Structural Transitions of Deoxyribonucleic Acid in Aqueous Electrolyte Solutions. I. Reference Spectra of Conformational Limits†

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ABSTRACT: The circular dichroism properties of calf thymus DNA have been examined at 27° over the wavelength range of 215–300 nm in aqueous solutions of NaCl, KCl, LiCl, CsCl, and NH_4Cl at pH 7. The concentrations of these electrolytes were varied from 0.01 to ca. 5–10 *m*. The spectral changes induced by changes in concentration of NaCl and KCl and all but the highest concentrations of NH_4Cl as well as lower concentrations of CsCl and LiCl could be represented by a common two-state transition involving the conversion of the typical conservative spectrum commonly seen in dilute solutions of these salts to a nonconservative spectrum similar to that obtained by Tunis-Schneider and Maestre ((1970), *J. Mol. Biol. 52,* 521) for the C form of DNA. At higher concentrations of CsCl, LiCl, and NH_4Cl, an additional component, resembling an A type spectrum, was required to account for the observed CD changes with changing concentration of electrolyte. Relying on the published spectra of the B, the C, and the A forms of DNA by Tunis-Schneider and Maestre for identification and approximate values of the molecular ellipticities of these forms, we have analyzed these spectral transitions by two least mean squares methods in order to obtain accurate reference spectra of aqueous "B", C, and "A" conformations of calf thymus DNA. The results obtained suggest that although the C form in solution is identical with that obtained in film, the aqueous B conformational limit is not identical with the crystallographic Watson–Crick structure. In addition, the A form generated in solution under our experimental conditions appears to be more similar to that assumed by low molecular weight *Escherichia coli* DNA at 75% relative humidity rather than calf thymus DNA at the same relative humidity.

The original observations of Cheng (1965) and Tunis and Hearst (1968) that the optical rotatory dispersion (ORD) of DNA is profoundly affected by high concentrations of relatively innocuous electrolytes led to the conclusion by these authors that these salts were inducing a structural transition in DNA. Later investigations on the effects of various neutral electrolytes on the circular dichroism (CD) spectral properties reinforced this early conclusion: the CD transitions appeared to reflect a transformation on the secondary structure of the macromolecule rather than the denaturing effect observed in solutions of certain salts with highly polarizable anions (Hamaguchi and Geiduschek, 1962; Emanuel, 1960; Hanlon, 1966). With the advent of the identification of the CD spectra appropriate for the B, the C, and the A forms of DNA by Tunis-Schneider and Maestre (1970), it was concluded by these authors as well as ourselves (Hanlon et al., 1972) and others (Nelson and Johnson, 1970; Studdert et al., 1972; Ivanov et al., 1973; Zimmer and Luck, 1973) that the effects of many of these electrolytes involved the conversion of the secondary structure of DNA from the B (or Watson-Crick form) to the C form or a variant thereof. In point of fact the CD spectra of calf thymus DNA in high concentrations (ca. 6 *M*) of NH_4Cl and LiCl closely resemble (Hanlon et al., 1972) the spectrum obtained by Tunis-Schneider and Maestre (1970) of a film of LiDNA under conditions of relative humidity (r.h.) close to that required to generate the C form in fibers (Marvin et al., 1961). It might be noted, however, that the spectra of DNA obtained at low concentrations of most electrolytes generally exhibit higher values of the molecular ellipticity in the positive band, above 260 nm, than that observed in the spectrum of DNA, presumably in the B form obtained by Tunis-Schneider and Maestre (1970). Thus the actual "B" form of DNA in aqueous solutions could be structurally different from the Watson–Crick form in fibers of the Na salt at 92% r.h. This suggestion has also been made by Bram (1971).

We have been interested in these structural transitions of DNA in electrolyte solutions, for several reasons. If the data of Tunis-Schneider and Maestre (1970) were used for the identification of the B, C, and A forms of DNA, a suitable analysis of the circular dichroism changes could, in principle, provide unequivocal evidence that structural transformations between either these forms or subtle variations thereof had ensued. Accurate reference spectra, freed of artifacts which might arise in the film spectra, could thus be extracted from the spectral data. These would correspondingly be the reference spectra appropriate for the molecularly dispersed form of DNA in aqueous solutions under a variety of conditions and would be useful in analyzing the conformation of DNA present in a variety of biologically important complexes (Maestre and Tinoco, 1965; Hanlon et al., 1972; Johnson et al., 1972; Dorman and Maestre, 1973). Since certain other properties of DNA, such as hydration, are dependent on the electrolyte content of the aqueous medium, it was reasonable to expect that these structural transitions, if quantitated, could then be correlated—and rationalized—with changes of other properties of the macromolecule in these solutions.

We correspondingly undertook a detailed analysis of the

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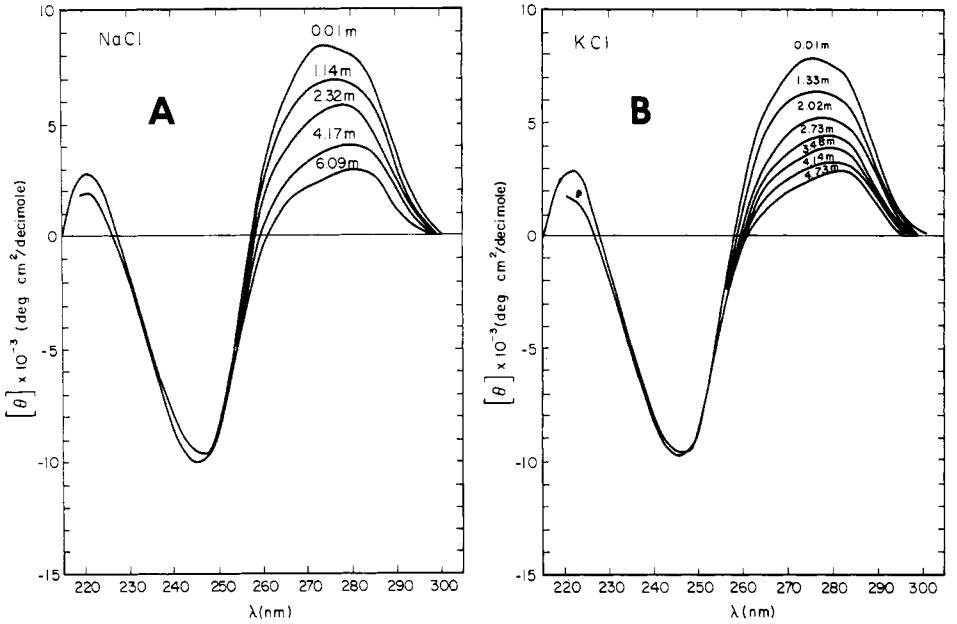

FIGURE 1: The circular dichroism spectra of calf thymus at pH 7 (27°) in aqueous solutions of varying concentrations of NaCl (A) and KCl (B). The molal concentrations of electrolyte appropriate for a given solution are printed above each spectrum.

transformation of the circular dichroism properties of calf thymus DNA in aqueous solutions containing varying concentrations of LiCl, NaCl, KCl, CsCl, and NH_4Cl. In this first paper, we describe the mathematical methods employed in analyzing the spectral transformations and the corresponding reference spectra extracted by these methods. The second paper in this series deals with the correlation of the extent of these transitions in secondary structure with the hydration of DNA in these solutions (Wolf and Hanlon, 1975). Finally a third paper will describe the further transformation of calf thymus DNA to a higher order structure at very high concentrations of LiCl. A preliminary report of these results has appeared previously (Hanlon et al., 1972; Wolf et al., 1972).

Experimental Section

A commercial preparation of calf thymus DNA (Sigma, Lot 802184) was employed in these experiments. Its protein content was determined by the Lowry method (Lowry et al., 1951) to be ≤1%. The RNA content determined by the orcinol method (Clark, 1964) was 0.6%. A concentrated stock solution was generally prepared by dissolving 10 mg in 10 ml of 0.02 *M* electrolyte. Concentrations of these stock solutions (in moles of P/l.) were determined spectrally by dilution with 0.10 *M* NaCl and 0.05 *M* NaH_2PO_4–Na_2HPO_4 buffer at pH 7.0. A molar extinction coefficient at 260 nm, ϵ_p^{260}, of 6600 M^{-1} cm^{-1} in this solvent was employed (Johnson et al., 1972).

All salts employed in the preparation of the DNA solutions were reagent grade. Solutions were prepared by weight on a Mettler H20T semi microbalance from a concentrated DNA stock solution (pH 7) in 0.02 *M* salt. A stock solution of the appropriate electrolyte at pH 7 and water were weighed in as diluents in that order. The pH of the prepared solutions was readjusted to 7.0, if necessary. No corrections were made for the salt or water activity error of the combination glass electrode used. In concentrated electrolyte solutions, this correction could be appreciable although at pH 7 it should not amount to more than 1 pH unit (Bates, 1964). The pH range of stability for DNA in these solutions is quite broad, however, and a change in pH of ±1 should lead to no appreciable effects on conformation. In fact, a few experiments conducted at lower pH (5–6) showed no difference from the pH 7 results. The concentration of the electrolyte component in these solutions was calculated as molality (*m*) and is reported as such.

Absorption spectra were obtained between 400 and 210 nm in a 1-cm cell with a Cary 14 recording spectrophotometer equipped with thermostated adaptors. Solutions were always read against a matching reference solvent. Temperature of the cell contents and adaptors was maintained at 25.0 ± 0.1° by a Haake circulating water bath. Temperature was monitored by a Yellow Springs Instrument Company bridge and thermistor assembly.

