Cohen and Eisenberg) differ somewhat quantitatively from each other. Since we had no way of ascertaining which was the more accurate, we have analyzed our data using both sets, individually, in order to rule out the possibility that the patterns observed in the transformation of the secondary structure and the hydrodynamic properties of DNA as a function of the net hydration are attributable to errors in the estimation of Γ at a given a_w by a single laboratory.

Experimental Section

The same commercial preparation of calf thymus DNA (Sigma, Lot 802184) described in paper I was also utilized in these experiments. Experimental methods, equipment, reagents, and precautions observed in preparing solutions of DNA are identical with that given in paper I (Hanlon et al., 1975). The fractions of bases in the "B", C, and A forms are based on the experimental CD data reported in that paper. The average C and A reference spectra presented in Tables IV and V, and the experimentally observed B spectrum in dilute solutions of NaCl given in Table III, were used together with the observed CD spectra to calculate fractions of bases in the "B", the C, and the A forms by method I. Calculations of the normalized B content, referred to as % B′, utilized the spectra of DNA in dilute solutions of each individual salt, presented in Figure 6 of paper I. These data are appropriate for pH 7 and 27°.

The concentration of the electrolyte component in the DNA solutions was calculated in molal units as previously described in paper I. Water activity, a_w, in the two component solvents, electrolyte and water, was calculated from the osmotic coefficients, ϕ, according to eq 1 taken from

$$-\ln a_w = \frac{\phi \nu m_3}{55.5} \quad (1)$$

Robinson and Stokes (1965), where m_3 is the formal molal concentration of the electrolyte and ν is the number of dissociable ionic species. For the 1:1 electrolytes employed in these studies, $\nu = 2$. The values of ϕ at 25° were obtained from Robinson and Stokes (1965). Densities and viscosities of the solvents were found in Robinson and Stokes (1965), The International Critical Tables of Numerical Data (1933), and The Handbook of Chemistry and Physics (1952).

The net hydration, Γ (in moles of H_2O/mole of nucleotide), at 25° was obtained from the data reported in the literature by Hearst and coworkers (Hearst and Vinograd, 1961a,b; Hearst, 1965; Tunis and Hearst, 1968a) as well as Cohen and Eisenberg (1968). A curve of Γ vs. a_w at 25° could be calculated from the data supplied by the latter authors in Tables I and II of their publication. The NaCl values could be similarly calculated from the data in Table II of the publication by Hearst (Hearst, 1965). The remainder of the data of Hearst and coworkers was provided in graphical form (Hearst and Vinograd, 1961a,b; Tunis and Hearst, 1968a). Values of Γ in reproductions of various graphs found in the series of articles quoted above were obtained by measurements made on the coordinate positions with a metal ruler graduated in 0.5-mm divisions. These values were then plotted against the appropriate water activity of the solvent on a larger scale which permitted interpolating and averaging. Although this procedure obviously has inherent errors of its own, this could not have amounted to very much. Comparison of the values so obtained in this manner with those reported in the single publication (Hearst, 1965) in which tabulated data were also available revealed that the discrepancies between the estimated value and the calculated value were always less than ±0.25 mol of H_2O/mol of nucleotide.

A common curve for each set of hydration data taken from the two sources, Cohen and Eisenberg (Γ_C) and Hearst and coworkers (Γ_H), was employed for all electrolytes. Both laboratories had reported negligible differences in the functions defining Γ as a function of a_W for salts of Na^+, Li^+, and Cs^+. In utilizing these common curves, therefore, we have made the assumption that they are equally valid for solutions of DNA in KCl and NH_4Cl. Except for values at water activities near 1.0, Γ_C was less than Γ_H. Below Γ of 15, the difference amounted to 2–4 mol of H_2O.

No corrections of the data of Hearst and coworkers were made for the difference in G–C content between the T-4 bacteriophage DNA for which the data were obtained, and calf thymus DNA to which the data were applied. Calculation based on a difference in hydration of 2 H_2O per A–T pairs (Tunis and Hearst, 1968b) showed the expected correction to be less than 0.2 water molecule/nucleotide.

Sedimentation velocity experiments were conducted at 24–26° in top loading cells with a Spinco Model E analytical ultracentrifuge equipped with absorption optics. The films taken with the latter were scanned with an Analytrol densitometer. Absorbances of solutions fell within the range required for a linear response of the film/densitometer combination to concentration. At the concentration of DNA employed in these experiments (ca. 0.003% or 1×10^{-4} M), the sedimentation coefficients were independent of DNA concentrations at or above ionic strengths of 0.1 M. Thus, the values reported represent those at infinite dilution.

The median sedimentation coefficient was obtained by following the half-height position of the boundary. These values were corrected to the conventional anhydrous standard state corresponding to a medium with the density, $\rho_{w,20}$, and viscosity, $\eta_{w,20}$, of water, w, at 20°, using eq 2a or 2b.[2] In the above expressions, the terms are defined as fol-

[2] In principle, this equation should only be applied to monodisperse preparations of DNA, since an average value of Γ determined by hydration methods need not be appropriate for the particular average measured in the sedimentation velocity method. The characteristic polydispersity in molecular weight and composition of calf thymus DNA should have negligible influence on the value of the hydration per nucleotide residue, however, and hence the use of 2a and 2b for these solutions should be valid.

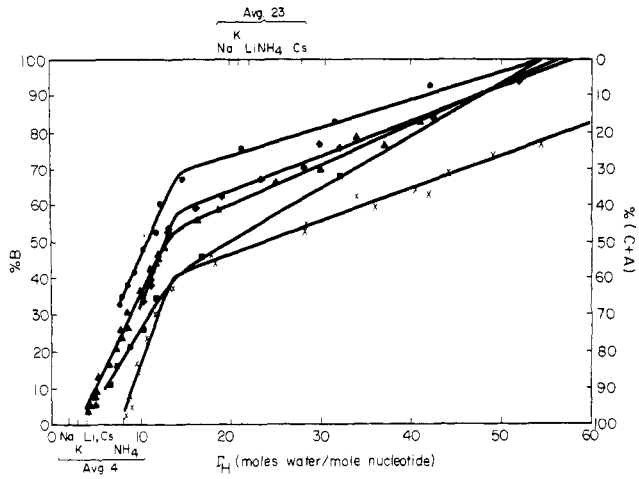

FIGURE 1: Behavior of the fractional "B" content of DNA as a function of the net hydration data of Hearst and coworkers. The fractional "B" content, % B, calculated as described in the text for solutions of DNA in NaCl (●), KCl (♦), LiCl (▲), CsCl (■), and NH_4Cl (×), is plotted on the left-hand ordinate against the net hydration, Γ_H, of DNA in the same solution.

