Conformational Characteristics of Deoxyribonucleic Acid–Butylamine Complexes with C-Type Circular Dichroism Spectra. 1. An X-ray Fiber Diffraction Study†

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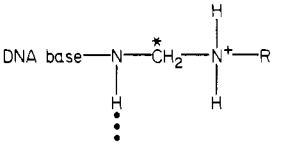
ABSTRACT: A set of complexes of calf thymus DNA and *n*-butylamine, covalently cross-linked to the DNA bases with CH_2O, has been examined by X-ray diffraction. The attachment of this amine to DNA has been previously shown to result in a profound reduction of the rotational strength of the positive band above 260 nm in the circular dichroism (CD) spectrum in 20 mM NaCl, pH 7, without changing any of the properties which are characteristic of a native base-stacked duplex [Chen, C., Kilkuskie, R., & Hanlon, S. (1981) *Biochemistry 20*, 4987]. In 20 mM NaCl, pH 7, substitution levels of 0–0.15 mol of amine/mol of nucleotide are sufficient to produce a family of CD spectra which range from the conservative one normally seen for protein-free DNA in this solvent to the nonconservative one ascribed to the C form of DNA [Hanlon, S., Brudno, S., Wu, T. T., & Wolf, B. (1975) *Biochemistry 14*, 1648–1660]. Fibers of the DNA·amine complexes at 79% relative humidity (rh) and 33% rh do indeed show C-type X-ray patterns whereas the controls exhibit A forms under the same conditions. The same preparations, however, exhibit only B patterns when the fibers are examined at 98% rh and in the wet fiber form. Although we cannot rule out the possibility that molecular interactions in the fiber have imposed conformational restraints on the DNA structure that are not present in solution, these present results suggest that the conformation of DNA giving rise to the "C" CD spectrum is a variant of the B form.

The conformation of DNA in chromatin and in core particles of chromatin has been a matter of controversy for some time. One aspect of the problem resides in the interpretation of the circular dichroism (CD)[1] spectral properties of the DNA constituent. In chromatin, the rotational strength of the positive band above 260 nm, due solely to the DNA constituent, is severely depressed in comparison to its value in protein-free DNA in the same solvent (Shih & Fasman, 1970; Johnson et al., 1972). The rotational strength of the corresponding band is slightly negative in the CD spectrum of the core particles (Rill & Van Holde, 1973; Cowman & Fasman, 1978).

Similar effects can also be observed in the CD spectra of DNA if the electrolyte content of the solvent is increased (Studdert et al., 1972; Ivanov et al., 1973; Hanlon et al., 1972, 1975). In very concentrated electrolyte solutions (6 *m* LiCl and 8 *m* NH_4Cl), the CD spectrum of protein-free DNA resemble that of DNA in core particles. These spectra are also similar to one obtained by Tunis-Schneider & Maestre (1970) on an isotropic film of LiDNA at 76% rh which these workers assigned to a C-DNA conformation.

On the basis of the latter assignment, Hanlon and coworkers (Hanlon et al., 1972, 1975; Johnson et al., 1972) have concluded that the conformation of DNA in concentrated electrolyte solvents and in core particles is predominantly a C structure. This conclusion has been challenged by a number of investigators who have examined both nucleosomes (Zama et al., 1978; Goodwin & Brahms, 1978) and protein-free DNA (Maniatis et al., 1974; Sprecher et al., 1979; Baase & Johnson, 1979). The most recent study is that of Zimmerman & Pheiffer (1980), who obtained only B-type X-ray patterns from fibers of DNA immersed in solvents which produce a C CD spectrum in dilute solutions of DNA. These authors have concluded that a relative humidity of 76% in the experiments of Tunis-Schneider and Maestre was insufficient at the salt content of the isotropic film of DNA to convert the B to the C form. Thus, the original basis for assigning the C CD spectrum to the C form of DNA was invalid.

In principle, this apparent conflict could be resolved in a direct manner by examining conformational properties of DNA *in solution* in C-type solventts by techniques which can yield unequivocal results. In practice, the nature of the solvent required to generate a C-type CD spectrum creates special problems for other methods. Recently, however, Hanlon and co-workers (Chen et al., 1981) have found that this same type of CD spectrum can be induced by covalently coupling amines to DNA via CH_2O, as in the following proposed structure:



where the –N–H... represents an exocyclic amino group of a base involved in an intact Watson–Crick hydrogen bond and the $*CH_2$ unit is derived from CH_2O. This structure is reasonably stable at pH 7 and room temperature in the absence of free amine and CH_2O. The extinction coefficient at 259 nm, the cooperative character of its melting profile and the hyperchromic increase upon melting, the sedimentation coefficient, the intrinsic viscosity, and the electron microscopic appearance of the derivatized DNA differ insignificantly from the native base-stacked control which contains no amine. The derivatized DNA is thus a base-stacked duplex and is *not* collapsed or aggregated in aqueous solutions of modest elec-

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[1] Abbreviations: CD, circular dichroism; EDTA, ethylenediaminetetraacetate; rh, relative humidity.

