Structural Transitions of Calf Thymus DNA in Concentrated LiCl Solutions†

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ABSTRACT: The solubility, sedimentation, circular dichroism, and absorption spectral characteristics of calf thymus DNA have been examined in concentrated solutions of LiCl (6–13 *m*) at 25 to 27 °C. At all concentrations of LiCl, the DNA is base stacked and exhibits normal hypochromicity. At the upper end of this range of LiCl concentrations, DNA aggregates and ultimately precipitates completely from solution between 13 and 14 *m* LiCl. This aggregation process is dependent on concentration, base composition, and molecular weight of DNA. The sedimentation velocity data taken together with the absorbance spectral data suggest that the aggregation process leading to the formation of *large* structures begins at ~9 m. Prior to the onset of aggregation, the circular dichroism (CD) spectra can be adequately fitted by a linear combination of contributions of the B, C, and A forms of DNA (Hanlon, S., Brudno, S., Wu, T. T., and Wolf, B. (1975), *Biochemistry 14,* 1648). Above 9 m LiCl, both factor analysis and a primitive version of matrix rank order analysis indicate that *at least* one additional spectral component is required to account for the observed CD spectra above 260 nm. The general shape of this additional component or distortion resembles the ψ form of DNA.

In previous publications, we have reported on our investigations of the effects which simple 1:1 electrolytes have on the spectral properties of DNA at pH 7 and 27 °C (Hanlon et al., 1972; 1975; Wolf and Hanlon 1975). Using the spectral data of Tunis-Schneider and Maestre (1970) as references, we demonstrated that the changes in the circular dichroism spectrum of calf thymus DNA induced by increasing concentrations of NaCl, KCl, LiCl, CsCl, and NH_4Cl could be interpreted mainly in terms of a transformation of a form resembling the Watson–Crick B structure to the C conformation, which is a helical variant of the B form. An additional minor component, whose spectrum resembled that of an A form, appeared in the more concentrated solutions of LiCl, CsCl, and NH_4Cl.

The extent of these transformations could be correlated quantitatively with the magnitude of the preferential hydration of DNA in these electrolyte solutions. The B conformation appears to be associated with a primary hydration shell of ca. 18 mol of H_2O/mol of nucleotide. Removal of all but three to four of these water molecules (presumably from the grooves of the helix) in concentrated solutions of the alkali metal salts leads to complete conversion to a set of conformations consisting of 83% C and 17% A. It was speculated that the latter fraction of A forms corresponds to the heavy satellite species of calf thymus DNA (Kurnit et al., 1973) whose GC content is too high to permit the assumption of a stable C form (Pilet and Brahms, 1973).

On the basis of this model, one would expect the conformational transitions to be complete at ca. 9 *m* LiCl, where the preferential hydration of DNA is on the order of 3 to 4 mol of H_2O/mol of nucleotide. The spectrum of DNA, however, continues to change as the LiCl concentration exceeds this value. Initial experiments revealed that this further transformation cannot be accounted for satisfactorily by a linear combination of the reference spectra of the A, B, and C forms used for the analysis of the data below 9 *m*. Finally, the extent of the additional transformation is roughly correlated with the appearance of a fast-moving species in sedimentation velocity experiments, although the parallel is not exact (Hanlon et al., 1972).

In an effort to elucidate the nature of this final transformation of the DNA structure, we have undertaken the current detailed analysis of the optical and sedimentation properties of DNA in concentrated LiCl solutions. Our results are interpreted in terms of a transition to an ordered micellar structure, as dehydrating conditions in the LiCl solvent become extreme.

Experimental Section

The commercial preparation of calf thymus DNA (Sigma, Lot 802184) examined in these experiments was that used in previous studies (Johnson et al., 1972; Hanlon et al., 1975; Wolf and Hanlon, 1975) and its properties have been reported in these cited publications. Briefly, its RNA content was 0.6% and its protein content was ≤1%. Its extinction coefficient at the maximum (259 nm) was 6600 M^{-1} cm^{-1} on a molar phosphorus or molar nucleotide basis in 0.10 M NaCl, 0.05 M NaH_2PO_4/Na_2HPO_4 buffer at pH 7. This extinction coefficient was employed to estimate the concentration of stock solutions of DNA. The latter were routinely prepared at a concentration of ca. 1 mg/mL in 0.02 *m* LiCl, adjusted to pH 7 by small additions of HCl or NaOH, and then diluted precisely with 0.1 M NaCl, 0.05 M NaH_2PO_4, Na_2HPO_4, pH 7, for purposes of estimating concentrations of the stock solution. All concentrations of DNA quoted in the text are on a residue basis (i.e., moles of nucleotide/liter).

Dilute solutions of DNA in LiCl were prepared by weight on a Mettler H2OT semimicrobalance from appropriate weights of the concentrated DNA stock solution, water, and a concentrated stock solution of LiCl (15 *m*) whose pH had been adjusted to 7 prior to use. Weighing and transfer operations were performed as quickly as possible in order to avoid extensive absorption of atmospheric water by solutions of high LiCl concentration. The LiCl was dry reagent-grade material

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obtained from Fisher Scientific Chemical Co. The LiCl concentrations in these DNA solutions are uniformly given in molal units (mol of LiCl/1000 g of H_2O). The pH of the DNA solutions so prepared was readjusted to 7 if necessary, and the solutions were stored for 24 h, prior to use, in glass-stoppered flasks sealed with parafilm.