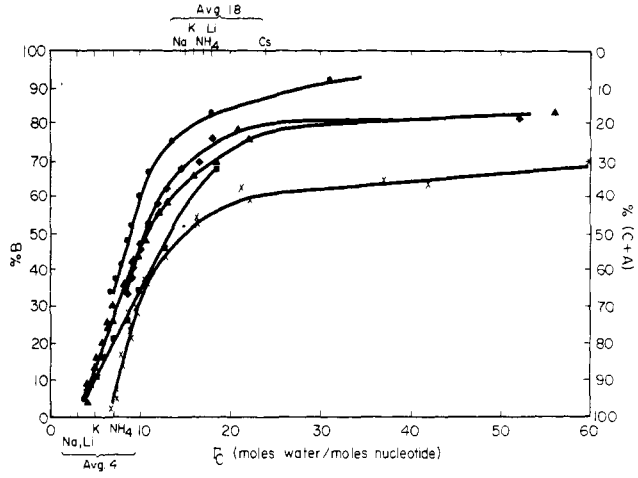

FIGURE 2: Behavior of the fractional "B" content of DNA as a function of the net hydration data of Cohen and Eisenberg. The fractional "B" content, % B, calculated as described in the text for solutions of DNA in NaCl (●), KCl (♦), LiCl (▲), CsCl (■), and NH_4Cl (×), is plotted on the left-hand ordinate against the net hydration, Γ_C, of DNA in the same solution.

$$s_{20,w}^{0} = s_{obsd}^{0}\left[\frac{\eta_{solv,T}}{\eta_{w,20}}\right]\left[\frac{(1 - \bar{v}_2\rho_{w,20})}{(1 - \bar{v}_2\rho) + \Gamma'(1 - \bar{v}_1\rho)}\right] \quad (2a)$$

$$s_{20,w}^{0} = s_{obsd}^{0}\left[\frac{\eta_{solv,T}}{\eta_{w,20}}\right]\left[(1 - \bar{v}_2\rho_{w,20})\Big/\left(\frac{\partial\rho}{\partial C_2}\right)^{0}_{\mu_1,\mu_3}\right] \quad (2b)$$

lows: $s_{20,w}^{0}$ is the median sedimentation coefficient at infinite dilution, corrected to the standard state. s_{obsd}^{0} is the median sedimentation coefficient at infinite dilution measured in a mixed solvent of viscosity, η_{solv}, and density, ρ, at temperature T.

The index numbers 1, 2, and 3 refer to the components, water, DNA, and salt, respectively. The symbol, $\bar{v}$, represents the partial specific volume. $\bar{v}_1$ was taken as 1.00. The partial specific volumes of the DNA salts, $\bar{v}_2$, were taken from the literature. Values of $\bar{v}_2$ for NaDNA and CsDNA were taken from Tables I and II, respectively, in the paper of Cohen and Eisenberg (1968). The $\bar{v}_2$ of the ammonium salt of DNA, in water (0.538 cm^3/g), was calculated from the data given by Rinehart and Hearst (1972a). Values of $\bar{v}_2$ for NH_4DNA at varying concentrations of NH_4Cl were adjusted according to the incremental differences observed for $\bar{v}_2$ of CsDNA as a function of salt concentration given in Table II of Cohen and Eisenberg. Similar adjustments were made for $\bar{v}_2$ of LiDNA, whose limiting value of 0.561 cm^3/g at high salt was taken from Hearst and Vinograd (1961).

Γ' is the weight of water preferentially solvated per weight of nucleotide residue. For these values we used the common curves of Γ_H and Γ_C taken from the previously cited literature references, and converted to a weight basis by use of

$$\Gamma' = \frac{\Gamma(18)}{309 + M_{cation}} \quad (3)$$

The value of 309 represents the average molecular weight of the nucleotide residue of calf thymus DNA minus the counterion, and M_{cation} is the appropriate atomic weight of the cation of the electrolyte employed.

For the Na and Cs salts of DNA, density increment data, $(\partial\rho/\partial C_2)_\mu°$, were available as numerical values in Tables I and II of Cohen and Eisenberg (1968). Equation 2b was correspondingly used for the buoyancy correction for those two salts in the data presented in Figure 7B.

It should be mentioned that we did not apply the preferential solvation correction, $[\Gamma'(1 - \bar{v}_1\rho)]$, to the sedimentation coefficients measured in 0.1 *m* salt, as we did not have sufficiently accurate hydration data at this low concentration of electrolyte. The error inherent in this omission is estimated to be on the order of 2–4%. These values in 0.10 *M* salt, after applying the standard corrections for viscosity and density of solvent were 16.5 S, 16.4 S, and 25.0 S, for the Na, NH_4, and Cs salt of the DNA sample, respectively, which we employed in these experiments.

Statistical analyses were performed by standard methods (Snedecor and Cochran, 1967). For the reported results of the linear regression analyses, the various fractions of secondary structure were taken as the error free variable. No significant difference in the values of Γ for the various intercepts was observed, however, if the reverse procedure was followed.

Results

Effects of Hydration on Secondary Structure. The fraction of bases in the "B" conformation (i.e., that conformation of DNA characterized by a limiting CD spectrum in 0.01–0.04 *m* NaCl) in five salts has been plotted in Figures 1 and 2 against the preferential hydration of DNA in the same solution. Figure 1 utilizes the net hydration data of Hearst and coworkers (Hearst and Vinograd, 1961a,b; Hearst, 1965; Tunis and Hearst, 1968) and Figure 2, that of Cohen and Eisenberg (1968). Although the curves for the five electrolytes are not coincident, they do share certain common characteristics. In both plots, there is a range of high hydration values above Γ of ca. 12–14 mol of water/mol of nucleotide in which the B character is lost relatively slowly. Below this value, the dependence of the B content on Γ is more marked. In subsequent discussions (below), these two ranges of values are differentiated by referring to the former as "region II" and the latter as "region I".

When the conformational data are plotted against the

Table I: Characteristics of the Least Mean Squares Linear Relationships between Secondary Structure of DNA and Net Hydration, Γ_H (mol of H_2O/mol of Nucleotide).