Circular dichroism spectra were measured in a 1-cm cell in the 350–200 nm range with a Cary 60 recording spectropolarimeter equipped with a 6001 CD unit. Slight drifts in the base-line positions of solutions which exhibited little light scattering were compensated by translating the base line to the experimental spectra between 350 and 330 nm where the ellipticity of DNA in solution should be 0. Results are reported in terms of molecular ellipticity, $[\theta]$, in (deg cm^2)/dmol. The calibration of the instrument was checked with an aqueous solution of *d*-10-camphorsulfonic acid, as recommended in the Cary 60/6002CD User's Manual. Prior to use, the *d*-10-camphorsulfonic acid (Eastman Kodak) was purified by recrystallization from hot 95% ethanol and dried to constant weight in vacuo at 40°.

All computations were performed with an IBM 370/155 computer. Programs for the two least mean squares regression analyses, written in Fortran IV, can be obtained from the authors upon request.

Results and Discussion

The effects of increasing the concentration of the chloride salts of Na^+, K^+, Li^+, NH_4^+, and Cs^+ on the CD spectrum of calf thymus DNA are shown in Figures 1–3. The final spectra shown in all but Figure 2A (LiCl) were obtained at saturating concentrations of the given electrolytes.

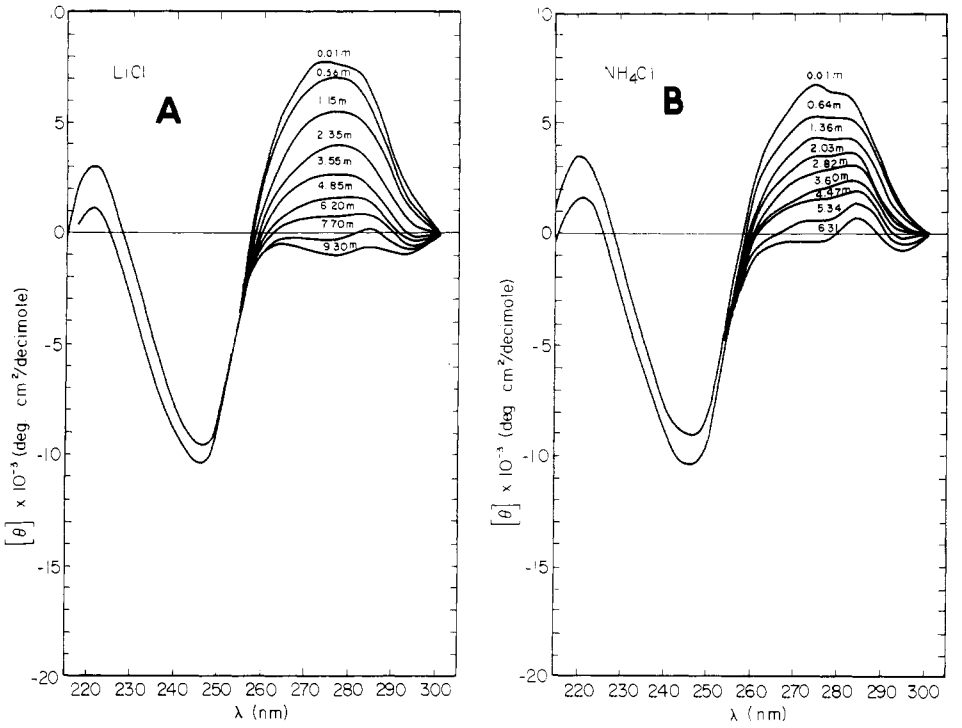

FIGURE 2: The circular dichroism spectra of calf thymus DNA at pH 7 (27°) in aqueous solutions of varying concentrations of LiCl (A) and NH_4Cl (B). The molal concentrations of electrolyte appropriate for a given solution are printed above each spectrum.

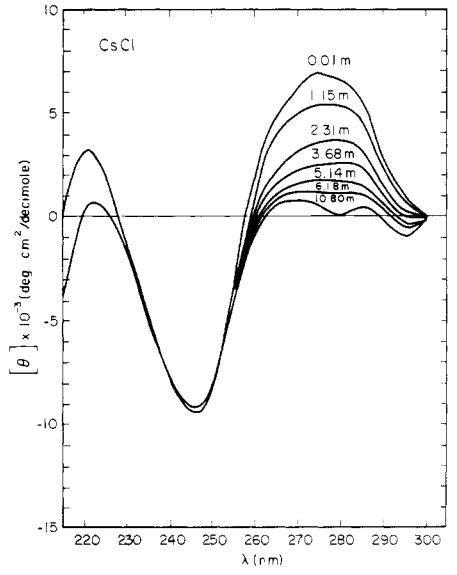

FIGURE 3: The circular dichroism spectrum of calf thymus at pH 7 (27°) in aqueous solutions of varying concentrations of CsCl. The molal concentrations of CsCl appropriate for a given solution are printed above each spectrum.

Increases in the LiCl concentrations above 8.5 *m* LiCl to saturation resulted in an additional transformation of the DNA structure in these salt solutions which will be discussed in the third paper in this series. In all cases these spectra are identical in the presence or absence of 0.005 *M* NaEDTA and hence the transformations effected by changing the concentration of electrolyte were not due to heavy metal ion contamination. These effects are also not attributable to denaturation—or base unstacking—as the extinction coefficients of DNA at 259 nm in these solutions did not vary by more than 3% over the entire range of electrolyte concentrations. This confirms a similar report by Studdert et al. (1972). Gruenwedel et al. (1971) has reported a lowering of the T_m of calf thymus DNA in concentrated solutions of four of these salts, but in all cases his values for the T_m of DNA in the presence of these salts were appreciable above 27°, the temperature at which we obtained our CD data.

The most reasonable interpretation, as discussed in the introduction, is that DNA is being transformed from a form similar to the B structure to one or more of the other secondary structures which it assumes in fiber form. (The spectra in 6.3 *m* NH_4Cl and 7.7 *m* LiCl are very close, in fact, to the spectrum of films of LiDNA at 75% r.h., published by Tunis-Schneider and Maestre (1970).) As a first step in testing this hypothesis, we had to first ascertain whether the transitions observed in the presence of varying concentrations of a given electrolyte represented a simple two-state transition or would more than two components be required to describe the observed effects. Although more sophisticated methods are available for such determinations, the method which we utilized was a simple linear least mean squares analysis of our data. We chose this approach because a slight variation of it also permitted us to extract accurate versions of the various reference spectra of the secondary structures of components in solution without changing the basic computer program.

In formulating our equations for this analysis, we began with the assumption, common to all spectral analyses, that the observed optical signal at a given wavelength λ_j, represents a linear combination of the contributions of the various components present in the solution. In this present case, the observed molecular ellipticity in solution i, $[\theta]_{\lambda_j}^{\text{obsd},i}$, is the sum

$$[\theta]_{\lambda_j}^{\text{obsd},i} = \sum_k f_k [\theta]_{\lambda_j}^{K} \qquad (1)$$

where f_k is the mole fraction of the kth structural compo-

nent whose reference spectrum (at $f_k = 1.00$) has a ellipticity of $[\theta]_{\lambda_j}^{K}$ at λ_j.

If only two structural components are present—the B and the C forms of DNA, for example—then eq 1 is simply

$$[\theta]_{\lambda_j}^{\text{obsd},i} = f_B[\theta]_{\lambda_j}^{B} + f_C[\theta]_{\lambda_j}^{C} \quad (2)$$

In dilute solutions of these various electrolytes, presumably we should be starting out with all of the bases in one form—the B. As the salt concentration increases with consequent conversion of the B to the C form, f_c should increase at the expense of f_B. A series of i intermediate spectra—1, 2, ... n—should thus be obtained whose shape confirms this conversion.

The simplest confirmation of a two-state transition is the existence of a common isoelliptic point. Unfortunately, no isoelliptic points were detected in any of our spectra except those at the highest LiCl concentrations. The lack of a common intersection point thus required that a somewhat more complicated analysis be employed to test for the presence of a two-state transition.

It can be shown that if only two forms are present and the intermediate spectra can be expressed as a linear combination of the contributions of the reference spectra of these two forms, then intermediate spectra 2 through $(n - 1)$ can also be expressed as a linear combination of the first, 1, and the last, n, intermediate spectrum, even though these observed spectra, 1 and n, do not represent the true reference spectra. Furthermore, the hypothetical fractions, or coefficients, in the expression

$$[\theta]_{\lambda_j}^{\text{obsd},2\leq i\leq n-1} = f_B'[\theta]_{\lambda_j}^{1} + f_C'[\theta]_{\lambda_j}^{n} \quad (3)$$

should also sum to 1.00, within the limitation of experimental error, if the observed transformation obeys all of the criteria of a two-state transition.

The coefficients, f_B' and f_C', could be obtained from the observed experimental data, without superimposing the requirement that they sum to 1.00, in the following manner. Equation 3 can be rearranged and summed in order to obtain a residual, R', for a conventional two component least mean squares analysis

$$R' = \sum_j (f_{B_i}'[\theta]_{\lambda_j}^{1} + f_{C_i}'[\theta]_{\lambda_j}^{n} - [\theta]_{\lambda_j}^{\text{obsd},2\leq i\leq n-1})^2 \quad (4)$$

R' is then differentiated with respect to f_{B_i}' and f_{C_i}' and set equal to 0 in order to minimize the sum of the squares. The resulting two simultaneous equations

$$0 = f_B' \sum_j ([\theta]_{\lambda_j}^{1})^2 + f_C' \sum_j ([\theta]_{\lambda_j}^{n})([\theta]_{\lambda_j}^{1}) - \sum_j ([\theta]_{\lambda_j}^{\text{obsd},2\leq i\leq n-1})([\theta]_{\lambda_j}^{1}) \quad (5)$$

$$0 = f_B' \sum_j ([\theta]_{\lambda_j}^{1})([\theta]_{\lambda_j}^{n}) + f_C' \sum_j ([\theta]_{\lambda_j}^{n})^2 - \sum_j ([\theta]_{\lambda_j}^{\text{obsd},2\leq i\leq n-1})([\theta]_{\lambda_j}^{n}) \quad (6)$$

can then be solved unequivocally for f_{B_i}' and f_{C_i}' for each of the designated i solutions $(2 \leq i \leq (n - 1))$.