All pH measurements were performed with a Radiometer 4 pH meter equipped with a combination glass electrode. No corrections were made for the salt and water activity errors of the glass electrode. For many of the solutions examined, this correction may be appreciable, amounting to perhaps ±1 at pH 7. Because DNA's pH range of stability is quite broad in these solvents, however, errors in pH measurements of this sort were not thought to be a serious problem.

Absorption spectra were obtained between 350 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 of the same LiCl concentration. Temperature of the cell contents was maintained constant by a circulating water bath (Haake) and was monitored by a Yellow Springs Instrument bridge and probe assembly. Except for the thermal melting profiles, spectra were generally obtained at 25.0 ± 0.1 °C. Melting profiles of DNA were obtained at a concentration of 0.15 mM in the solvent of interest by measuring the absorbance between 25 and 95 °C. In these experiments, the reference solution was also heated to a matching temperature.

Solubility studies of DNA between 12 and 13 *m* LiCl were conducted with 1 mM DNA solutions, gently agitated at 25 °C for 20 min, a period of time sufficient to ensure that an equilibrium state had been achieved at this temperature. These solutions were then centrifuged in a Sorvall bench-top centrifuge at 10 000 rpm for 10 min, in order to separate the precipitated DNA from the supernatant. The precipitate, after re-solution, and the supernatant fractions were dialyzed back to 0.02 M NaCl, pH 7. After a final clarification by low-speed centrifugation (2000 rpm, 10 min), these various fractions were examined for several properties as described in the text. Their base compositions were determined by analyzing their absorption spectra at 25 °C by the method of Hirschman and Felsenfeld (1966).

Sedimentation coefficients of DNA solutions in top-loading cells of 12- and 1.5-mm thickness were obtained with a Spinco Model E analytical ultracentrifuge equipped with absorption optics. Films taken with the latter system were scanned with an Analytrol densitometer. The DNA concentration in these experiments was routinely 0.13 mM, and the salt concentration ranged from 0.10 *m* NaCl to 12 *m* LiCl. Under these conditions, the values reported for the sedimentation coefficients of DNA represent those at infinite dilution. In an effort to establish a concentration dependence of the fractional amounts of fast and slow components, a few experiments were performed at a concentration of 0.28 and 1.2 mM.

When single values of the sedimentation coefficient are reported, these correspond to the median sedimentation coefficient, s_{50}, obtained by following the half-height position of the boundary. Sedimentation-distribution profiles were also calculated, using three time frames, by the method of Schumaker and Schachman (1957). In measuring fractional amounts of the fast and slow components, corrections were made for radial dilution.

All of the observed sedimentation data, (s^0_{obsd}), both median values and distributions, were corrected to the conventional anhydrous state, $s^0_{20,w}$ corresponding to a medium with the density, $\rho_{20,w}$, and viscosity, $\eta_{20,w}$, of water, w, at 20 °C, using eq 1 below:

$$s^0_{20,w} = s^0_{obsd}\left(\frac{\eta_{solv,T}}{\eta_{w,20}}\right)\left(\frac{(1-\bar{\nu}_2\rho_{w,20})}{(1-\bar{\nu}_2\rho)+\Gamma'(1-\bar{\nu}_1\rho)}\right) \quad (1)$$

where ρ and $\eta_{solv,T}$ represents the viscosity and density, respectively, of the solvent at the temperature, T, of the experiment, $\bar{\nu}_2$ is the partial specific volume of DNA in the solvent, $\bar{\nu}_1$ is the partial specific volume of the component, water, (taken as 1.000), and Γ' is the preferential hydration of DNA (in g of H_2O/g of nucleotide) in the specific solvent. For estimates of the latter, we used the preferential hydration data of Hearst and co-workers (Hearst and Vinograd, 1961a,b; Hearst, 1965; Tunis and Hearst, 1968) in the manner previously described (Wolf and Hanlon, 1975). The preferential solvation correction was not applied, however, to the data in 0.1 M NaCl, as the values of Γ' in that solvent were too uncertain. Neglect of this correction for this solvent results in an error of 2 to 4%.

Circular dichroism spectra were measured at 27 °C in the manner previously described (Hanlon et al., 1975) in a 1-cm cell between 350 and 210 nm with a Cary 60 recording spectropolarimeter equipped with a 6001 CD unit. DNA concentrations were 0.26 mM. Spectral data are reported in terms of the mean residue ellipticity, $[\theta]$, in deg cm^2/dmol. Spectra were analyzed in terms of the contributions of the A, B, and C secondary structures by the multicomponent linear-regression method (method I) described in paper I of this series (Hanlon et al., 1975). The reference spectra of the A form in LiCl, the "B" form determined experimentally at low salt (0.02 *m* NaCl), and the C form derived by method I, described in our previous work, were employed as values of the error-free independent variables in this analysis.

The same linear-regression analysis was also employed for the procedure described as "LMS Minimum Component" analysis. The other method of component analysis, factor analysis, utilized the program options (in Fortran IV) described by Nie et al. (1975). This analysis was based on an *R*-type matrix which was extracted by a principal-component method (principal factoring without iteration) as well as Rao's canonical factoring solution (common factor solutions). The results were rotated to orthogonal factors by the Varimax rotation method.