		Fractional "B" Content				Fractional C Content				Fractional A Content	
Salt	Region	(d% "B"/ dΓ_H)	Γ_H 100% "B"	Γ_H 0% "B"	$\Gamma_{intersect}$[a]	d% C/dΓ_H	Γ_H 0% C	Γ_H 100% C	$\Gamma_{intersect}$[a]	d% A/dΓ_H	Γ_H 0% A
NaCl	I	5.63	19.6	1.9	14.1	−5.66	19.4	1.7	13.8		
	II	0.76	54.9			−0.78	54.6				
KCl	I	5.93	21.2	4.4	14.1	−6.14	20.9	4.6	14.1		
	II	0.96	57.8			−0.96	57.8				
LiCl	I	5.39	22.0	3.4	13.2	−4.04	24.4	0.3	13.7	−1.6	14
	II	1.09	56.5			−0.92	60.6				
CsCl	I	4.02	28.3	3.5	13.8	−2.66	34.0	−3.6	13.5	−1.8	15
	II	1.43	54.5			−1.36	53.6				
NH_4Cl	I	6.16	21.8	7.8	13.5	−5.57	24.1	6.1	13.2	−1.2	15
	II	0.89	79.8			−0.95	77.9				
Av	I		22.6	4.2	13.7		24.6		13.7		15
SD			±3.3	±2.2	±0.4		±5.7		±0.3		

[a] The symbol $\Gamma_{intersect}$ represents the value of Γ_H at which the linear relationship defining regions I and II intersect.

Table II: Characteristics of the Least Mean Squares Linear Relationships between Secondary Structure of DNA and Net Hydration, Γ_C (mol of H_2O/mol of Nucleotide) in Region I (below Γ_C of 15).

	Fractional B Content				Fractional C Content				Fractional A Content	
Salt	(d% "B"/ dΓ_C)	Γ_C 100% "B"	Γ_C 0% "B"	$\Gamma_{intersect}$[a]	d% C/dΓ_C	Γ_C 0% C	Γ_C 100% C	$\Gamma_{intersect}$[a]	d% A/dΓ_C	Γ_C 0% A
NaCl	8.99	14.5	3.3	12	−7.76	16.1	0.2	12		
KCl	8.79	16.0	4.6	12	−8.79	16.0	4.6	12		
LiCl	6.92	17.6	3.2	12	−4.85	20.3	−0.4	11	−1.6	12
CsCl	4.68	24.0	2.7	14	−2.91	30.1	−4.2	10	−2.0	13
NH_4Cl	9.23	17.4	6.5	11	−7.24	19.0	5.3	10	−1.5	12
Av		17.9	4.1	12		20.3		11		12
SD		±3.6	±1.5			±5.8				

[a] The symbol $\Gamma_{intersect}$ represents the value of Γ_C where the data points begin to deviate from the linear relationship defining region I.

values of Γ_H, the preferential hydration data of Hearst and coworkers, the % B vs. Γ_H relationships are clearly linear in both regions of the graph below Γ_H of ca. 50. Above this hydration all of the curves, with the exception of NaDNA, level off, and even in dilute concentrations (0.01–0.04 *m*) of salt do not attain 100% "B" character, due to the ionic interactions mentioned previously. The intersection points of the straight lines defining the relationships in regions I and II fall at ca. 14 mol of H_2O/mol of nucleotide. When the data are plotted against the values of Γ_C, the hydration data of Cohen and Eisenberg (1968), the linearity in region II is not as apparent, but the points defining region I are linear up to about 12 mol of water/mol of nucleotide. If the least mean squares relationships for region I in both Figure 1 and Figure 2 are extrapolated to 100% B (0% C + A) and 0% B (100% C + A), variable results are obtained for the individual salts, and for the two sets of hydration data (Γ_H and Γ_C) employed. The values average around 18–23 mol of H_2O for the 100% B intercept and ca. 4 mol of H_2O for the 0% B intercept. Intercepts for region II at 100% B average at ca. 56–65 for the alkali metal salts. Values for the NH_4DNA are significantly higher.

These various average values together with the values for the individual salts are given in Tables I and II together with other salient features of the linear relationships calculated by a least mean squares analysis of the data. Included in these tables are the characteristics of the linear relationships observed for the C and, in some cases, the A content as well. The dependence of all three secondary structures on Γ is shown graphically for the individual salts in Figures 3 and 4 as least mean squares lines without points. In no case does the transformation in any of these secondary structures appear to be cooperative. It is notable that the A form does not appear until hydration levels have dropped below 12–15 mol of H_2O.

As noted previously, there is a significant lowering of the "B" content of DNA in dilute solutions of KCl, LiCl, CsCl, and NH_4Cl, relative to NaCl, well before significant gross dehydration has ensued. In order to normalize for this initial effect of the individual cations, we have used for each electrolyte the spectrum obtained in the individual salt solution between 0.01 and 0.04 *m* as the reference B spectrum in that salt. When the fractional B content in each electrolyte is now recalculated using these new limits, the plots of % B vs. Γ for Na^+, K^+, Li^+, and Cs^+ now fall on common curves with only the NH_4^+ data still deviant. This is illustrated by the data presented in Figures 5 and 6, which are plots of the normalized B content, % B′, against Γ_H (Figure 5) and Γ_C (Figure 6). The solid lines represent the common curves defined by the data points for NaCl, KCl, LiCl, and CsCl. A covariance analysis reveals no difference between the linear characteristics of the individual plots for each of