Some typical results of this analysis are presented in Table I for a few solutions of DNA in the various electrolytes employed in these experiments. The concentrations of salt employed as the reference limits, $[\theta]_{\lambda_j}^{1}$ and $[\theta]_{\lambda_j}^{n}$, are also given in this table. For NaCl, KCl, low concentrations of LiCl, and all but the highest concentration of NH_4Cl, the sum of $(f_B' + f_C')$ was found to be 1.00 ± 0.06. In CsCl, LiCl, and NH_4Cl, however, the use of solutions containing high concentrations of electrolyte for values of $[\theta]_{\lambda_j}^{n}$ resulted in a failure of these fractions to sum to 1.00, thus revealing that in the higher concentrations of these electrolytes, at least, more than two components were required to account for the observed data.

Table I: Test Linear Least Mean Squares of a Two-State Transition of DNA in Aqueous Solutions of Electrolytes.

Electrolyte	Molal Concn of Salt in Solutions Analyzed (m)	Molal Concn of Salt in "Reference" Solutions (m)		Sum Test ($f_B' + f_C'$)
		$[\theta]^1$ $f_B' = 1.00$	$[\theta]^n$ $f_C' = 1.00$	
NaCl	0.57	0.050	6.09	0.96
	1.14	0.050	6.09	1.00
	1.68	0.050	6.09	0.94
	3.55	0.050	6.09	1.02
	4.17	0.050	6.09	1.00
	4.84	0.050	6.09	0.97
KCl	1.11	0.56	4.73	1.01
	2.02	0.56	4.73	0.98
	2.93	0.56	4.73	1.02
	4.14	0.56	4.73	0.98
NH_4Cl	0.52	0.10	2.82	1.02
	0.65	0.10	2.82	0.96
	1.36	0.10	2.82	0.98
	0.52	0.10	4.47	1.06
	0.65	0.10	4.47	1.01
	1.36	0.10	4.47	1.06
	2.11	0.10	4.47	1.04
	2.87	0.10	4.47	0.96
	5.40	0.10	7.38	1.13
LiCl	1.68	0.86	2.88	1.00
	1.73	0.86	2.88	0.98
	2.32	0.86	2.88	0.95
	2.88	0.86	7.40	1.23
	3.58	0.86	7.40	0.48
	5.03	0.86	7.40	0.29
	6.41	0.86	7.40	0.09
CsCl	2.31	1.15	10.8	0.69
	3.68	1.15	10.8	0.69
	5.14	1.15	10.8	0.66
	6.18	1.15	10.8	0.74
	9.14	1.15	10.8	0.81

Method I. Having established which of these electrolyte solutions could be analyzed in terms of two spectral components, we next attempted to extract accurate reference spectra from these data. In the first approach, referred to herein as method I, we simply used a variant of the linear least mean squares procedure described above, in which we transformed f_B and f_C into $1/f_C$ and (f_B/f_C), or, α and β, respectively. In this case, the expression for the residual, R, becomes

$$R = \sum_j \left\{ \left(\frac{1}{f_C}\right)[\theta]_{\lambda_j}^{\text{obsd},i} - \left(\frac{f_B}{f_C}\right)[\theta]_{\lambda_j}^{B} - [\theta]_{\lambda_j}^{C} \right\}^2 \quad (7a)$$

or

$$R = \sum_j \{\alpha[\theta]_{\lambda_j}^{\text{obsd},i} - \beta[\theta]_{\lambda_j}^{B} - [\theta]_{\lambda_j}^{C}\}^2 \quad (7b)$$

In this form, $[\theta]_{\lambda_j}^{\text{obsd},i}$ and $[\theta]_{\lambda_j}^{B}$ are now cast as the error-free independent variables, with $[\theta]_{\lambda_j}^{C}$ as the dependent variable whose approximate values are known. R is then differentiated with respect to α and β, and these expressions for the partial derivatives are set equal to 0 in order to minimize the sum of the squares. The two simultaneous equations, similar in structure to eq 5 and 6, are then solved for

α and β and the best fitting C spectrum correspondingly was calculated. As was previously the case for f_B' and f_C', no restriction is placed on the relationship between α and β, or f_B and f_C. If, however, the system can indeed be represented by a two-state transition, and a reasonable set of approximate values have been substituted for $[\theta]_{\lambda_j}^{C}$ (i.e., a set of values with no systematic deviations) then $(\alpha + \beta)$ or $(f_B + f_C)$ for each individual solution should sum to 1.00. If the sum is indeed 1.00, one can also be reasonably confident that the effects of the individual cation on the actual reference spectra are either negligible or else can be accounted for in terms of the two extreme references employed. For those solutions which did not meet the requirements of a two-state transition, this same approach could be expanded (as described in subsequent sections) to accommodate additional spectral components.

Method II. The above described Method I could utilize an approximate version of the C spectrum, but needed an accurate version of the B spectrum, for the calculations. A different least mean squares analysis referred to herein as method II could avoid this latter requirement in those cases which met the criteria of a two-state transition. Using this method we could obtain both the best fit for the B reference spectrum as well as the C spectrum, by substituting the "approximate" values of both.

In this approach, the residual, G, to be minimized is a double summation of values over both wavelength, j, and

$$G = \sum_{i=1}^{n} \sum_{j=1}^{m} \{[\theta]_{\lambda_j}^{\text{obsd},i} - f_{B_i}[\theta]_{\lambda_j}^{B} - (1 - f_{B_i})[\theta]_{\lambda_j}^{C}\}^2 \quad (8)$$

solution, i, indices. The residual, G, is differentiated with respect to f_{B_i}, $[\theta]_{\lambda_j}^{B}$, and $[\theta]_{\lambda_j}^{C}$, and set equal to 0 to give $(2m + n)$ equations. That is

$$\left.\begin{matrix}\text{set of}\\ n\\ \text{equations}\end{matrix}\right\} \left(\frac{\partial G}{\partial f_{B_i}}\right) = 0 = 2\sum_{j=1}^{m} \{[\theta]_{\lambda_j}^{\text{obsd},i} - f_{B_i}[\theta]_{\lambda_j}^{B} - (1 - f_{B_i})[\theta]_{\lambda_j}^{C}\}\{-[\theta]_{\lambda_j}^{B} + [\theta]_{\lambda_j}^{C}\} \quad (9)$$

$$1 \le i \le n$$

$$\left.\begin{matrix}\text{set of}\\ m\\ \text{equations}\end{matrix}\right\} \left(\frac{\partial G}{\partial [\theta]_{\lambda_j}^{B}}\right) = 0 = 2\sum_{i=1}^{n} \{[\theta]_{\lambda_j}^{\text{obsd},i} - f_{B_i}[\theta]_{\lambda_j}^{B} - (1 - f_{B_i})[\theta]_{\lambda_j}^{C}\}(-f_{B_i})\} \quad (10)$$

$$1 \le j \le m$$

$$\left.\begin{matrix}\text{set of}\\ m\\ \text{equations}\end{matrix}\right\} \left(\frac{\partial G}{\partial [\theta]_{\lambda_j}^{C}}\right) = 0 = 2\sum_{i=1}^{n} \{[\theta]_{\lambda_j}^{\text{obsd},i} - f_{B_i}[\theta]_{\lambda_j}^{B} - (1 - f_{B_i})[\theta]_{\lambda_j}^{C}\}\{-(1 - f_{B_i})\} \quad (11)$$

$$1 \le j \le m$$

These equations can be simplified as

$$\left.\begin{matrix}\text{set of}\\ n\\ \text{equations}\end{matrix}\right\} 0 = \sum_{j=1}^{m} \{[\theta]_{\lambda_j}^{\text{obsd},i} - f_{B_i}[\theta]_{\lambda_j}^{B} - (1 - f_{B_i})[\theta]_{\lambda_j}^{C}\}\{[\theta]_{\lambda_j}^{B} - [\theta]_{\lambda_j}^{C}\} \quad (12)$$

$$1 \le i \le n$$

$$\left.\begin{matrix}\text{set of}\\ m\\ \text{equations}\end{matrix}\right\} 0 = \sum_{i=1}^{n} f_{B_i}\{[\theta]_{\lambda_j}^{\text{obsd},i} - f_{B_i}[\theta]_{\lambda_j}^{B} - (1 - f_{B_i})[\theta]_{\lambda_j}^{C}\} \quad (13)$$

$$1 \le j \le m$$

Table II: Standard Deviations of Simulated Spectral Data Used in and Calculated by Methods I and II.