Essentially what the program does is to form a correlation matrix, $R_{i,i'}$, between the measured variables, $[\theta]_{\lambda_j}$, in which the general element, $r_{i,i'}$, is defined as

$$r_{i,i'} = \frac{\sum_{j=1}^{m}([\theta]_{\lambda_j}{}^{i} - [\bar{\theta}]^{i})([\theta]_{\lambda_j}{}^{i'} - [\bar{\theta}]^{i'})}{\sum_{j=1}^{m}([\theta]_{\lambda_j}{}^{i} - [\bar{\theta}]^{i}) \sum_{j=1}^{m}([\theta]_{\lambda_j}{}^{i'} - [\bar{\theta}]^{i'})} \quad (2)$$

$[\theta]_{\lambda_j}{}^{i}$ is the mean residue ellipticity at wavelength λ whose index is "j" in a solution of LiCl whose concentration is indexed by the letter "i". The letter i' is used to designate some other LiCl solution in the series which may or may not be identical to i. There are m values of j index for wavelength and n values of the i solution index. The mean value, $[\bar{\theta}]^{i}$, is given by

$$[\bar{\theta}]^{i} = \frac{\sum_{j=1}^{m}[\theta]_{\lambda_j}{}^{i}}{m} \quad (3)$$

The correlation matrix is a *nxn* square matrix which normalizes the variance of each individual variable to 1 and locates it on the appropriate diagonal. For n variables, the total variance is simply n.

If f new variables ($f < n$), $F_{\lambda_j}{}^{1}, F_{\lambda_j}{}^{2} \ldots F_{\lambda_j}{}^{f}$, can be found such that each of the original variables, $[\theta]_{\lambda_j}{}^{i}$, can be expressed as a linear sum of these new, $F_{\lambda_j}{}^{f}$, variables (which are referred

TABLE I: Properties of the Supernatant and Precipitate Fractions of Calf Thymus DNA Obtained in 12 to 13 *m* LiCl.

	Supernatant					Precipitate				
LiCl concn (*m*)	% DNA	% GC[a]	$\delta^{a,b}$	$s^0_{20,w}$ (S)	T_m (°C)	% DNA	% GC[a]	$\delta^{a,b}$	$s^0_{20,w}$ (S)	T_m (°C)
11.98	84	40.3 ± 0.6	0.07	17.3		16	46.3 ± 0.8	0.12	15.8	
12.35	68	41.2 ± 0.3	0.06	14.9	75.5	32	44.6 ± 0.3	0.10	17.4	77.2
12.56	42	41.1 ± 0.7	0.07	13.3		58	41.1 ± 0.2	0.09	18.0	
12.73	20	40.5	0.06	12.7		80	41.2 ± 0.3	0.08	18.6	
13.16	6					94	41.2 ± 0.5	0.09	18.5	
Controls	100	41.0	0.08	17.5						
	100	41.1	0.08							
Average		41.1 ± 0.4								

[a] Calculated according to the procedure of Hirschman and Felsenfeld (1966). [b] The "randomness" parameter.

to in the analysis as "factors") then, *in an ideal case,* three consequences follow.

(1) A matrix equation (eq 3) can be constructed such that

$$R_{i,i'}\mathbf{F}_{\lambda_j} = E\mathbf{F}_{\lambda_j} \quad (4)$$

In the above equation $\mathbf{F}_{\lambda_j}$ is a vector, referred to as an "eigenvector". It is not formally identical to the variable F_{λ_j} described above, but there are the same number, f, of eigenvectors as there are underlying irreducible factors, or variables. E is an ordinary scalar constant, of which there are f solutions, called "eigenvalues", whose magnitudes reflect the relative contributions of the underlying variables, F_{λ_j}.

(2) The sum of the eigenvalues equals n, the total variance. The percentage of the total variance contributed by a given eigenvalue is simply

$$\% \text{ variance} = \frac{100E}{n} \quad (5)$$

(3) The smaller number, f, of variables (or factors) required to account for the original variables, $[\theta]_{\lambda_j}{}^i$, can be arrived at by simply counting the number of eigenvalues required to account for n, the total variance.

In the nonideal case, random and systematic errors inject spurious eigenvalues. When dealing with limited data, there is generally no easy method of discounting these in a manner which is not arbitrary. A characteristic of these artifactual components, however, is their small contribution to the total variance. We have therefore chosen an arbitrary cutoff value of 1.0% as the lower limit of acceptable eigenvalues.

The factor analyses as well as the linear-regression analyses were performed with IBM 370/155 and 370/168 computers.

Results and Discussion

The range of LiCl concentrations covered in this study was ca. 6 to 13 *m*. At this latter electrolyte concentration, dilute DNA solutions were actually turbid, with a substantial amount of nucleic acid precipitating out upon standing. We undertook an investigation of this salting out process between concentrations of 12 and 13 *m* LiCl, in an effort to delineate the experimental conditions which would ensure the fact that we were not dealing with aggregates of such a size that our results would be obscured by problems of light scattering and the loss of material by sedimentation at 1*g*.

As one might expect, the formation of such large aggregates was a sensitive function of the DNA concentration. At 1 mM, a significant amount of DNA (16%) was lost from a 12 *m* LiCl solution by a brief low-speed centrifugation, whereas this fraction fell to 6% at 0.27 mM and 0 ± 5% at 0.1 mM DNA at the same concentration of LiCl. At 13 *m* LiCl, almost complete precipitation (94%) was observed at 1 mM DNA, whereas only 23.5% of the DNA was lost at 0.27 mM.

A detailed study was conducted on the properties of the supernatant and precipitate fractions of 1 mM DNA solutions prepared at LiCl concentrations between 11.98 and 13.16 *m*. The results of these experiments together with control solutions of DNA in 0.02 *m* NaCl are summarized in Table I. The fractions of DNA precipitating at 11.98 and 12.35 *m* show a small but significant increase in the GC content, as determined by both spectral analysis at room temperature as well as the value of T_m in the thermal melting profile. The randomness parameter, which reflects the degree of randomness of the base sequence, is slightly elevated in the two fractions which show increases in GC content. The increase is small, however, and is still well below the value which reflects severe deviations from randomness in the sequence.