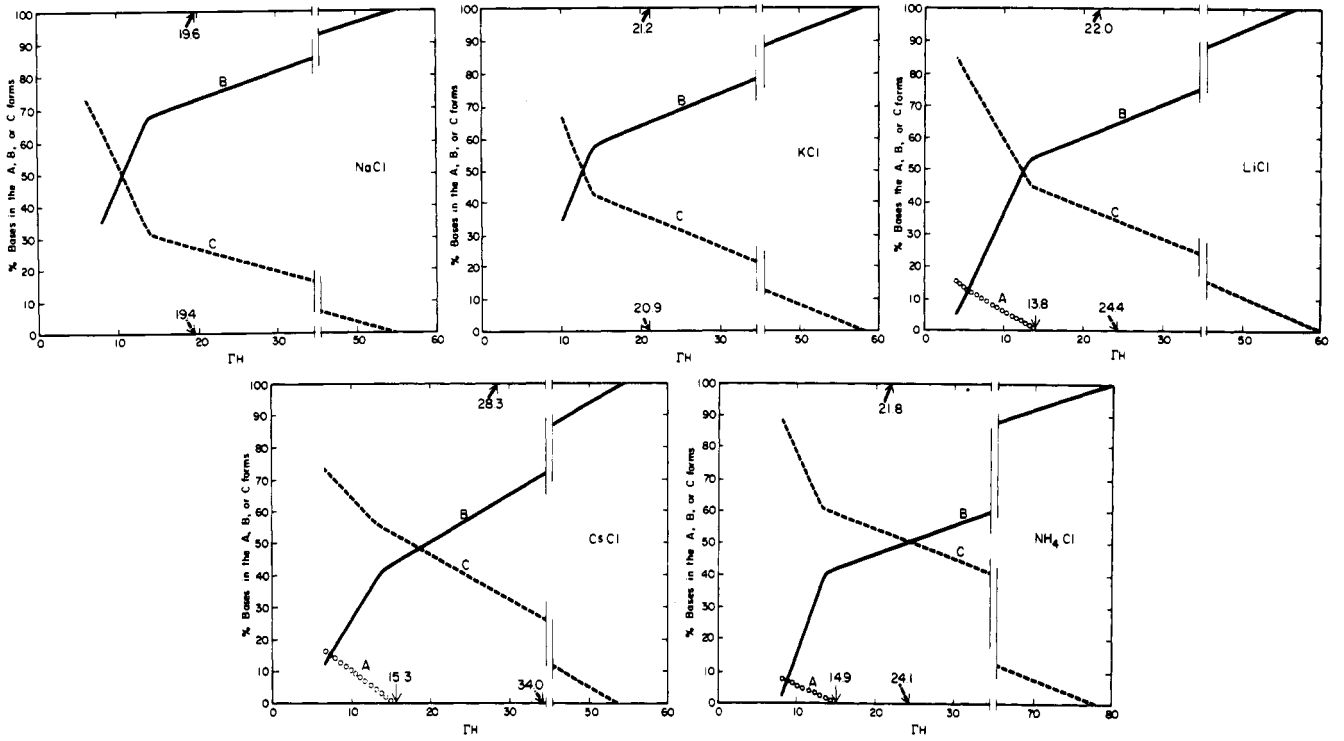

FIGURE 3: Relationship between the fractional content of the "B", the C, and the A forms of DNA in various electrolytes as a function of the net hydration, Γ_H, taken from the data of Hearst and coworkers.

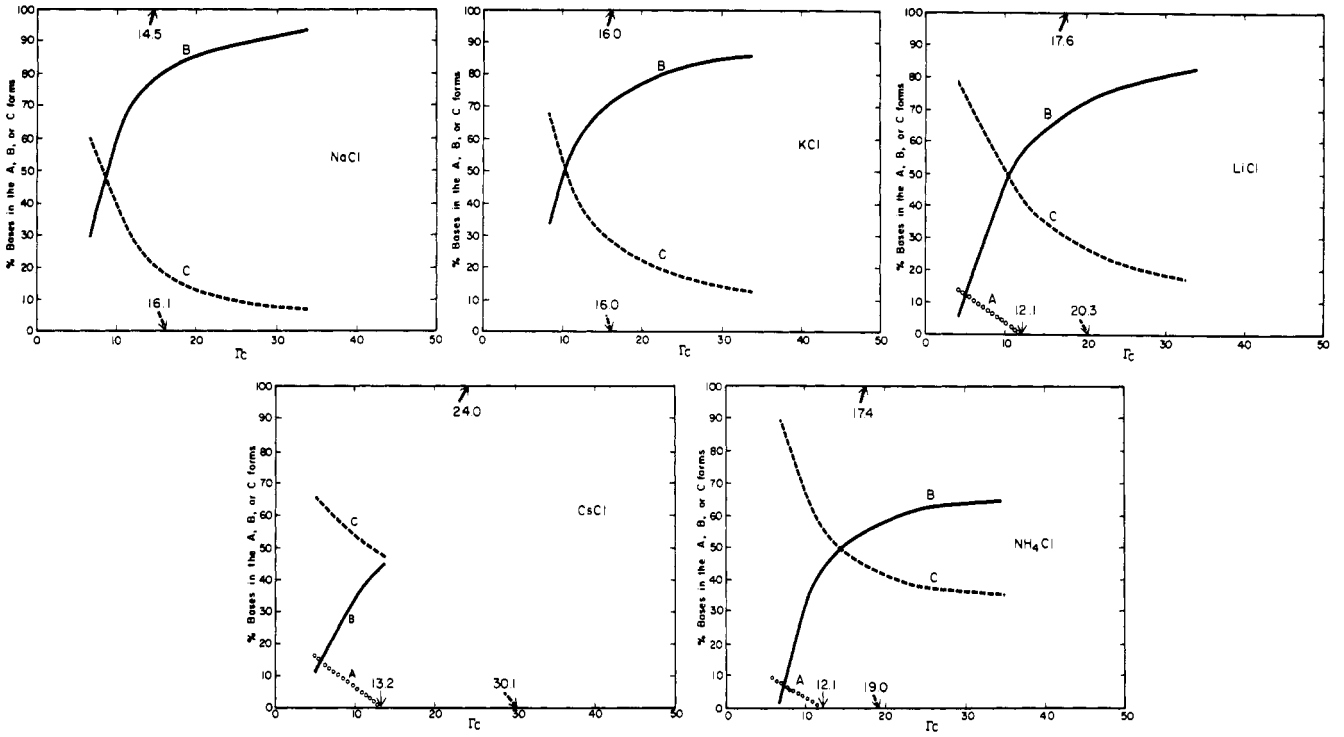

FIGURE 4: Relationship between the fractional content of the "B", the C, and the A forms of DNA in various electrolytes as a function of the net hydration, Γ_C, taken from the data of Cohen and Eisenberg.

these salts although there is a possibility that the intercepts for NaCl points might be lower than the average by ca. 1 H_2O molecule. There are, however, significant differences between this group and the NH_4Cl data. The linear relationships observed for the latter salt are represented by dashed curves. The characteristic intersection points for the lines defining regions I and II in these plots, as well as the intercepts at 100% B′ and 0% B′ for the linear relationships in region I are given in Table III. The intersection points for these plots are comparable to those observed in Figures 1 and 2, ranging from ca. 12 mol of H_2O for the Γ_C data to 14 mol of H_2O for the Γ_H data. The intercepts at 0% B for the region I data are also comparable, being 3–4 mol of H_2O for the alkaline earth cations and 6–8 mol of H_2O for