Spectrum	Maximum Error $\times 10^{-3}$ [(deg cm²)/dmol]	D_{used}[a] Methods I	D_{used}[a] Methods II	D_{calcd}[b] Methods I	D_{calcd}[b] Methods II
Q	±0.1, random	0.07	0.07		0.08
K	±0.5, random	0.33	0.33	0.19	0.19
	±0.5, alternating	0.34	0.34	0.08	0.06
K	±1.0, random	0.61	0.61	0.15	0.08
	±1.0, alternating	0.61	0.61	0.12	0.06
B (expt)	±0.3, random	0.19	0.19		0.31
C (method I)	±2.0, random	1.39	1.39	0.64	0.32

[a] Symbol defined by eq 15 in text. [b] Symbol defined by eq 16 in text.

and, finally, subtracting eq 11 from 10, we obtain a third equation

$$\left.\begin{matrix}\text{set of}\\ m\\ \text{equations}\end{matrix}\right\} 0 = \sum_{i=1}^{n} \{[\theta]_{\lambda_j}^{\text{obsd},i} - f_{B_i}[\theta]_{\lambda_j}^{B} - (1 - f_{B_i})[\theta]_{\lambda_j}^{C}\} \quad (14)$$

$$1 \le j \le m$$

The resulting $(2m + n)$ equations containing $(2m + n)$ unknowns (m accurate values each of $[\theta]_{\lambda_j}^{B}$ and $[\theta]_{\lambda_j}^{C}$ and n values of f_{B_i}) can be solved by iteration, starting with approximate values of $[\theta]_{\lambda_j}^{B}$ and $[\theta]_{\lambda_j}^{C}$.

Tests of Methods I and II Using Simulated Data. In order to estimate the reliability of methods I and II in yielding accurate reference spectra, we created and tested an artificial set of spectral data which resembled, to a certain extent, the B and the C reference spectra in magnitude and shape. Two exact reference spectra, Q and K, shown in Figure 4, were first drawn as smooth curves without marked structure. The values of $[\theta]_{\lambda_j}^{Q}$ and $[\theta]_{\lambda_j}^{K}$ were tabulated at 16 equispaced wavelength positions. These values, referred to as $[\theta]_{\lambda_j}^{Q,\text{true}}$ and $[\theta]_{\lambda_j}^{K,\text{true}}$, were then combined in various fractional contributions in order to create a series of exact intermediate spectra with known contributions of the Q and the K components (i.e., 90% Q, 80% Q, 60% Q, 50% Q, 30% Q, and 10% Q) which always summed to 100.0% (i.e., $f_Q + f_K = 1.00$). Errors were then imposed on the exact values of $[\theta]_{\lambda_j}$ of the Q, K, and the intermediate spectra, appropriate to the random experimental errors which we anticipated. In the case of the Q and intermediate spectra, this amounted to a variable increment in ellipticity, whose maximum value was $\pm 0.10 \times 10^3$ (deg cm²)/dmol. The actual incremental amount added to any individual value of $[\theta]_{\lambda_j}$ was indicated by a table of random numbers and the sign of the increment by a flip of a coin. Larger maximum errors of $\pm 0.5 \times 10^3$ and $\pm 1.0 \times 10^3$ (deg cm²)/dmol were assigned in a similar manner to the K spectrum, which was the C spectrum analog. In the latter error assignments, the sign of the increment added was alternated in a systematic fashion in one series and determined randomly by a flip of a coin in another.[1]

[1] With relatively few intermediate spectra and only 16 points per spectrum to manipulate, this procedure could hardly be expected to result in a completely random error assignment. The systematic deviations introduced by this lack of ideality, however, were not found to be greater than that anticipated in the actual experimental situation.

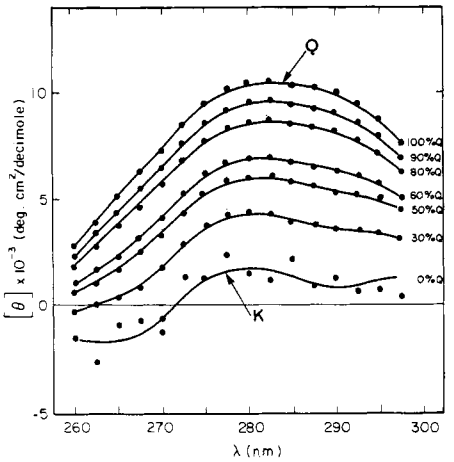

FIGURE 4: Simulated spectral data used in testing methods I and II. Two continuous artificial reference CD spectra, designated as Q and K, are displayed as the extreme limits of 100% Q and 0% Q (100% K) together with the expected intermediate spectra consisting of varying proportions of the Q and the K components. The points on the curve represent the values of $[\theta]_{\lambda_j}$ with added random errors of maximally $\pm 0.1 \times 10^3$ [(deg cm^2)/dmol] in the case of the Q and the intermediate spectra, and $\pm 1.0 \times 10^3$ [(deg cm^2)/dmol] for the K spectrum. These points represent the data employed in the tests of methods I and II, as described in the text.

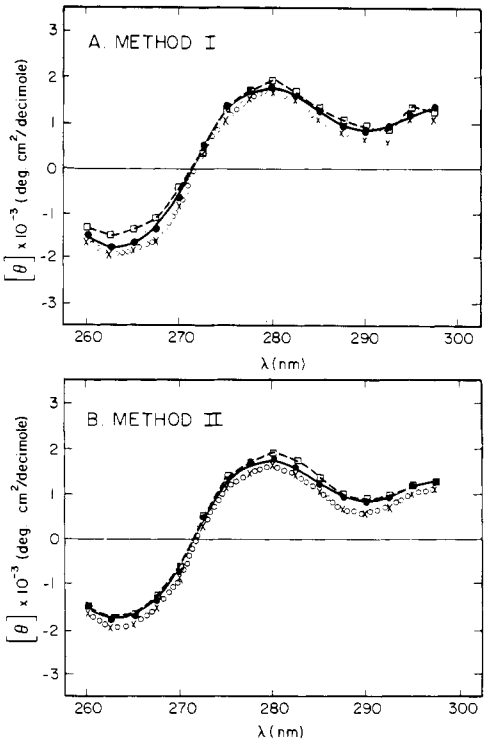

FIGURE 5: Comparison of the K spectra calculated by methods I and II with the actual K spectrum, prior to addition of errors. The actual K spectrum, before added errors (●) is displayed together with the calculated spectra using the Q spectrum and the generated intermediate spectra with maximal errors of $\pm 0.1 \times 10^3$ [(deg cm^2)/dmol] together with approximate K spectra containing random maximal errors of $\pm 0.5 \times 10^3$ (deg cm^2)/dmol (○) and $\pm 1.0 \times 10^3$ [(deg cm^2)/dmol] (□). Frame A represents the results of method I and frame B represents the results of method II.

These spectral data with the incorporated errors were then analyzed by methods I and II. Figure 4 presents the spectral data, as points on the smooth curves, used in the analysis and Figure 5 shows the calculated K spectra obtained from the two methods. Values of a standard deviation function, D, of the data employed ($[\theta]_{\lambda_j}^{\text{used}}$) and those calculated ($[\theta]_{\lambda_j}^{\text{calcd}}$) are shown in Table II. These two deviations are

$$D_{\text{used}} = \sqrt{\frac{\sum_j ([\theta]_{\lambda_j}^{\text{used}} - [\theta]_{\lambda_j}^{\text{true}})^2}{j}} \quad (15)$$

$$D_{\text{calcd}} = \sqrt{\frac{\sum_j ([\theta]_{\lambda_j}^{\text{calcd}} - [\theta]_{\lambda_j}^{\text{true}})^2}{j}} \quad (16)$$

$[\theta]_{\lambda_j}^{\text{true}}$ are the known values of $[\theta]_{\lambda_j}$ for the K and the Q spectra prior to superimposing the described errors.

A more stringent test of the two methods, for our particular situation, could be made after reasonably accurate versions of the B and the C reference spectra were available. The actual calculated results for the C spectrum obtained by method I together with the aqueous B spectrum determined experimentally, as described in the subsequent part of this section, were taken to be the true values, and used for the construction of an artificial set of intermediate spectra. Errors were then imposed on all spectral values in the manner previously described. Our error limits for this second test were somewhat more generous, being maximally $\pm 0.3 \times 10^3$ (deg cm^2)/dmol for the B and the intermediate spectra, and $\pm 2 \times 10^3$ (deg cm^2)/dmol for the C spectrum. The results of this second test, in terms of the standard deviation functions, are also given in Table II. These standard deviations represent upper limits to our expected error in the generation of these two reference spectra.

These results demonstrated that the two methods could be expected to yield reasonably accurate versions of the C spectrum, as well as the B spectrum. In both these tests, method II yields a somewhat better fitting C spectrum, but only at the expense of accuracy in the B spectrum. The extent of the error introduced into the calculated B spectrum depends on the distribution and magnitude of the errors of all spectra analyzed. In the first test, the increase in the standard deviation of the analog of the B spectrum was negligible and would certainly point to method II as the method of choice in generating the C spectrum. In the second test, however, the actual B spectrum calculated by method II is substantially more inaccurate than the input spectrum, and one is hard put to choose which of the two methods should be employed for generating the C spectrum under these error conditions. Ideally, however, the two methods should be used in conjunction, since agreement between the calculated reference spectra obtained will confirm the absence of systematic errors in the approximate reference limit spectra employed. For the purpose of calculating the fractions of residues in each conformation, use of either method yields comparably accurate values. For these latter calculations, it does not matter basically whether the error in the reference spectra is contained in only one limit, or is proportioned between the two limits employed.

Limiting Reference Spectrum of the Aqueous B Form. In order to obtain the accurate values of $[\theta]_{\lambda_j}^{\text{B}}$ of the aqueous B spectrum, required by method I, we extrapolated the spectral data gathered at fairly low concentrations of salt (ca. 0.06–0.02 *m*) to 0 concentration of the given electrolyte. In this range, the changes in the ellipticities of the positive band leveled off and, in fact, the spectra became independent of electrolyte concentration between 0.04 and 0.01 *m*. Surprisingly, however, the spectra in this range were different for the five cations employed in these experiments.