The circular dichroism spectra of all fractions were identical and indistinguishable from the control in the same solvent, 0.02 *m* NaCl. Thus, the transformations in the CD[1] spectra induced by increasing concentrations of LiCl (described subsequently) were completely reversible.

With the exception of the 11.98 *m* LiCl solution, an increase in the sedimentation coefficient of the precipitate fraction coupled with a concomitant decrease in the sedimentation coefficient of the supernatant fraction were uniformly observed. This would imply that the higher-molecular-weight fragments of DNA are preferentially precipitated at concentrations above 11.98 *m*.

From these results it would appear that two factors are involved in the aggregation and subsequent precipitation of DNA in the range of LiCl concentrations examined in these experiments. At the lower end of the concentration scale (11.98 *m*) the fragments bearing a higher GC content are preferentially driven out of solution. As the LiCl concentration increases, however, discrimination on the basis of this factor disappears, and the more usual discrimination on the basis of size takes its place—that is, the higher molecular weight fractions are preferentially precipitated. Even this differential disappears at 13.2 *m* LiCl which is sufficient to precipitate virtually all of the nucleic acid present, under the conditions of the experiment.

As previously mentioned, obvious precipitation and turbidity are not observed below 13 *m* LiCl when the concentration of DNA is 0.27 mM and below. The absorption properties of DNA in LiCl solutions under these nonprecipitating conditions are given in Table II. The extinction coefficients of DNA

[1] Abbreviations used are: CD, circular dichroism; NaEDTA, the sodium salt of ethylenediaminetetraacetic acid.

TABLE II: Properties of the Absorption Spectra of Calf Thymus DNA in LiCl Solutions.

LiCl (m)	ϵ_{259}[a] (l/mol cm.)	Light-scattering indices $(A_{340}/A_{259}) \times 10^3$	$(A_{320}/A_{259}) \times 10^3$
0.10 (NaCl)	6600	4	6
5.03	6566	7	13
6.41	6531	8	13
7.40	6641	7	12
8.53	6635	10	10
9.51	6588	17	24
11.03	6624	23	21
12.04	6480[b] 6200[c]	19	27

[a] Determined at concentrations of 0.15 and 0.27 mM, unless otherwise noted. [b] Determined at concentrations of 0.15 mM. [c] Determined at concentrations of 0.27 mM.

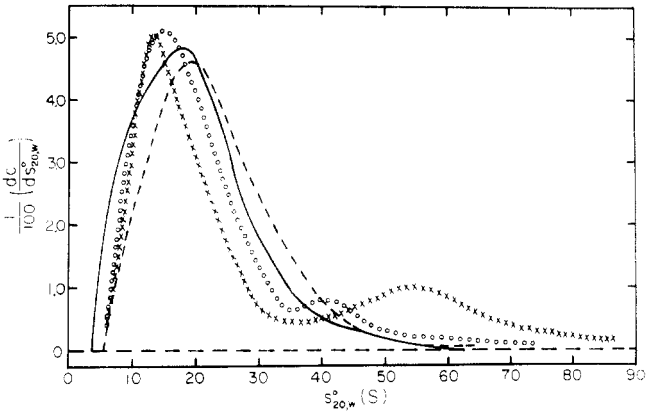

FIGURE 1: The sedimentation coefficient distribution of calf thymus DNA in LiCl solutions at 0.77 m (—), 7.40 m (- - -), 9.51 m (○), and 11.03 m (x).

(given in column 2), determined relative to the value in 0.10 M NaCl, were uniformly around 6550 ± 50 M^{-1} cm^{-1}, indicating that no substantial base unstacking had occurred in the LiCl solvents. This was also confirmed by examining the melting profile of a sample in 11.03 m LiCl. Although the DNA precipitated from solution before the melting process was complete, the hyperchromic increase achieved just prior to precipitation was 1.35, a value which compares favorably with a value of 1.39 for a control sample in 0.02 M NaCl.

A measure of the light-scattering properties is given in this table in columns 3 and 4 in the form of ratios of the absorbances at 320 and 340 nm (A_{320} and A_{340}) to the absorbance at the maximum (A_{258}). There appears to be a small but significant increase in the scattering properties between 8.5 and 9.5 m LiCl. Since it is unlikely that the effective refractive index increment for DNA is undergoing an *abrupt* increase in this concentration range due to preferential solvation effects, we conclude that this increase reflects an increase in the average particle size of the DNA. The particle size, however, is not sufficient to cause visible turbidity.

The sedimentation properties of DNA also undergo changes in this range of LiCl concentrations. These properties, determined between 22 and 24 °C at DNA concentrations of 0.13 mM, are given in Table III, and the sedimentation distributions are shown in Figure 1. With increasing concentration of LiCl, the continuous distribution of sedimenting species shown in Figure 1 (reflecting, more or less, a continuous distribution of molecular weights) broadens and the entire distribution shifts to higher $s^0_{20,w}$ values. This trend continues up to 9.5 m LiCl at which point a definite second peak (fast component) appears at the leading edge of the boundary. The distribution curve for the slower-moving component at this LiCl concentration is correspondingly narrower. The fast-moving species continues to increase in magnitude and its distribution broadens and moves to higher $s^0_{20,w}$ values, as the LiCl concentration increases up to 12.04 m. In contrast, the distribution of the slow-moving species moves to lower $s^0_{20,w}$ values and sharpens, as the concentration of LiCl increases from 9.51 to 12.04 m.