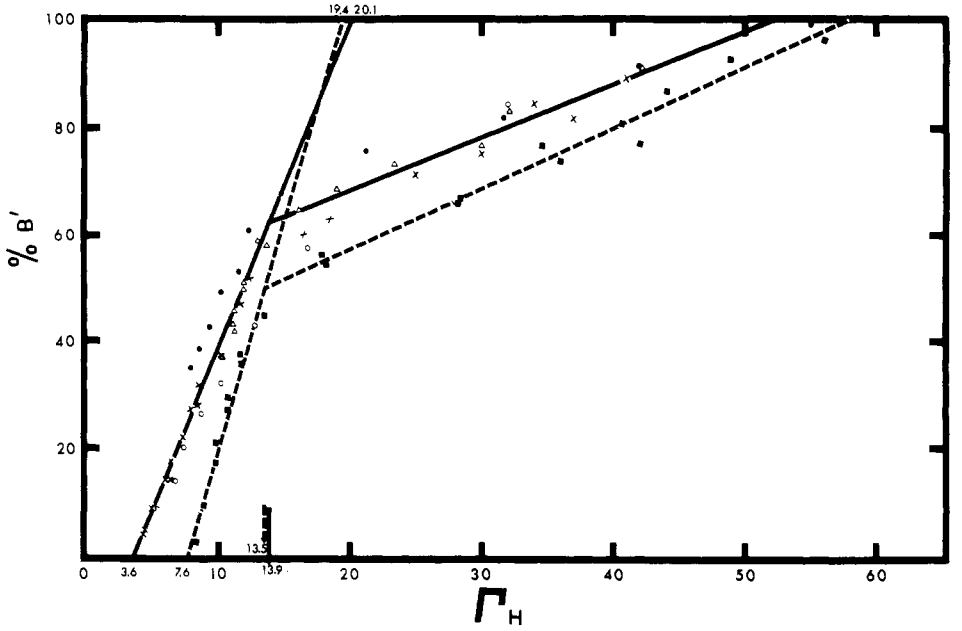

FIGURE 5: Behavior of the normalized B content of DNA as a function of the net hydration data of Hearst and coworkers. Values of % B′, calculated as described in the text, for solutions of DNA in NaCl (●), KCl (△), LiCl (×), CsCl (○), and NH_4Cl (■), are plotted on the ordinate against Γ_H on the abscissa. The solid line represents the common linear regression of the alkali metal salts, and the dashed line that for NH_4Cl.

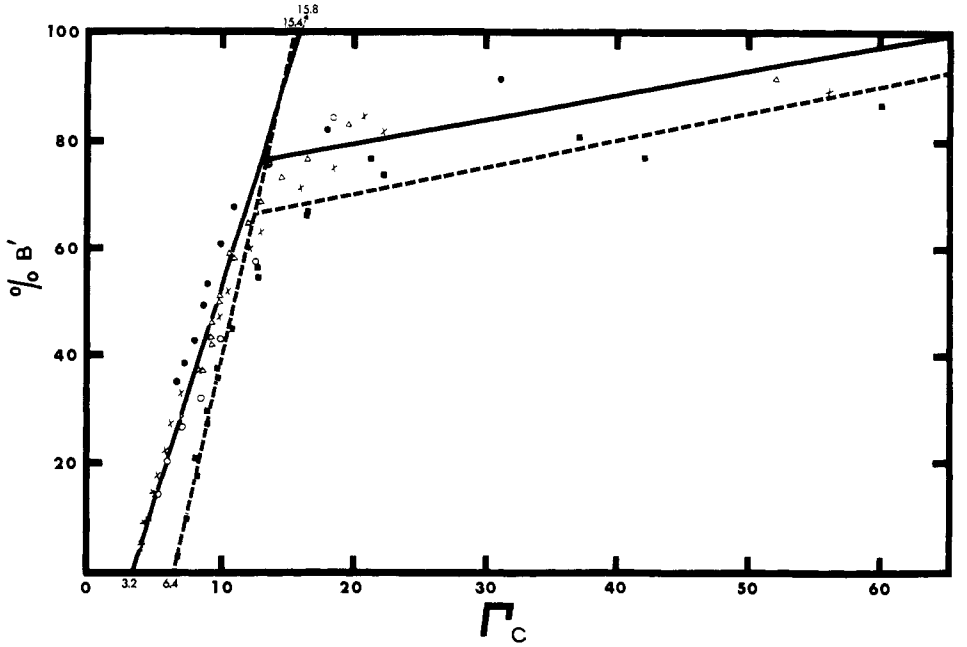

FIGURE 6: Behavior of the normalized B content of DNA as a function of the net hydration data of Cohen and Eisenberg. Values of % B′, calculated as described in the text, for solutions of DNA in NaCl (●), KCl (△), LiCl (×), CsCl (○), and NH_4Cl (■), are plotted on the ordinate against Γ_C on the abscissa. The solid line represents the common linear regression of the alkali metal salts, and the dashed line that for NH_4Cl.

NH_4Cl solutions. Both groups of points averaged ca. 16 mol of H_2O for the Γ_C intercept at 100% B′, and 20 mol of H_2O for the Γ_H intercept at 100% B′. This is somewhat lower than the equivalent averages shown in Tables I and II if Cs^+ is included. If Cs^+ is not included, however, these values are quite comparable. A value of 18 ± 2 mol of H_2O, representing an average of the results from the two sets of hydration data, would seem to be a reasonable value for this intercept freed of the initial ionic pertubation effects.

Sedimentation Velocity Studies. The behavior of the sedimentation coefficients, corrected for buoyancy effects in these electrolyte solutions, exhibits certain features which parallel the transformation of the secondary structure. In order to compare our results with those of others, we have plotted the ratio ($s_{0.1m}/s_{xm}$) as a function of hydration in Figure 7, where $s_{0.1m}$ is the corrected sedimentation coefficient, $s_{20,w}{}^0$, observed in 0.1 *m* concentration of the given electrolyte, and s_{xm} is the sedimentation coefficient in *x* molal concentration of the same electrolyte, corrected by eq 2a and 2b using the preferential solvation data taken from the literature. (Our choice of the 0.1 *m* concentration as a reference point was dictated by the fact that at the optimal concentration of DNA employed in these sedimentation velocity experiments (0.003%), the dependence of the sedimentation coefficient on DNA concentration did not become negligible until this salt concentration had been at-

Table III: Characteristics of the Linear Relationships between the Normalized B Content (%B') and the Net Hydration of DNA.

Chloride Salts (XCl)	Region	%B' vs. Γ_H (d% B'/dΓ_H)	Γ_H 100% B'	Γ_H 0% B'	Γ_H[a] Intersect	%B' vs. Γ_C (d% B'/dΓ_C)	Γ_C 100% B'	Γ_C 0% B'	Γ_C[b] Intersect
Na^+, K^+, Li^+, Cs^+	I	6.05	20.1	3.6	13.9	7.36	16.5	2.9	12
	II	1.00	51.8						
NH_4^+	I	8.51	19.4	7.6	13.5	10.9	15.6	6.4	12
	II	1.13	57.7						

[a] The symbol Γ_H intersect represents the value of Γ_H at which the two linear relationships defining regions I and II intersect. [b] The symbol Γ_C intersect represents the value of Γ_C at which the data points depart from the linear relationship defining region I.