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trolyte content (ca. 20–100 mM NaCl).

The CD spectra of the derivatives, however, are profoundly altered in a manner reminiscent of the effects seen in solutions of high salt concentration and in chromatin and core particles [see Figure 9 in Chen et al. (1981)]. The effect is especially striking in the positive band above 260 nm which is particularly responsive to conformational effects and relatively insensitive to compositonal effects (Johnson et al., 1981). The extent of the reduction of the rotational strength of this positive band above 260 nm is a function of the amount of amine attached. At ca. 0.12 mol of *n*-butylamine/mol of nucleotide, the spectrum of the DNA complex in 20 mM NaCl, pH 7, is similar to one obtained on underivatized DNA in 8 *m* NH_4Cl which, in turn, is similar to one assigned to the C conformation.

These derivatives are ideal for resolving the question of whether the lowered rotational strength of the positive band of the CD spectrum of DNA does indeed reflect C geometry of the duplex. In this paper, we report the results of X-ray diffraction studies on oriented fibers prepared from these derivatives. The following paper in this series (Fish et al., 1983) reports the results of Raman spectroscopic studies on these derivatives.

Experimental Procedures

The calf thymus DNA used in these experiments was a high molecular weight preparation (lot 900007) obtained from Calbiochem. The properties of this sample have been previously described (Hanlon et al., 1976). Further purification of this sample for the X-ray and Raman experiments (Fish et al., 1983) is described below. Absolute ethanol was obtained from the Aaper Alcohol and Chemical Co. Reagent-grade *n*-butylamine was a product of Aldrich Chemical Co. The stock 37% formaldehyde solution used in the preparation of the DNA·amine derivatives was a product of Fisher. All salts used in the preparation of solutions were reagent grade.

Two sets of derivatized DNA and controls were prepared for the X-ray diffraction studies. The DNA used in the preparation of these sample sets was first purified by either precipitation at 1.25 mg/mL in 3 M NaCl with 2 volumes of ice-cold 95% ethanol (sample set 2) or phenol extraction by the procedure of Marmur & Doty (1959) and ethanol precipitation (sample set 1). The precipitated DNA was collected by winding on a glass rod and rinsed with cold 95% ethanol and distilled H_2O (sample set 2) or rinsed twice with 70% ethanol and dried with N_2 (sample set 1). The precipitates were redissolved in 20 mM NaCl, pH 7, at a concentration of ca. 1 mg/mL and dialyzed exhaustively against 20 mM NaCl, pH 7.

A stock solution of this DNA at ca. 2.1–2.7 mM nucleotide concentration was then used for the preparation of controls and derivatized DNA samples by the procedure previously described in Chen et al. (1981). A 37% formaldehyde solution, adjusted to pH 7, and 0.20 M NaEDTA, pH 7, were added to bring the solvent composition to 1.9% CH_2O, 10 mM EDTA, and 16 mM NaCl, pH 7.0. An aliquot of this mixture was removed as a control and allowed to stand at room temperature for the maximum duration of the reaction (2.0–3.5 h). The remainder was made 7.3 mM (sample set 1) or 26.4 mM (sample set 2) in *n*-butylamine by the addition of 0.1 volume of an appropriate stock solution of *n*-butylamine hydrochloride at pH 7. The amine stock solutions were prepared, and their concentrations were established by titrating aqueous *n*-butylamine solutions to pH 7 with a standard solution of HCl.

The reaction was followed by CD and absorbance spectroscopy. Aliquots were removed from the reaction vessel at several times up to 3.5 h: sample set 1 aliquots were removed at 20 min, 55 min, and 3.5 h and sample set 2 at 2 h. These were dialyzed overnight in the cold (7 °C) against 20 mM NaCl, pH 7, to remove unreacted CH_2O and butylamine. This step was followed by two subsequent dialyses against 1 mM NaCl, pH 7, for 24 h each at 7 °C. This lower salt concentration was chosen as the final solvent in order to ensure that the salt content of the fibers prepared for the X-ray diffraction experiments would be no greater then 0.6%, which, upon wetting, would yield a NaCl concentration of 0.02–0.1 M (Zimmerman & Pheiffer, 1979). The control aliquots were all dialyzed in an identical fashion. This procedure resulted in a set of DNA·butylamine complexes whose level of substitution ranged from 0 to 0.13 mol of amine/mol of nucleotide, as determined from the CD spectral properties of the dialyzed products (see below).