Estimates of the fractional amounts of fast and slow components based on a more direct analysis of the shapes of the sedimenting boundaries are presented in Table III together with the median sedimentation coefficients of both the components and the entire distribution. In order to rule out the possibility that these effects were due to heavy-metal contamination, we repeated the experiment in 11.1 m LiCl in the solvent, 11.10 m LiCl, 0.05 M EDTA. The results demonstrated that the presence of NaEDTA had no significant effect in the magnitude of the sedimentation coefficients and the distribution of DNA species between the fast and the slow components. That the presence of the fast-moving species is at least, in part, attributed to an aggregation process was demonstrated by the fact that increases in the concentration of DNA in solutions of fixed LiCl concentration result in increases in the fraction of fast species present. In 11.03 m LiCl, a doubling of the DNA concentration resulted in an increase in the fast component up to 50% of the population. Higher concentrations (1.2 mM) in this same solvent led to substantial

TABLE III: Median Sedimentation Coefficients of Calf Thymus DNA in LiCl Solutions.

Solvent (m LiCl)	Γ_H	Slow	$s^0_{20,w}$[a] (S)	Fast	Fractions, in % (±5%) Slow	Fast
0.1 M (NaCl)						
0.77	37.0		17.3			
5.03	7.9		20.7			
6.41	6.3		20.3			
7.40	5.2	18.5		31.8	93	7
8.53	4.3	18.5		36.8	85	15
9.51	3.7	16.3		45.2	76	24
11.03	2.9	15.7		51.2	60	40
11.10	2.9	14.0		51.8	58	42
11.10 m + 0.05 M EDTA	2.9	13.9		51.5	56	44
12.04	2.4	14.4		52.9	56	44

[a] Determined at 0.13 mM. Solutions were prepared initially at 0.26 mM for CD studies and diluted just before examination in the ultracentrifuge.

TABLE IV: Distribution of Structural Forms of DNA in LiCl Solutions.

LiCl (m)	Γ_H (mol of H_2O/mol of nucleotide)	Conformational distribution			
		f_C	f_A	f_B	Σf
0.86	30	0.31		0.70	1.01
2.88	10.2	0.51	±0.05	0.43	0.96
5.03	7.9	0.61	0.09	0.25	0.95
6.41	6.3	0.72	0.12	0.13	0.97
7.40	5.2	0.79	0.14	0.09	1.02
8.53	4.3	0.77	0.15	0.04	0.96
9.28	3.8	0.82	0.17	−0.02	0.97
9.51	3.7	0.71	0.15	−0.02	0.84
9.85	3.5	0.54	0.15	−0.03	0.66
10.96	2.9	0.75	0.16	−0.14	0.77
11.03	2.9	0.63	0.14	−0.12	0.65
11.77	2.5	0.51	0.16	−0.20	0.47
12.04	2.4	0.96	0.17	−0.27	0.86

losses of material at speeds below 10 000 rpm.

The behavior of the median sedimentation coefficients of the two components—fast and slow—as a function of the fraction of the fast-moving component is shown in Figure 2. Extrapolation of the sedimentation coefficient of the slow component to 0 concentration of fast species yielded a value of 19.7 S, virtually identical to the average value obtained prior to the appearance of the fast species. A similar extrapolation of the data for the fast-moving species, however, gave a significantly higher value of 29.3 S.

This discrepancy in the extrapolated values of the sedimentation coefficients coupled with the fact that no change in the scattering properties was observed until 9 m suggest that a portion of the DNA population first collapses to a more compact structure which ultimately aggregates to form higher-molecular-weight particles as the concentration of LiCl increases. The initial collapse probably occurs between 7 and 8 m LiCl, while the subsequent aggregation does not ensue until 9 m LiCl. The latter step appears to involve preferentially the higher-molecular-weight species of DNA.

The interpretation of a collapse preceding the aggregation step is not unequivocal, since the aggregation process may be rapid and reversible under the conditions of the experiment. If this is the case, the higher value of the extrapolated sedimentation coefficient of the fast species is simply the minimum value required to achieve separation of the aggregate from the nonaggregated monomers before significant reequilibration can occur, given the effective rate constants for the forward and reverse reactions, under the conditions of the centrifuge experiment (Gilbert, 1955). The decrease in the sedimentation coefficient of the slow-moving species as the magnitude of the fast-moving species increases can, with somewhat more assurance, be interpreted as a preferential incorporation of the higher-molecular-weight species of the polydisperse collection of DNA molecules in the aggregate.

The effects of LiCl on the circular dichroism (CD) properties of DNA at a concentration of 0.27 mM are shown in Figure 3A. (For purposes of comparison, we have also included spectra at 0.02 and 5.06 m salt. The properties of spectra between 0 and 5.06 n LiCl have been discussed in our earlier publications (Hanlon et al., 1975; Wolf and Hanlon, 1975).) At the very highest concentration of LiCl shown (13 m), the solution was visibly turbid and the spectrum is distorted by light-scattering artifacts, as evidenced by the appearance of an optical signal in regions outside the absorption band. Some of the same symptoms are suggested in the solution in 12.04