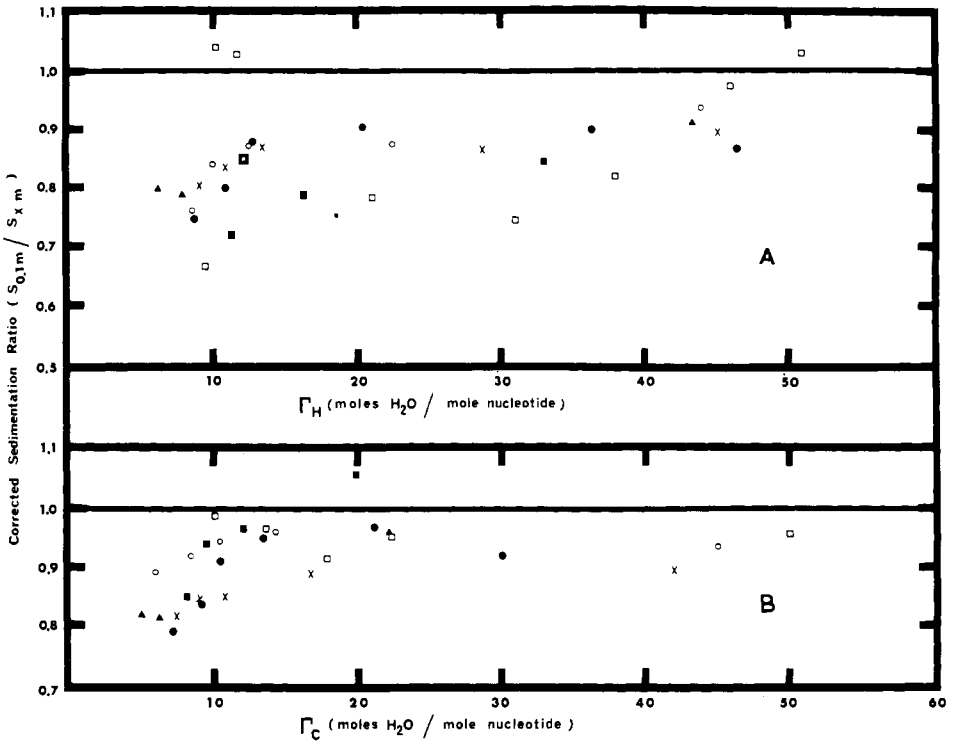

FIGURE 7: Behavior of the sedimentation coefficient ratios as a function of net hydration in aqueous electrolyte solutions. Ratios of the sedimentation coefficient, $s_{20,w}$, corrected for solvent viscosity and preferential solvation effects at a given concentration, x molal, of electrolyte, relative to its value at 0.1 m in the same electrolyte (see text) are plotted against the net hydration data of Hearst and coworkers (Γ_H) in the upper part of this figure (A) and against the data of Cohen and Eisenberg (Γ_C) in the lower part (B). The closed symbols represent the data taken from our own experiments while the open symbols represent those data taken from Pouyet et al. (1965). The various electrolytes are coded as follows: NaCl, this work (●), LiCl, this work (▲), CsCl, this work (■), NH_4Cl, this work (×), NaCl, Pouyet et al. (1965) (○), and CsCl, Pouyet et al. (1965) (□).

tained.) If the data are plotted in this fashion, we can incorporate the results of others, notably those of Pouyet et al. (1965), in order to emphasize the fact that the same behavior has been observed by others using different samples of calf thymus DNA. In the upper part of this figure (A) we have used the preferential solvation data of Hearst and coworkers, whereas in the lower part (B) we have employed the data of Cohen and Eisenberg (1968).

Since

$$s_{20,w}{}^0 = \frac{M(1 - \bar{v}_2\rho_{w,20})}{Nf} \qquad (4)$$

the sedimentation ratios should be constant and equal to 1.00, in the absence of changes in molecular weight (M) or frictional coefficient (f) of the sedimenting species. Despite the scatter in the points displayed in Figure 7A and B, it is clear that this ratio is neither 1.00 nor, in fact, constant over the entire range of hydration values. The initial decrease has been noted before by Rinehart and Hearst (1972a-c) for various species of DNA in NH_4OAc and NaCl. These authors have interpreted this effect in terms of a decrease in the frictional coefficient of the DNA due to decreases in the excluded volume and stiffness of the coil, as electrostatic repulsions along the backbone phosphate groups are damped out by increasingly higher concentrations of salt (1972b). This effect more or less levels off at about 0.5-1.0 M salt although for DNA in NH_4OAc, there does appear to be a slight increase in frictional coefficient above this salt concentration (1972a). As is obvious from the drift of the points in Figure 7, however, another somewhat more precipitous decrease in the sedimentation coefficient ratio occurs at a hydration level of ca. 12-15 mol of H_2O/mol of nucleotide. The lower values observed below this point are statistically significant. As may be recalled, this was also the hydration level at which the dependence of the B character on the hydration of DNA became more marked.

This transformation in the sedimentation coefficient below ca. 15 mol of H_2O/mol of nucleotide could be a reflection of changes in the molecular weight or the frictional

coefficient. In concentrations of LiCl greater than a concentration corresponding to a Γ of 4.5 mol of H_2O, a process occurs in which there is enhanced turbidity of the solutions and a bimodal appearance of the sedimentation coefficient distribution. This is accompanied by a characteristic appearance of the CD spectrum which we have interpreted in terms of the formation of an asymmetric micelle structure of some type (see paper III in preparation). Although it is possible that DNA is undergoing the same aggregation process in this and other electrolyte solutions at hydration levels above 4–5 mol of H_2O, the obvious earmarks of such an event are absent. We thus tend to favor the interpretation that the observed decreases in the sedimentation coefficient ratio reflects decreases in the frictional coefficients of the sedimenting species in these concentrated electrolyte solutions.

It might be noted that these sedimentation ratios of DNA in NaCl solutions are approximately equal to those in NH_4Cl at the same level of hydration. Barring a fortuitous cancellation of effects, one may conclude from these results that the use of the common curve defining the hydration of DNA in Cs^+, Na^+, and Li^+ salts to estimate the values for DNA in NH_4Cl is justified.