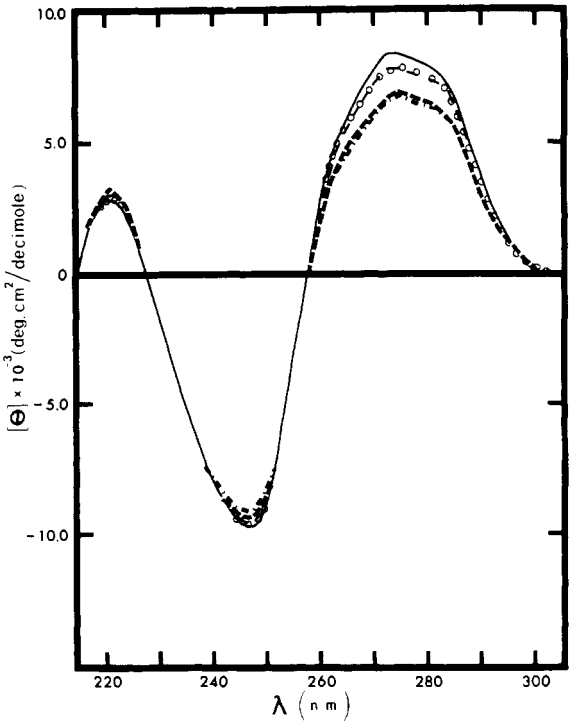

FIGURE 6: Circular dichroism spectra of calf thymus DNA at pH 7 (27°) in dilute concentrations of electrolytes. The spectra displayed above are the average obtained in 0.01 *m* and 0.04 *m* concentrations of NaCl (—), KCl (— —), LiCl (○), CsCl (■ • ■•) and NH_4Cl (■ ■ ■).

This point is demonstrated by spectra in 0.01 *m* salt solutions, reproduced in Figure 6 for comparison. Although the curves for Na^+, K^+, and Li^+ were very close, they were not identical, and those for NH_4^+ and Cs^+ were quite significantly different.

These differential effects, which, in fact, have been observed by others (Adler and Fasman, 1971; Rinehart and Hearst, 1972), could not be attributed to variations in the degree of base stacking or partial denaturation at these low ionic strengths. This was demonstrated by the fact that the extinction coefficients of DNA were unaffected by the nature and concentration of the five electrolytes in the range of concentration employed in these experiments. In addition, the molecular ellipticities of the negative band, centered at 247 nm, exhibited no significant change.

Rather, it seemed reasonable to assume that these differences in the positive CD band in these salts represented the differences in efficiency of the various cations in inducing a transformation in conformational character common to all the salt solutions. Because of electrostatic effects, a large fraction of the DNA phosphates should be neutralized by site binding even at low concentrations of the cations of the salts employed. For reasons which were not immediately apparent, Na^+, according to this hypothesis, appeared to be least efficient in distorting the B structure of DNA toward that of the C, whereas NH_4^+ was the most efficient.

In an effort to obtain a reference spectrum unperturbed by specific cationic interactions, the spectral properties of DNA were examined in the presence of tetraalkylammonium salts. Solutions of DNA in 0.1 *M* tetrabutylammonium chloride were slightly hyperchromic and the CD bands were distorted and shifted to red. The spectra obtained in this solvent were definitely not an extension of the C to B transition.

Table III: Reference Spectra of the B Form of DNA in Aqueous Solutions of Electrolytes.

	$[\theta]_{\lambda_j}^{B} \times 10^{-3}$ [(deg cm²)/dmol]				
λ_j (nm)	Experimental B Spectrum obtained from NaCl Experiments[a]	Result of Method II			
		NaCl	KCl	NH_4Cl	Av
297.5	0.55	0.77	0.64	0.86	0.74
295.0	1.34	1.16	1.37	1.56	1.42
292.5	2.40	2.50	2.69	2.37	2.31
290.0	3.94	4.08	3.89	4.10	3.99
287.5	5.48	5.36	5.68	5.40	5.39
285.0	6.83	6.88	6.71	6.95	6.86
282.5	7.90	8.15	7.76	7.96	7.90
280.0	8.30	8.55	8.58	8.25	8.31
277.5	8.54	8.45	8.80	8.51	8.51
275.0	8.56	8.51	8.98	8.68	8.74
272.5	8.35	8.63	8.64	8.48	8.57
270.0	7.90	7.70	7.84	7.85	7.90
267.5	7.23	6.99	6.77	7.02	7.00
265.0	6.23	5.92	5.61	6.12	6.04
262.5	4.95	4.86	4.89	4.98	5.03
260.0	2.83	2.97	2.46	2.65	2.69
255.0	−3.33				
250.0	−8.84				
245.0	−9.77				
240.0	−8.01				
235.0	−5.08				
230.0	−1.68				
225.0	1.86				
220.0	2.86				
215.0	−0.01				
SD[b]	±0.11 (297.5–260 nm)				±0.16
	±0.14 (297.5–215 nm)				

[a] Average of four determinations. [b] SD is the mean average of the individual standard deviation calculated for each wavelength. That is

$$SD = \sqrt{\frac{\left\{[\theta]_{\lambda}{}^{B} - \left(\frac{[\theta]_{\lambda}{}^{B}}{n}\right)\right\}^2}{(n-1)}}\Big/m$$

where n is number of solutions analyzed, and m is the number of wavelength values over which the average was taken.

In 0.2 *M* tetramethylammonium chloride, however, the hypochromicity was normal and the CD curves were identical with those obtained at low concentrations of NaCl. The average of the latter were correspondingly used as the limiting reference spectrum of the aqueous "B" form. These values are tabulated in column 2 of Table III, and displayed as a spectrum in Figure 7A. After an accurate C spectrum was obtained (see below), the validity of using these data from the NaCl and tetramethylammonium chloride experiments for analyzing the transitions in the other electrolyte solutions could be tested by the sum criterion: use of an inappropriate B limit spectrum in the method I analysis of those solutions undergoing a two-state transition would yield fractions of the two spectral components which failed to sum to 1.00.

The Approximate C Reference Spectrum. Both methods I and II required approximate values of the C spectrum which exhibited no systematic deviations. A spectrum was available for unoriented films of LiDNA, obtained by Tunis-Schneider and Maestre (1970) under conditions of r.h. (75%) sufficient to generate the C form (Marvin et al., 1961). We took the spectral values from Figure 5 in the publication of these authors and attempted a further refinement of the data using an approach which was a qualitative version of method II. In those electrolyte solutions which appeared to be undergoing a two-state transition, we calculated values of f_{B_j} at each wavelength, λ_j, for 16 wave-

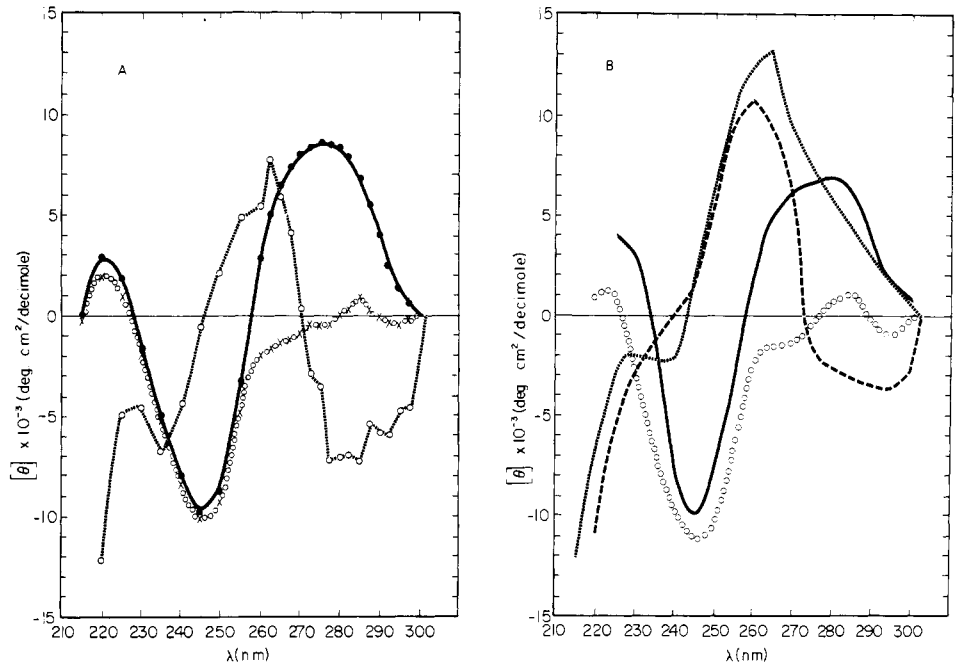

FIGURE 7: Circular dichroism spectra of DNA in the B, C, and A secondary structures. (A) The circular dichroism spectra obtained in this work. (● — ●) Spectrum of calf thymus DNA extrapolated to 0 concentration of NaCl, and used as the aqueous B spectrum in methods I and II; (×O O×) average C spectrum calculated by method I; (O ■ ■ O) the A spectrum of DNA, calculated by method I using data from the LiCl solutions. (B) Circular dichroism spectra taken from Tunis-Schneider and Maestre (1970). (—) The Na salt of a film of calf thymus DNA at 92% r.h., taken from Figure 2 of the cited paper and identified as the B form spectrum; (O O O) the Li salt of a film of calf thymus DNA at 75% r.h., taken from Figure 5 of the cited paper and identified as the spectrum of the C form. (■ ■ ■) the Na salt of calf thymus DNA at 75% r.h., taken from Figure 3 of the cited paper, and identified as the spectrum of the A form of calf thymus DNA; (- - -) the Na salt of a film of *E. coli* DNA at 75% r.h., taken from Figure 4 of the cited paper and identified as the spectrum of the A form of *E. coli* DNA.