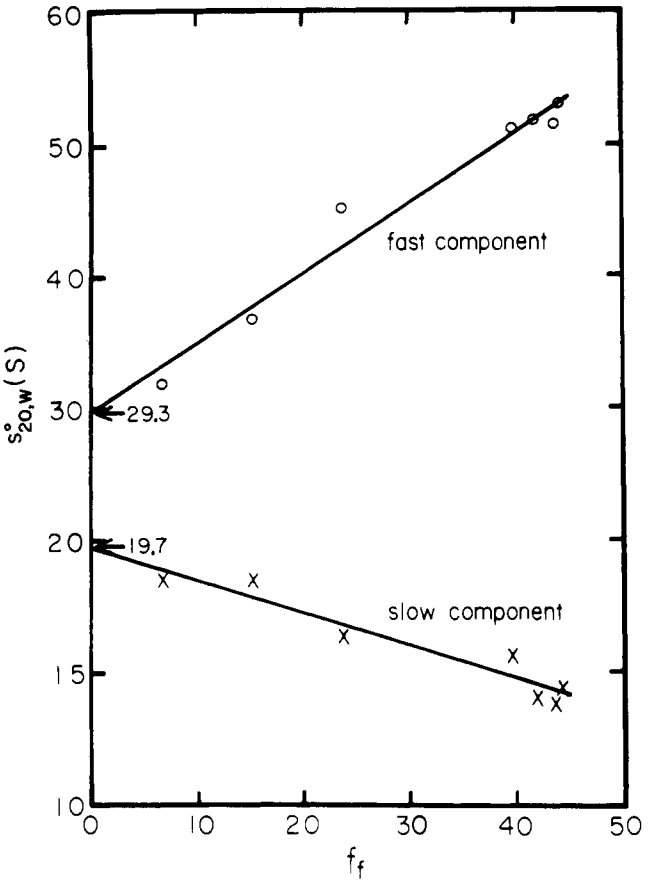

FIGURE 2: Behavior of the sedimentation coefficient ($s^0_{20,w}$) of the fast and slow component as a function of the fraction, f_f, of the fast-sedimenting species.

m, which had lost ca. 6% of its DNA content by precipitation at the concentration of DNA at which the experiment was performed. The other solutions evidenced only the light-scattering properties reported in Table II.

In Figure 3B, we have reproduced the reference spectra of the B, C, and A forms of calf thymus DNA, determined in our previous study (Hanlon et al., 1975). Comparison of the shapes of these reference spectra with the spectra shown in panel A reveals that the transformation in LiCl solutions should be accountable in terms of a conversion of the B form to the C and A forms. The results of our spectral analysis, shown in Table IV, indicates that this prediction is verified up to concentrations of LiCl of 9.28 m. Above that concentration, however, the spectra can no longer be fitted by a linear combination of the three reference spectra shown in Figure 3B. This means that either the reference spectra have changed or that new spectral components are required to account for the observed data. (The hydration data, Γ_H, in the second column of this table reveals no abrupt changes that might account for these effects.)

An analysis of the minimum number of independent spectral components present in various concentration ranges of LiCl confirmed this observation. For this task, we employed two types of component analysis of the complete set of spectral data in LiCl reported here and in our previous paper. The first of these was a factor analysis which proved to be relatively insensitive to the presence of the C spectral component, as this spectrum is very close to 0 in the range of wavelengths employed in the analysis (260–297.5 nm). The results of this analysis is shown in Table V. Over the entire range of LiCl concentrations in which no visible turbidity was manifest (0.77–11.8 m), three factors accounted for 98% of the variance.

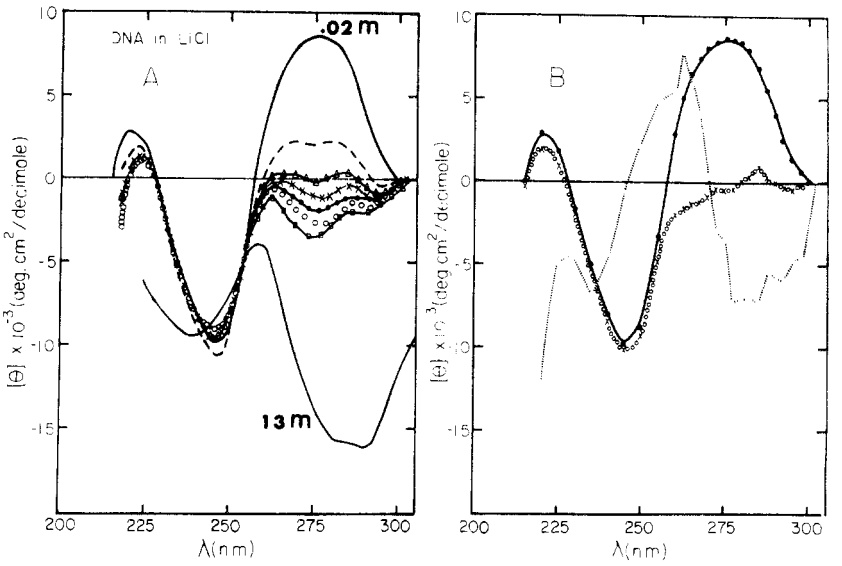

FIGURE 3: Circular dichroism spectra of calf thymus DNA. (A) Spectra in LiCl at 0 *m* (0.02 *m* NaCl) (—), 5.06 *m* (- - -), 7.40 *m* (▲), 9.51 *m* (x), 11.03 *m* (●), 11.80 *m* (○), 12.04 *m* (□), and 13.00 *m* (—). (B) Spectra of the B (●), C (x ○ ○ x), and A (○ · · · ○) conformations of DNA in LiCl Solution. (Taken from Hanlon et al. (1975).)

TABLE V: Component Analyses of the Circular Dichroism Spectra of DNA in LiCl Solutions.