Conclusion

A simple inspection of these data reveals that two factors appear to be of primary importance in effecting the transconformational reactions which occur in these electrolyte solutions. One of these was apparent from the description of the experimental results given in paper I (Hanlon et al., 1975). That is, the structural conversions appeared to depend on specific interactions with the cation in the medium, as even at high hydration levels and relatively dilute solutions of these ions, the % "B" character varied from one salt to another. If the ionically unperturbed spectrum is taken as the standard reference for the "B" form (i.e., the spectrum in 0.2 *m* $(CH_3)_4NCl$ or that extrapolated to 0 *m* NaCl) then the relative efficiencies of the ions in inducing the structural transformation to a more C like state at dilute concentrations of salt will vary from ca. 0 effectiveness in the case of Na^+ to maximal effectiveness (in this series) in the case of NH_4^+ and Cs^+. The other ions fall in the order: $Na^+ < K^+ \leq Li^+ < Cs \leq NH_4$. The nature of these differences is puzzling since the order bears no relationship to ionic size and hydration (Pauling, 1960; Robinson and Stokes, 1959; Harned and Owen, 1958), relative affinities of the ion for the phosphate groups of DNA, and the fraction of phosphate sites occupied at a given ionic concentration (Ross and Scruggs, 1964a,b; Strauss et al., 1967) or any other single ionic property in aqueous solutions at room temperature. Clearly, the interaction leading to the transformation of the secondary structure of DNA by these specific ion effects involves a combination of two or more ionic properties which dictates where in the macromolecular structure they bind, and, once bound, how they affect the other important factor in these reactions, the hydration of the DNA macromolecule in solution.

Before interpreting the effects of this second factor, hydration, we would first like to review the salient features of the hydration of DNA. Based on the data and discussions of Wang (1955), Hearst (1965), Tunis and Hearst (1968a,b), Cohen and Eisenberg (1968), and Falk et al. (1962, 1963a,b), the following picture emerges: the hydration of DNA can be most usefully considered in terms of two discrete layers, a primary and a secondary shell. The primary layer consists of those water molecules immediately adjacent to the DNA duplex. This layer is in a chemically different state compared to liquid water: Its infrared spectrum indicates that the OH frequencies are not identical with those of liquid water, and it is impermeable to electrolyte in the sense that ions cannot randomly diffuse about in this layer with the same activation energy as is characteristic of liquid water.

Within this primary shell, the water molecules fall into two classes. One of these, which we shall refer to as class A, consists of those that are directly bound to sites in the DNA duplex, according to the scheme of Falk et al. (1962, 1963a,b). If the quantitative data of Falk et al. are applicable to the solution form of DNA, the number of molecules in this class should number 11–12. Five of these are associated with base sites in the grooves of the helix and have a lower affinity than the remaining six. The four water molecules bound at furanose oxygens and phosphodiester linkages are somewhat more tightly bound then those associated with the base sites. The remaining two waters associated at the ionic phosphate site as part of its hydration shell have the highest affinity. Removal of these last two generally results in complete structural disruption of DNA (Falk et al., 1963b).

The other class of water molecules (B) found in the primary hydration layer are distinguishable only insofar as the hydration layers of DNA do not evidence the infrared spectral characteristics of liquid water until about 20 water molecules have been adsorbed per nucleotide residue. Since only 11–12 of these 20 are directly site bound to DNA, this leaves about 8–9 water molecules in an interface region which is hydrogen bonded to the site bound waters, and is spectrally perturbed as a consequence. Presumably, the entire set of these class B molecules would be expected to have lower affinity for DNA than the class A molecules bound directly to the DNA sites.

Surrounding the primary shell is a secondary shell whose water structure is essentially that of liquid water found in the bulk solvent. This layer is freely permeable to the ions of the electrolyte. Most of this secondary layer may be more apparent than real since it would include the domain of the solvent in which the electrolyte content is lower than it is in the bulk solvent due to the Donnan effect.

It is anticipated that the apparent dehydration of this model, as reflected in the decreased values of the preferential solvation as the electrolyte content of the solution increases, should proceed by several mechanisms. The simplest of these is an effect on the Donnan contribution to the preferential solvation (Hearst, 1965; Cohen and Eisenberg, 1968) due to increased electrolyte concentration. This should be a nonspecific effect (as contrasted to the site binding mechanism discussed below) and would influence only the secondary shell.

Another mechanism involves a direct interaction of the cation of the electrolyte with the negatively charged phosphates, thus partially neutralizing the charge borne by each. This process should release the more loosely bound water (class B) from the primary shell in the vicinity of the phosphate groups because of the usual reversal of electrostrictive effects found for low molecular weight solutes. Although Cohen and Eisenberg disclaim this as a possibility, it is likely that at least a part of the increase in the partial specific volume of DNA with increase in electrolyte concentration (Cohen and Eisenberg, 1968) is attributable to this effect. In addition, the increase in the charge neutralization of the

polyelectrolyte will also decrease the Donnan contribution to the preferential hydration (Hearst, 1965; Cohen and Eisenberg, 1968). As such, the same interaction which displaces water from the primary shell will also have a profound effect on the "secondary" shell as well. This mechanism should clearly be a function of the identity of the cation and its affinity for the DNA phosphates.

A third mechanism which should be operative in this solution involves the removal of water from the primary shell due to the reduction of the water activity in these concentrated electrolyte solutions. As has been extensively discussed by Hearst and coworkers (Hearst and Vinograd, 1961a,b; Hearst, 1965; Tunis and Hearst, 1968a,b) and Cohen and Eisenberg (1968), the degree of hydration of all species in solution is modulated by a_w, and the hydration of the polymer component decreases with decreasing a_w, as has been experimentally observed by these workers.

With this hydration model and mechanisms of dehydration together with the specific ion effects, we can rationalize the observed changes in secondary structure by proposing that the maintenance of the "B" conformation in aqueous solution is dependent not only on the absence of perturbing cationic influences but also on the preservation of an intact primary hydration shell consisting of both A and B classes of water molecules. As this primary shell is stripped away conformational adjustments of both an inter- and intranucleotide nature ensue leading mainly to the C structure with some A structure formed in the presence of some salts.

With this additional proposal, we can now explain the differences in the slopes of the plots of % B or % B′ vs. Γ in the following manner. At high water activities in the absence of perturbing ionic effects of added electrolyte, both the secondary and the primary hydration shells are intact. As the salt concentration increases, with increasing site binding of cations, the dehydration of DNA probably initially proceeds by all of the mechanism described in the preceding paragraphs. The secondary shell is primarily affected but there is probably also some removal of the more weakly bound class B molecules of the primary layer because of ion pair formation and the lowering of the water activity of the solvent. The primary layer water molecules account for only a small fraction of total water removed, however, and hence the slopes of the plots of % B vs. Γ are relatively small in this region II.