lengths across the positive band (where the difference between the spectrum of the B and the C forms is most marked) using

$$f_{B_j} = \frac{[\theta]_{\lambda_j}^{obsd,\,i} - [\theta]_{\lambda_j}^{C'}}{[\theta]_{\lambda_j}^{B} - [\theta]_{\lambda_j}^{C'}} \qquad (17)$$

The symbol $[\theta]_{\lambda_j}^{C'}$ represents the values that were obtained from the data of Tunis-Schneider and Maestre (1970) and $[\theta]_{\lambda_j}^{B}$ were the experimental values of the B spectrum obtained as described above. These individual values of f_{B_j} were then inspected for systematic drifts across the band of a number of spectra of DNA solutions in the given electrolyte series. Depending on the direction and magnitude of these trends in f_{B_j}, increments were then added or subtracted to both the B reference spectrum and the approximate C spectrum, until more or less self-consistent values of f_{B_j} had been obtained across the positive band for several solutions whose average f_B values differed significantly. The f_{B_j} at λ_j was then mean averaged for each solution ($\bar{f}_{B_i}$) and values of $[\theta]_{\lambda_j}^{C,i}$ of each ith solution were calculated as

$$[\theta]_{\lambda_j}^{C,\,i} = \frac{[\theta]^{obsd,\,i} - \bar{f}_{B_i}[\theta]_{\lambda_j}^{B}}{(1 - \bar{f}_{B_i})} \qquad (18)$$

These values of $[\theta]_{\lambda_j}^{C,i}$ were also mean averaged at each wavelength position and substituted as the approximate values of $[\theta]_{\lambda_j}^{C}$ in the equations of method I and method II. These approximate values are given in column 2 of Table IV.

As has been previously noted, the most marked differences in the spectral characteristics of the B and C forms occurred in the wavelength range between 260 and 297.5 nm. Correspondingly, the analyses of the spectral data by methods I and II were restricted to this range. Values of $[\theta]_{\lambda_j}^{obsd,i}$ taken at 2.5-nm intervals in this range were substituted together with values of $[\theta]_{\lambda_j}^{B}$ and $[\theta]_{\lambda_j}^{C}$ (obtained as described above) in the equations of methods I and II. The results in terms of the best fitting B and C reference spectra are shown in Figures 7A and 8 and in Tables III and IV. Table III gives the B spectral data, determined both experimentally from the NaCl data as well as that determined by method II. In Table IV, the best fitting values of the C spectrum obtained by method I are given for the individual salt solutions in columns 3, 4, and 5 with the grand average of all three presented in column 6. In columns 7, 8, and 9 the best fitting C spectra are presented for the different salt series calculated by method II together with the grand average for all electrolytes in column 10. Figure 8A displays the positive band of the B spectra, determined experimentally and calculated by method II. Figure 8B compares the two calculated grand average C spectra with that obtained by Tunis-Schneider and Maestre (1970), and the spectrum of DNA in ethylene glycol obtained by Nelson and Johnson (1970).

The B spectrum determined experimentally and that obtained by method II are essentially identical within the standard deviations expected. As anticipated, methods I and II yield comparable results for the calculated C spectra. In fact, their averages are identical within the limitations of the standard deviations of the salt series and the standard deviations predicted by the simulated data. Use of the C spectrum obtained in method I for the approximate C spectrum in method II leads to no noticeable improvement in the number of cycles of iteration required by method II to yield convergent values of $[\theta]_{\lambda_j}^{B}$ and $[\theta]_{\lambda_j}^{C}$. These facts taken together rule out the possibility that significant sys-

Table IV: Reference Spectra of the C Form of DNA in Aqueous Solutions of Electrolytes.

		$[\theta]\lambda_f^C \times 10^{-3}$ [(deg cm²)/dmole]							
		Results of Method I				Results of Method II			
λ_f (nm)	Approximate C Spectrum Used	NaCl	KCl	NH_4Cl	Av	NaCl	KCl	NH_4Cl	Av
297.5	−0.50	−0.05	−0.34	−0.33	−0.24	−0.24	−0.38	−0.68	−0.42
295.0	−0.70	−0.38	−0.60	−0.63	−0.54	−0.22	−0.63	−0.87	−0.61
292.5	−0.68	−0.11	−0.34	−0.72	−0.39	−0.19	−0.62	−0.59	−0.30
290.0	−0.30	−0.02	−0.12	−0.28	−0.14	−0.13	−0.11	−0.42	−0.19
287.5	0.39	0.35	0.28	−0.01	0.21	0.42	0.15	0.24	0.35
285.0	0.80	0.79	0.89	0.74	0.81	0.74	0.96	0.62	0.78
282.5	0.45	0.48	0.73	0.52	0.57	0.26	0.81	0.43	0.57
280.0	−0.05	0.06	0.05	0.00	0.04	−0.17	−0.21	0.02	0.00
277.5	−0.42	−0.52	−0.44	−0.64	−0.52	−0.47	−0.67	−0.59	−0.50
275.0	−0.65	−0.54	−0.59	−0.43	−0.52	−0.56	−0.90	−0.61	−0.70
272.5	−0.74	−0.53	−0.73	−0.30	−0.53	−0.75	−0.88	−0.57	−0.75
270.0	−0.80	−1.01	−1.05	−0.84	−0.97	−0.81	−1.03	−0.81	−0.99
267.5	−0.80	−1.23	−1.21	−1.27	−1.29	−1.07	−0.95	−1.07	−1.09
265.0	−1.00	−1.38	−1.44	−1.41	−1.41	−1.21	−1.05	−1.36	−1.34
262.5	−1.80	−1.92	−1.67	−1.59	−1.73	−1.88	−1.63	−1.62	−1.79
260.0	−2.80	−2.05	−2.10	−1.94	−2.03	−2.25	−1.77	−1.84	−1.95
				SD[a]	±0.12			SD[a]	±0.18
		Data Given below were Calculated by Eq 18 in Text							
255.0		−4.90	−4.80	−5.17	−4.96				
250.0		−9.45	−9.06	−9.58	−9.36				
245.0		−10.43	−9.92	−10.08	−10.14				
240.0		−9.00	−8.66	−8.04	−8.56				
235.0		−5.36	−5.33	−5.23	−5.31				
230.0		−2.21	−2.02	−1.79	−2.01				
225.0		0.40	0.44	1.79	0.88				
220.0		1.40	1.75	2.45	1.87				
215.0		−0.35		−0.26	−0.31				
				SD[a]	±0.19				

[a] SD function defined in Table III. Values above represent variation between the three electrolyte series.

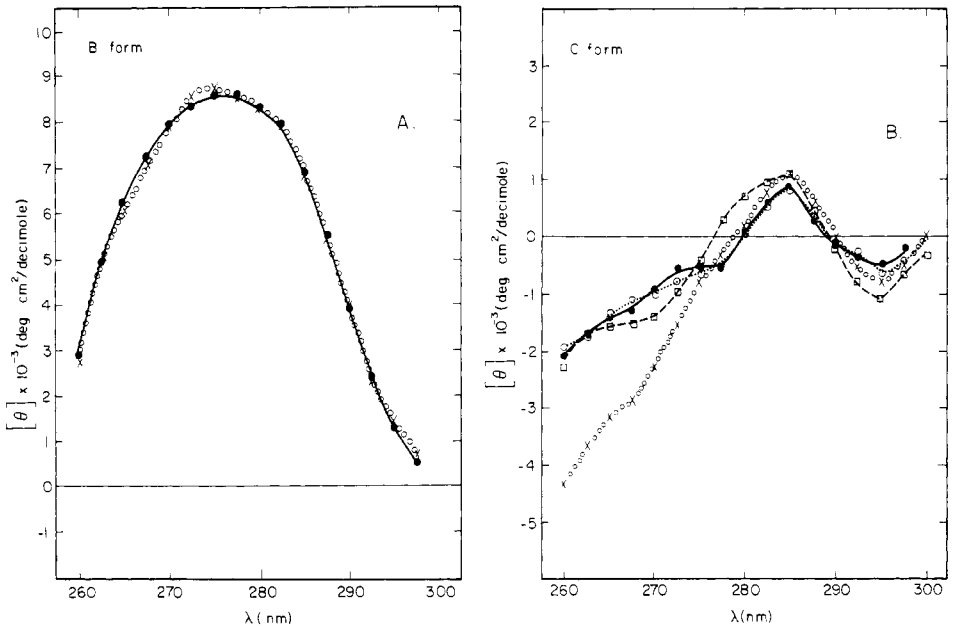

FIGURE 8: Comparison of the reference spectra of calf thymus DNA between 260 and 297.5 nm, calculated by methods I and II. (A) The B reference spectra of DNA obtained by extrapolation of the NaCl data at low concentrations of salt (●—●) and the average spectrum calculated by Method II (×OO×). (B) The C reference spectra of DNA: (●—●) and (O - - - O) The average C spectra obtained in this work by methods I and II, respectively; (□ - - □) the data for a film of Li DNA at 75% r.h., taken from Figure 5 of Tunis-Schneider and Maestre (1970); (×OO×) the spectrum of calf thymus DNA in 100% ethylene glycol, taken from Figure 1 of the paper by Nelson and Johnson (1970).

tematic errors were present in the approximate reference spectra employed in these calculations.