	Factor Analysis							
	Factor 1		Factor 2		Factor 3			
LiCl Concn Range (*m*)	Eigenval	% Var	Eigenval	% Var	Eigenval	% Var	Σ % Var	LMS minimum No.
0.77–11.77	23.95	74.8	6.89	21.5	0.37	1.2	97.5	>3
0.86–2.88	7.92	99.0					99	2 $\Sigma f = 1.0 \pm 0.05$
0.77–7.40	19.11	95.5	0.61	3.0			98.4	3 $\Sigma f = 1.0 \pm 0.05$
3.56–7.40	11.39	94.9	0.42	3.5			98.4	
7.67–12.03	6.85	57.1	4.41	36.8	0.28	2.4		>3
					(F4) 0.16	1.3		
					(F5) 0.14	1.2	98.7	

Examination of the Varimax matrix revealed that the first factor remained relatively constant up to a concentration of 3 to 4 *m* LiCl, at which point the contribution of the second factor assumed significance. The latter continued to increase until a concentration of ca. 9 *m* LiCl, at which point the third factor began to significantly increase with a concomitant decrease in the second. The first factor whose positive contribution has been continuously decreasing, since 4 *m*, actually began to make negative contributions at this point. An examination of the structural analysis of the CD spectra in terms of the A, B, and C components reveals that these concentration points, 2.9 and 9.3 *m*, correspond to (1) the appearance of the A form (2.9 *m*) and (2) the failure to be fit adequately by a linear combination of the A, B, and C reference spectra alone (9.3 *m*).

When these LiCl ranges of concentration were processed separately, the results shown in the last four entries of Table V were obtained: in the LiCl concentration range of 0.86 to 2.9 *m*, where the spectral data were adequately fit with only the B and C spectrum, one eigenvalue accounted for 99% of the variance.[2] If the range of LiCl data was extended to 7.4 *m*, then another eigenvalue appears, whose contribution becomes significant at 2.9 *m* LiCl. Finally, if the analysis is conducted with those solutions from 7.4 to 12.03 *m*, the concentration range in which the sedimentation anomaly appears, the solution yields two major eigenvalues and a number of lesser ones. Furthermore, the coefficients of factor 1 become increasingly more negative in the final Varimax rotated matrix as the LiCl concentration increased above 10 *m*. Clearly, the spectral behavior in this latter range of concentrations requires additional variables to account for the observed variance.

Another type of component analysis which we employed is a primitive version of a matrix rank-order analysis. Although it has a number of disadvantages, its use permits one to detect independent components of small magnitude which factor analysis tends to overlook. Essentially, one attempts to analyze a series of spectra in terms of a linear combination of members of the same series which are also made up of linear combinations of the same independent spectral components present in all members of the series. In this procedure, the observed optical signal, $[\theta]_{\lambda_j}^{\text{obsd},i}$, at the wavelength λ_j of the *i*th solution can, in principle, be expressed as

$$[\theta]_{\lambda_j}^{\text{obsd},i} = f_q[\theta]_{\lambda_j}^{q \neq i} \tag{6}$$

where f_q are arbitrary coefficients. Since there are experimental errors, this equation is rearranged as

$$\mathbf{R} = \sum([\theta]_{\lambda_j}^{\text{obsd},i} - \sum f_q[\theta]_{\lambda_j}^{q \neq i})^2 \tag{7}$$

for a least-mean-square analysis. The residual, *R*, is differentiated with respect to each of the f_q values and set equal to 0. The set of *q* simultaneous equations can be solved for the values of f_q without imposing any restraints on the value of Σf_q. It can be shown that the minimum number of *q* spectra of the series which results in $\Sigma f_q = 1.00 \pm 0.05$ (where ±0.05 cor-

[2] This is a particularly dramatic example of the failure of this analysis to pick up the C component. At 2.9 *m*, the intensity of the positive band had been lowered by 45% compared to the value in 0.02 *m* LiCl.

responds to our estimated error of analysis) with an acceptable standard deviation corresponds to the minimum number of independent spectral components giving rise to the series of i spectra.[3]

The results of this "LMS minimum number" component analysis is also shown in Table V in the last column. As previously shown (Hanlon et al., 1975), the spectra between 0.02 and 2.88 m LiCl are adequately accounted for in terms of two components. Beyond 2.88 m up to 9.28 m, a third component is required. Above 9.28 m, however, the spectra could not be fitted satisfactorily with any number of linear combinations of "basis" spectra at lower LiCl concentrations.

Both methods of component analysis thus indicated the presence of an additional spectral component or distortion in LiCl solutions above 9 m. In an effort to delineate the shape of such a distortion or additional band, we used an approach which relied on two major assumptions. The first of these was that the transformation of the B to the C + A forms continued to be linearly dependent on the preferential hydration of DNA in these solvents, until the conversion was complete. Our previous analysis, reported in the paper by Wolf and Hanlon (1975), revealed that this latter point corresponded to a preferential hydration of 3 to 4 mol of H_2O/mol of nucleotide, which is the preferential hydration of DNA in 9 m LiCl. In this solvent, under these conditions of hydration, the distribution of the C and the A structural forms should correspond to 83 ± 1% C and 17 ± 1% A.

We further assumed that any structural transformation beyond this point would result in higher-order structures without disturbing the secondary structural features of the DNA molecules. The last assumption was that both levels of structure—secondary and higher order—would contribute in a linear manner to the observed signal, $[\theta]_{\lambda_j}^{obsd}$, such that the latter could be represented by

$$[\theta]_{\lambda_j}^{obsd} = 0.83[\theta]_{\lambda_j}^{C} + 0.17[\theta]_{\lambda_j}^{A} + g_x[\theta]_{\lambda_j}^{x} \quad (8)$$

where $g_x[\theta]_{\lambda_j}^{x}$ is the systematic deviation of the spectrum resulting from the contribution of the higher-ordered structure(s). Thus, a plot of

$$[\theta]_{\lambda_j}^{obsd} - 0.83[\theta]_{\lambda_j}^{C} - 0.17[\theta]_{\lambda_j}^{A} = g_x[\theta]_{\lambda_j}^{x} \quad (9)$$

would reveal the shape of the additional component. If our assumptions are incorrect, of course, the function, $g_x[\theta]_{\lambda_j}^{x}$, will consist not only of contributions of the additional component—or components—but of the other forms, B, C, and A, as well. If the aggregate giving rise to the additional signal is asymmetric, the function, $g_x[\theta]_{\lambda_j}^{x}$, could also reflect unequal weights of the anisotropic forms of the spectra of the three conformations (Chung and Holzwarth, 1975).