This process continues until a Γ of 13 ± 1 mol of H_2O/mol of nucleotide is reached, at which point all of the secondary shell and most of the class B water molecules have been removed, leaving only the class A molecules of the primary shell. As the latter are removed by the further lowering of the water activity of the solvent, the fractional B content falls more precipitously, reflecting directly the sensitivity of the transformations to the state of integrity of the primary hydration shell. Since only the latter is being removed in this process, an extrapolation to 100% B yields the magnitude of the primary hydration shell minimally required to maintain the "B" conformation observed in ionically unperturbed solutions. This value, which can be estimated from the normalized % B′ data, is on the order of ca. 18 water molecules per nucleotide residue. This corresponds reasonably well with the estimate of Falk et al. (1962, 1963a,b) of the number of water molecules in the hydration shell of DNA whose infrared spectrum differed from that of liquid water.

Extrapolation of this same linear function of % B vs. Γ below Γ of 12-14 water molecules (region I) yields an average value for the alkali metal salts of 3-4 water molecules. We conclude that the C and A structures of these salts retain only the water molecules associated with the phosphate group in these media. The value of 6-8 mol of water/mol of nucleotide for the 0% B intercept of the ammonium salt is significantly higher. This together with the greater effectiveness of the ion in inducing C structure and the fact that the intercept at 100% B′ for the linear relationship in region II is also higher suggests that some rather unique ways of binding to the DNA structure may be available to this cation. It is conceivable that it is able to participate in a rather complex set of hydrogen bonded interactions with water molecules, forming bridges between base sites and phosphate oxygens, with the net effect of creating a final C or A structure which carries more water than the alkali metal salts of DNA.

Although this picture conforms to the general trend of the data, there are a couple of points which require further comment. The continuous nature of the transconformational reactions is not surprising when one considers the B → C transition. These two forms are very similar (Langridge et al., 1960; Marvin et al., 1961) and this transition involves relatively minor adjustments in the internucleotide conformational arrangements. The B → A or the C → A transitions are more profound, however, and actually require more drastic conformational changes in the furanose ring of the nucleotide (Fuller et al., 1965). Thus, it is puzzling that such a transition is not cooperative. If our supposition is correct, however, that the A form produced in these solutions is enriched in GC content (see paper I, Hanlon et al., 1975) then it is possible that the cooperativity of the transition to the A structure is masked by the polydispersity in base composition.

A second point which we would like to discuss is the discrepancy which exists between our conclusions and those of Maniatis et al. (1974). On the basis of their wide angle X-ray scattering experiments with DNA gels in 6 *M* LiCl, these authors have concluded that calf thymus DNA at this electrolyte concentration is in the B conformation. In contrast, our results reveal that at this concentration of LiCl (6.37 *m*), DNA at the dilute concentration of ca. 2×10^{-4} *M* has only 13% "B" character with the remainder of the nucleotide residues in the C (72%) and the A (12%) forms. Even the normalized B content (%B′) is only 15%. We cannot give an unequivocal explanation for this contradiction, at the present time. Certainly, the data presented for the DNA gel in 6 *M* LiCl in Figure 16 in the paper of Maniatis et al. (1974) do not appear particularly convincing, especially in view of the errors in locating scattering maxima, and calculating reference curves for the B, the C, and the A forms of DNA. In fact, the positions of the maxima in these latter reference curves of DNA presented by these authors do not agree with the previously published data of Bram (1971).

There is another factor, however, that could conceivably account for the difference between our results and those of Maniatis et al. (1974). The concentration of DNA in the gels examined by these workers was 350 mg/ml, or ca. 1 *M* in nucleotide concentration. The DNA concentration was thus not quite an order of magnitude less than the LiCl concentration, and might successfully have competed for the available water in the medium. The hydration of DNA in these gels may thus be significantly higher than it is in a dilute solution of DNA in 6 *M* LiCl. The fractional B content, since it depends on DNA hydration, might be expected

to be higher in these gels, and could conceivably approach that level found in 1 *M* NaCl, which Bram (1971) has identified as salt concentration required to generate the Watson-Crick B form.

Accompanying this transformation in secondary structure, as assessed by the CD changes, is an increase in the average sedimentation coefficient of the DNA macromolecule in these electrolyte solutions. For reasons which we have discussed, we think that this change in the sedimentation coefficient, corrected for the preferential solvation, is a reflection of a decrease in the average frictional coefficient of the DNA coil rather than an aggregation to a higher molecular weight species. The presence of the A form is clearly not a necessity for this hydrodynamic change since the data points for NaDNA and the NH_4DNA are very close. The change in the frictional coefficient is most marked in the region in which the incremental change in the hydration is very small. Thus, simple removal of water from the coil at this level of hydration should have little direct effect on the hydrated frictional coefficient unless there is an accompanying increase in the mass/unit length, flexibility, or compactness of structure.

The first of these factors, the increase in mass per unit length, is inadequate to account for the observed decreases in the frictional coefficient. Hence, this leaves one or both of the latter factors—increased flexibility and/or compactness. An attractive hypothesis is that the C and A structures lend themselves to some more compact tertiary structure which is appropriate for the packaging of DNA in in vivo structures. The actual facts, however, need not be so exotic. It may well be that the dehydration process which leads to these changes in secondary structure also induces a contraction of the DNA coil by a mechanism similar to that which, in general, occurs for an uncharged polymer in a poor solvent. Concentrated electrolyte solutions are indeed poor solvents for DNA, insofar as the available water for solvating the DNA is limited. (In fact, DNA can be salted out of very concentrated LiCl solutions.) In addition, the great numbers of cations and anions in these concentrated electrolyte solutions simultaneously provide extensive shielding of the repulsive interactions of the negatively charged phosphate groups along the DNA backbone, thus permitting parts of the DNA coil to approach itself more closely and interact in an attractive manner than would be energetically permissible at more dilute concentrations of the electrolyte.

In any event, the transformation in secondary structure reported in paper I (Hanlon et al., 1975) appears to be intimately related both to ionic interactions and the state of hydration of the nucleic acid. This relationship can be quantitatively expressed in terms of the minimal hydration of ca. 18 mol of water/mol of nucleotide required to maintain the ionically unperturbed "B" conformation. Reduction of this hydration to ca. 4 mol of H_2O leads for most salts to the production of the C and A forms, and a reduction of the frictional coefficient. Both of these changes should result in DNA molecules which are more easily packaged (Marvin et al., 1961), in biological structures such as chromosomes and phage heads. This suggests that physiological mechanisms controlling the hydration of nuclear constituents may be involved in regulating cell division processes in eucaryotes.

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