In addition to giving the best fitting reference spectra between 260 and 300 nm, methods I and II also give the best coefficients α and β, or f_{C_i} and f_{B_i}, for the individual solutions. For a given solution, the values obtained by the two methods agreed with ±2%. The correlation between these values and other properties of the DNA solutions will be discussed in paper II. These fractional values of the B and the C forms were used together with eq 18, and the experi-

mentally determined reference spectrum for the B form to calculate the remainder of the C reference spectrum below 260 nm. The entire C spectrum calculated in this manner is also displayed in Figure 7A. (For consistency among the spectra presented in this figure, data from method I was used.)

Some characteristic values of f_B and f_C for individual solutions analyzed by method I are given in Table VI. The sum of $(f_B + f_C)$ for those solutions which could be analyzed as two-state transitions was found to be 1.00 ± 0.04, thus confirming the fact that the limiting "B" spectrum obtained from the NaCl data was appropriate for the other electrolytes. This automatically means that the limiting spectra obtained at low concentrations of the other electrolytes can be represented as linear combinations of the limiting B and the C reference spectra. That this is indeed the case is demonstrated by the initial entries in Table VI which give the fractions of spectral components found in 0.01–0.04 *m* concentrations of the individual salts.

Analysis of Solutions Which Did Not Conform to the Two State Criterion. In most solutions of LiCl and CsCl, as well as the highest concentrations of NH_4Cl, the observed spectra could not be fitted within the standard error of the analysis with the average values of $[\theta]_{\lambda_j}^B$ and $[\theta]_{\lambda_j}^C$ determined by methods I and II. In LiCl, part of the spectral distortions at very high concentrations could be attributed to the formation of a higher molecular weight aggregate (see paper III in preparation), which at ca. 13 *m* was large enough to create visible turbidity and sediment out of solution at 1*g*. In CsCl and high concentrations of NH_4Cl, there was no evidence of any aggregation process occurring in these solutions, and yet the spectra were also distorted.

The shape of the distortion in the 260-nm region of these spectra shown in Figures 2 and 3 suggested that perhaps an additional A structure was being formed at these high salt concentrations. In order to ascertain whether this was a feasible idea, we utilized the same approach as described for method I. A third term was added to the right-hand side of eq 2 and this expression was then rearranged as

$$R = \sum_{\lambda_j} \left\{ \left(\frac{1}{f_A}\right)[\theta]_{\lambda_j}^{obsd} - \left(\frac{f_B}{f_C}\right)[\theta]_{\lambda_j}^{B} - \left(\frac{f_C}{f_A}\right)[\theta]_{\lambda_j}^{C} - [\theta]_{\lambda_j}^{A} \right\}^2 \quad (19a)$$

$$R = \sum_{\lambda_j} \{\alpha'[\theta]_{\lambda_j}^{obsd} - \beta'[\theta]_{\lambda_j}^{B} - \gamma[\theta]_{\lambda_j}^{C} - [\theta]_{\lambda_j}^{A}\}^2 \quad (19b)$$

R was then differentiated with respect to α', β', and γ. The three equations expressing the partial derivatives were set equal to 0 and the values of α', β', and γ were solved for, as in the previous simpler case where only two coefficients were involved. In this analysis, the data over the wavelength range of 260–297.5 nm were again utilized. Values of $[\theta]_{\lambda_j}^B$ and $[\theta]_{\lambda_j}^C$, determined by method I (Tables III and IV), were substituted in the resulting equations.

The choice of the appropriate A reference spectrum presented a problem. Substitution of the data of Tunis-Schneider and Maestre (1970) for films of calf thymus NaDNA under conditions appropriate for the A form in fiber (Fuller et al., 1965) gave values of the sum of $(f_A + f_B + f_C)$ for individual solutions considerably different from 1.00. The spectrum of *E. coli* DNA, presumably in the A form, published by these same authors gave a sum closer to but still differing significantly from 1.00. To solve this dilemma, we made the assumption that this sum was indeed equal to 1.00 (±0.06), and then undertook an initial refinement process for generating this spectrum, similar to that described for our initial choice of the approximate C reference spectrum. As our starting values we used the A spectrum of *E. coli* DNA. The results of this trial and error approach is given in column 2 of Table V as the approximate A spectrum of DNA employed in the least mean squares analysis. Use of this spectrum resulted in an effective sum of 1.00 ± 0.06 for the A, B, and C components in NH_4Cl, CsCl, and up to 7.7 *m* LiCl, thus, verifying our assumption that the spectral data for DNA in these solutions required only three components to account for the transitions. The fractional amounts, as well as the sum, of these various components in NH_4Cl, LiCl, and CsCl are presented in Table VI. The results of the spectral analysis of the best fitting A spectrum in the LiCl, NH_4Cl, and CsCl solutions are shown in columns 3, 4, and 5 of Table V together with the grand average for all three salt solutions in column 6. As expected, the discrepancies between the several salt solutions are much greater than that observed for the C spectrum. This is probably due to accumulated error of the B and C values as well as the presence of only a small amount of A component in solution. The maximum value of f_A, for instance, in no case exceeds 16%. A plot of the average A spectrum for LiCl is shown in Figure 7A.

The remainder of the A spectrum below 260 nm was calculated in the same manner as has been described for the C spectrum below 260 nm. That is, the average values of f_B, f_C, and f_A, available from the least mean squares analysis of a given solution, *i*, together with the values of $[\theta]_{\lambda_j}^B$, $[\theta]_{\lambda_j}^C$, and $[\theta]_{\lambda_j}^{obsd}$ at λ_j were substituted in the expression

Table V: Reference Spectra of the "A" Form of DNA in Aqueous Solutions of Electrolytes Calculated by Method I.

	$[\theta]\lambda_j^A \times 10^{-3}$ [(deg cm²)/dmol]				
λ_j (nm)	Approximate A Spectrum	LiCl	NH_4Cl	CsCl	Grand Av
297.5	−2.70	−3.03	−6.65	−4.63	−4.77
295.0	−3.50	−3.47	−5.72	−5.08	−4.76
292.5	−5.60	−5.64	−5.94	−6.52	−6.03
290.0	−5.80	−5.76	−6.18	−5.62	−5.85
287.5	−5.30	−5.32	−5.51	−5.55	−5.46
285.0	−6.00	−6.03	−7.35	−8.38	−7.25
282.5	−5.90	−6.01	−6.53	−8.45	−7.00
280.0	−4.60	−4.23	−8.55	−8.59	−7.12
277.5	−2.80	−2.64	−4.51	−5.55	−4.23
275.0	−2.60	−2.37	−4.99	−3.61	−3.66
272.5	−1.20	−0.82	−6.62	−1.32	−2.92
270.0	2.20	2.33	−3.10	1.41	0.21
267.5	4.50	4.82	1.97	5.58	4.12
265.0	5.80	6.21	5.51	5.66	5.79
262.5	7.50	7.28	9.09	6.93	7.77
260.0	5.90	5.87	7.24	3.01	5.37
				SD[a]	±1.46
	Data Given below were Calculated by Eq 20 in Text				
255.0		3.79	6.25	4.57	4.87
250.0		3.73	1.87	0.77	2.12
245.0		1.15	−2.40	−0.30	−0.52
240.0		−1.55	−9.08	−2.54	−4.39
235.0		−4.62	−11.04	−4.68	−6.78
230.0		−5.72	−8.67	0.29	−4.70
225.0		−7.43	−13.09	0.82	−4.99
220.0		−11.52	−19.47	−5.32	−12.10
				SD[a]	±2.15

[a] SD function defined in Table III. Values represent variation between the three electrolyte series.

Table VI: Fractional Distribution of the A, B, and C Conformations of DNA in Various Electrolyte Solutions, as Calculated by Method I.

NaCl (m)	0.01	1.14	2.32	2.93	4.17	4.84	6.09			
f_B	0.99	0.82	0.68	0.61	0.49	0.43	0.35			
f_C	0.01	0.12	0.34	0.39	0.55	0.58	0.67			
Sum	1.00	0.94	1.02	1.00	1.04	1.01	1.02			
KCl (m)	0.01	0.56	1.38	2.73	3.50	4.14	4.73			
f_B	0.91	0.84	0.70	0.53	0.47	0.38	0.34			
f_C	0.09	0.19	0.28	0.48	0.51	0.64	0.69			
Sum	1.00	1.03	0.98	1.01	0.98	1.02	1.03			
LiCl (m)	0.01	0.77	1.68	2.62	3.56	4.54	5.52	6.41	7.40	8.53
f_B	0.93	0.76	0.59	0.44	0.35	0.26	0.21	0.13	0.09	0.04
f_C	0.07	0.19	0.46	0.51	0.61	0.64	0.67	0.72	0.80	0.77
f_A	0	$0 \leqslant f_A \leqslant 0.04$		±0.04	0.05	0.08	0.09	0.12	0.14	0.15
Sum	1.00	0.95	1.05	0.96	1.01	0.96	0.97	0.97	1.03	0.96
NH_4Cl (m)	0.01	0.10	0.52	1.10	2.03	2.82	3.67	5.34	6.42	7.38
f_B	0.80	0.77	0.70	0.61	0.44	0.36	0.30	0.14	0.07	0.02
f_C	0.20	0.25	0.34	0.39	0.55	0.62	0.65	0.83	0.82	0.93
f_A	0	0	0	0	0	0	$0 \leqslant f_A \leqslant 0.04$	0.05	0.06	0.09
Sum	1.00	1.02	1.04	1.00	0.99	0.98	0.95	1.02	0.96	1.04
CsCl (m)	0.01	1.15	2.31	3.68	5.14	9.14	10.8			
f_B	0.80	0.67	0.46	0.34	0.26	0.16	0.11			
f_C	0.20	0.23	0.51	0.54	0.72	0.77	0.73			
f_A	0	$0 \leqslant f_A \leqslant 0.04$		0.07	0.10	0.14	0.16			
Sum	1.00	1.00	0.97	0.95	1.08	1.07	1.00			

$$[\theta]_{\lambda_j}^{A,i} = \frac{[\theta]_{\lambda_j}^{obsd,i} - f_B[\theta]_{\lambda_j}^{B} - f_C[\theta]_{\lambda_j}^{C}}{f_A} \quad (20)$$

These values of $[\theta]_{\lambda_j}^{A}$ were then mean averaged at each wavelength for these solutions evidencing significant amounts of the A component. These results are displayed in Figure 7A as a continuation of the average A spectrum for LiCl below 260 nm.