The behavior of $g_x[\theta]_{\lambda_j}^{x}$ vs. λ as a function of salt concentration is shown in Figure 4. It should be noted that the ordinate represents a greatly expanded scale of $[\theta]$ values, and the points defining the average curve for 9.28 to 9.85 m LiCl lie just outside of predicted experimental error. The systematic deviations increase, however, at higher LiCl concentrations, ultimately resulting in a negative band which has a shape reminiscent of the curves of DNA at very low temperatures in 6 M LiCl (Studdert et al., 1972) and the ψ form of DNA (Jordan et al., 1972; Shapiro et al., 1969; Cheng and Mohr, 1975). It should be noted that the concentration range in which the systematic deviations become significant enough to be defined as an additional spectral component corresponds to the LiCl concentrations with a small but elevated increase in light scattering in the ultraviolet, and whose sedimentation distribution shows a definitely resolved fast component.

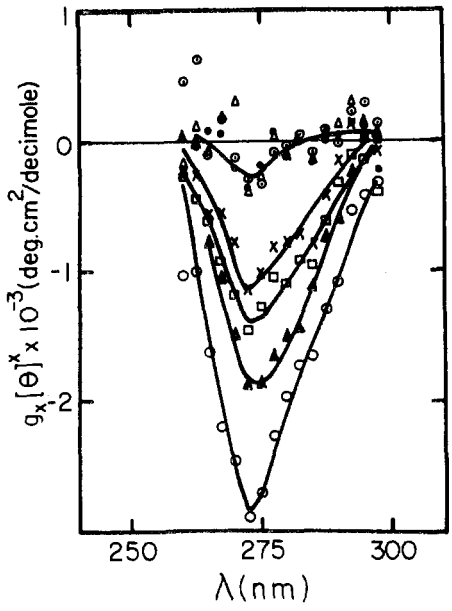

FIGURE 4: Shape of the additional spectral component in the circular dichroism spectra of DNA in concentrated solutions of LiCl. The function, $g_x[\theta]^x$, calculated as described in the text, is plotted against wavelength, λ, in nm, for 9.28 m (●), 9.51 m (△), and 9.85 m (⊙) LiCl, 11.03 m (x), 10.96 m (□), 11.77 m (▲), and 12.04 m (○).

The shapes of the curve obtained is a vague mirror image of the "B" spectrum of DNA. This accounts for the fact that negative values of f_B and the coefficients of factor 1 were obtained in the spectral analyses of the data above 9 m.

Conclusion

It is clear that at the highest concentrations of LiCl examined in this study an aggregation of DNA occurs, ultimately resulting in high-molecular-weight material which will sediment at 1g. DNA molecules of GC content and molecular weight greater than the average appear to be favored in this process. What is not certain, however, is the concentration of LiCl at which this process begins and at what point it provides a reasonable explanation for the effects observed in the sedimentation velocity and circular dichroism experiments. Although a fast component is detectable in the sedimentation velocity experiments at concentrations as low at 7.4 m LiCl, the distortions in the CD spectra and increases in light scattering do not become apparent until a concentration of 9 m LiCl. Although this would suggest an initial collapse or compaction of DNA molecules between 7 and 9 m, the sedimentation data taken alone can be explained equally well by aggregation without prior collapse.

The aggregation step appears to have given rise to an additional circular dichroism band. We cannot rule out the uninteresting possibility that this band is a light-scattering artifact which bears no relationship to the structure of the aggregate of DNA molecules. Except for the spectrum at 12.03 m, however, the spectra generally do not have the earmarks of those originating from solutions which are scattering appreciable amounts of light. Because of this, we favor the interpretation that the curve exhibited in Figure 4 reflects, for the most part, the contributions of an ordered array of DNA

[3] We stress the fact that this procedure yields a minimum number of independent components. There are obviously special cases where two or more components which, although orthogonal, nevertheless transform in a linked fashion under the particular circumstances of the process. In such a case, the linked components will appear as a single independent component in the analysis, as well as in other types of component analysis.

molecules, packed sufficiently closely to permit *intermolecular* interactions of the type frequently observed for liquid crystals (Holzwarth and Holzwarth, 1973). It is interesting that the shape of this distortion resembles the component of the CD spectrum of the C form of DNA, polarized perpendicular to the DNA helix axis (Chung and Holzwarth, 1975). Since this is the major secondary structure present in these highly concentrated LiCl solutions, it is possible that the super-structure consists of a side by side alignment of DNA molecules in which the component polarized parallel to the helix axis is effectively cancelled, and those components polarized perpendicular to the helix axis are preferentially enhanced, due to the enhanced polarizability of this arrangement. We suggest that the different shapes of ψ spectra which have been observed for DNA (Studdert et al., 1972; Jordan et al., 1972; Shapiro et al., 1969; Cheng and Mohr, 1975) can be attributed to similar types of micelle formation in which the alignment of the DNA molecules favors one or the other of the anisotropic spectra of the secondary structures present.

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