Conclusion

Figure 7B displays the reference spectra obtained by Tunis-Schneider and Maestre (1970) which have served as our identification spectra. Figure 7A, as previously indicated, shows an equivalent set of spectra obtained by method I. Obviously, if the assignments of Tunis-Schneider and Maestre (1970) are incorrect, then so are ours. There is no justification for assuming, however, that these authors are in error, or that the transitions which DNA undergoes in fiber do not occur in film. In fact, independent measurements by Brahms et al. (1973) and Pilet and Brahms (1973) using the technique of linear dichroism in the infrared have confirmed the fact that the B to C and B to A transitions do indeed occur reproducibly in films of DNA from a variety of sources (including calf thymus) as the r.h. is lowered if conditions are adequately defined and the r.h. is carefully controlled. Furthermore, once the transition has occurred, there is no further change in conformational parameters until that point is reached where the secondary structure undergoes total disorganization due to the extreme degree of dehydration of the macromolecule. Thus, both the C and the A structure in films appear to be end points in these transitions from the B form.

The B and the C spectra of DNA obtained by Tunis-Schneider and Maestre (1970) are more or less equivalent in shape to the ones which we have obtained from our data, although the values of $[\theta]_{\lambda_j}$ in some regions of the spectra differ significantly. Some of these differences are probably attributable to optical artifacts, such as light scattering, associated with the film spectra. The published B spectrum of Tunis-Schneider and Maestre (1970) differs most significantly and systematically from our limit "B" spectrum in the magnitude of the ellipticities of the positive band. Visual inspection of their B spectrum, reproduced in Figure 7B, suggests that the responsible conformation may contain a sizable fraction of C character, compared to the aqueous solution form. This would not be surprising in view of the tighter site binding and partial dehydration of the nucleic acid which may prevail at 92% r.h. (see discussion in paper II). Whatever the contributory factors may be, however, these data suggest that the structure of DNA in film or fiber at 92% r.h.—that is, the Watson–Crick, or crystallographic B form—is not identical with the electrostatically unperturbed "B" structure found in aqueous solution.

The differences in the positive CD bands of the Watson–Crick B and the aqueous "B" form suggest that the latter has a smaller winding angle (i.e., the rotational angle between base pairs), and perhaps a smaller tilt angle. This same conclusion has also been drawn by Ivanov et al. (1973) who pointed out the correlation between the magnitude of the ellipticity at 280 nm of DNA spectra in aqueous electrolyte solutions and the wide angle X-ray scattering results of Bram (1971). In these latter studies, Bram has suggested that in NaCl concentrations between 0.05 and 0.13 M, the winding angle is smaller than that of the Watson–Crick B form, and does not approximate it until an NaCl concentration of 1 M is reached. We agree with this conclusion and point out that in 1 M (or ca. 1 m) NaCl solutions, the magnitude of the positive CD band is comparable to that in the B spectrum of Tunis-Schneider and Maestre (1970).

In the case of the various A spectra, however, there is a marked discrepancy not only in the magnitude of $[\theta]_{\lambda_j}$ but in the actual shape of the spectra as well. As Figure 7B shows, however, the same order of discrepancy is also observed in the results of Tunis-Schneider and Maestre, as well. The A spectrum of the Na salt of calf thymus DNA differs appreciably from the one obtained for *E. coli* DNA. In addition, differences in the spectral properties of the NH_4 salt of *E. coli* DNA as a function of hydration have also been observed by Maestre (1970). He has suggested

that a given DNA is capable of assuming a variety of A-like geometries as the r.h. is varied.

The differences between these various A spectra could indeed reflect subtle differences in secondary structure between the various conformations, all of which could be classified as members of the A family. As Pilet and Brahms (1973) have shown, the r.h. at which Na salt of a given DNA undergoes the B → A transition is dependent on both the base composition and the ionic content of the environment. Once the transition has been effected, there are small differences in the geometry of the A structure of the several species of DNA examined. We assume that the differences in geometry between the various DNAs reflect the effects of small differences in base composition. Thus, the observed differences in the CD properties of the A form of calf thymus and *E. coli* DNA in films of similar electrolyte content exposed to the same r.h. might thus be rationalized on this basis.

Arguing along these lines, we propose that the fraction of base pairs undergoing the transition to the A form in these DNA solutions which we have examined is enriched in G-C content relative to the average of the calf thymus population (42% GC). It is possible that some of the heavy satellite DNA (Kurnit et al., 1973) is being converted to an A form, in preference to the C form, because of its high GC content. Thus the A spectrum which we obtain from our solution data is closer to that obtained in film for *E. coli* DNA whose average G-C content (50%) is higher than the average for calf thymus.

It might be argued, of course, that our A spectrum is not actually a contribution due to a different conformational component but rather reflects the correction of the C spectrum appropriate for the higher ionic concentrations of NH_4Cl, LiCl, and CsCl. This correction could conceivably be necessary due to changes in the refractive index of the medium surrounding the bases and/or specific cation association effects. A significant effect of refractive index is unlikely, in view of the similarity of the C spectrum observed in media of such vastly different refractive indices as found in film (Tunis-Schneider and Maestre, 1970), aqueous electrolyte solutions (this work), and organic solvents such as ethylene glycol (Nelson and Johnson, 1970) and ethanol (Girod et al., 1973). Although it is true that the spectrum observed in ethylene glycol, reproduced in Figure 7B, does exhibit rather significant differences in the 260–270 nm region, the values of $[\theta]_{260-270}$ in this solvent of higher refractive index are more negative, whereas the A spectrum is positive, and in fact, has a peak at 262.5 nm. The differences observed in the C spectrum obtained in ethylene glycol and that obtained in film and aqueous solutions are probably due to a small contribution of a super or folded form in that medium, whose spectral properties are similar to ψ DNA (Jordan et al., 1972) which is reported to be in a folded form (Maniatis et al., 1974).

In addition, one would also expect the effect of refractive index to be continuous, whereas a significant amount of the A spectral component does not appear until the ionic concentrations are quite high (see Table VI). As pointed out in the previous section, the B and the C spectra obtained from the data in NaCl, KCl, and NH_4CL are quite adequate for the analysis of the spectral data in LiCl and CsCl up to concentrations on the order of 3 *m*.

A specific cation association effect with a weak association constant is a somewhat more acceptable explanation for a C spectrum distortion at the higher ionic concentrations. We have no evidence for such associations, however. In principle, one would expect that the absorption spectrum of DNA in these media to be affected in the event of such associations. Although the absorption spectrum does indeed show a small red shift, and a slight (3%) depression of the extinction coefficient at 259 nm, these effects are more or less uniform over the range of electrolyte concentrations employed and are nonspecific with respect to the nature of the cation.

In the absence of any evidence for the fact that the A spectrum obtained by our analysis is a correction factor for the C spectrum at higher electrolyte concentrations, we favor the interpretation that the spectrum reflects the properties of a conformational state of DNA different from the B and the C secondary structures. Its shape is generally what would be anticipated for an A like structure, and hence we have concluded that this conformationally different form belongs to the A family.

It is, of course, possible that this conformationally different state represents a difference in both secondary and tertiary structure. This would mean that our A spectrum is actually a mixed spectrum consisting of the contribution due to an A secondary structure and the contribution due to a super or tertiary structure taken up by the molecule at the extremely high salt concentration. This point will be explored in paper III (in preparation).

In conclusion, we have established that calf thymus DNA in all of the electrolyte solutions examined undergoes a common structural transition at 27° from a form similar to but not identical with the Watson-Crick B structure to the C form, as the electrolyte concentration increases. In solutions containing high concentrations of LiCl, CsCl, and NH_4Cl, it is proposed that a small amount of an A type secondary structure appears as well, although the nature of its geometry is uncertain. The present analysis does not permit us to make a decision as to whether the structural conversions occur sequentially (i.e., B → C → A) or concomitantly (B → C; B → A).

We wish to emphasize the fact that the averaging procedures which we have employed in these studies automatically preclude the detection of changes in the fine structure of the CD spectra. Thus, marked asymmetries in the base composition of the *intermediate* stages in the structural conversions or *subtle* differences in the secondary structure of DNA in the presence of these various electrolytes will be overlooked if these changes do not produce profound effects on the optical properties. The limiting reference spectra which we report represent average spectra, reflecting average conformations of calf thymus DNA in these aqueous solutions under conditions where electrostatic charge repulsion is either minimized or nonexistent.

There is also the possibility, of course, that these reference spectra themselves represent linear combinations of contributions of more extreme hypothetical forms—for example, the T structure of Ivanov et al., (1973)—which have not been observed by other methods. Indeed, some of the extreme low temperature spectra of Studdert et al. (1972) and those obtained in mixed MeOH-electrolyte solutions by Ivanov et al. (1973) look as though they were simple extensions of the aqueous B → C transition. It is unlikely that these extreme forms occur at room temperature under normal aqueous conditions. Thus, the spectra provided by these analyses should be useful in quantitatively analyzing the conformation of DNA in biologically important complexes in dispersed form in aqueous solution.

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