In order to obtain gels whose concentrations were suitable for fiber preparation, control and amine-substituted preparations were spun for 1 h at modest speeds (ca. 7000*g*) to clarify the solutions and then pelleted by spinning at 50 000 rpm in a 65 rotor in a Spinco L 2 ultracentrifuge for 24 h. The pellets obtained were generally at nucleotide concentrations of 32–46 mM which corresponds to 10–14.2 mg of DNA/mL (mean residue weight of 309 daltons) or 11–15 mg of NaDNA/mL. Except for a brief 2-day transit period, all samples were maintained in a frozen state until used for the X-ray diffraction experiments.

The mole ratio of *n*-butylamine bound per mole of nucleotide (*R*) was determined in the manner previously described for $^{14}CH_2O$ and [1-^{14}C]ethylamine (Chen et al., 1981). Aliquots of *n*-[1-^{14}C]butylamine with a specific activity of 1.97 mCi/mmol (lot 810704, Pathfinder Laboratory) were mixed with a solution of cold amine to yield a solution of 0.273 M *n*-butylamine with a specific activity of 1.85×10^{-2} mCi/mmol. The ^{14}C-labeled complexes were prepared by using dilutions of this stock amine solution to 27 mM together with CH_2O (1.9%) and DNA (2 mM) in 16 mM NaCl/10 mM NaEDTA. Aliquots were removed from the reaction vessel at various times up to a maximum of 4 h and dialyzed exhaustively against 20 mM NaCl and 10 mM Na_2HPO_4/NaH_2PO_4 buffer, pH 7, to remove unreacted CH_2O and amine. The CD spectra of each sample were obtained while the remainder of the dialyzed aliquot was counted on a Beckman Model LS 9000 scintillation counter. The final dialysis fluid from each sample provided the appropriate background count.

Previous studies had revealed that a control mixture consisting of *n*-butylamine and $^{14}CH_2O$, but no DNA, did not result in the buildup of large nondialyzable polymers of CH_2O and amine which, in turn, would give rise to excess counts for the dialysis bag contents. This control was therefore omitted from our experiments. Only the two following controls were prepared: (1) DNA and CH_2O but no butylamine and (2) DNA and butylamine but no CH_2O. The counts for control 1 were insignificantly different from background. A small number of counts in excess of background appeared in control 2, to the extent of 0.003 ± 0.001 mol of ^{14}C-labeled amine/mol of nucleotide. Such excess has never been observed when similar controls are prepared with [1-^{14}C]ethylamine. In this present instance, it probably is due to a small amount of reactive impurity in the *n*-[1-^{14}C]butylamine. This value was used to correct the activities of the experimental samples containing all of the reaction components. The CD spectra of all controls after dialysis differed insignificantly from that of unreacted DNA which had never been exposed to CH_2O or *n*-butylamine.

Absorbance spectra in the ultraviolet were obtained in strain-free quartz cells in a Cary 14 CMR at 25 °C in the manner previously described (Chen et al., 1981). Path lengths of 0.1 cm were routinely used for monitoring the CH_2O/amine reaction. At lower concentrations, 1-cm paths were employed. The spectra of the gel samples were measured with thin path cells containing a 10-μm spacer, manufactured by Helma Cells, Inc. Spectra were taken in the latter at several rotational positions in the light path in order to establish the absence of inhomogeneity, orientation, and/or bubble artifacts. The concentrations of all DNA solutions were estimated from the absorbance at 259 nm together with an extinction coefficient of 6700 cm^{-1} (Chen et al., 1981).

Circular dichroism spectra were generally obtained at 27 °C in a Cary Model 60 spectropolarimeter equipped with a 6001 CD attachment. (A few spectra of the solutions of redissolved X-ray fibers at very low concentrations of DNA were obtained on a Jasco J40 instrument.) After the absorbance spectrum was measured, the solution in the same cell was transferred to the spectropolarimeter, and the CD spectrum was obtained between 350 and 210 nm. When the 10-μm path cell was used, we again obtained spectra at several rotational positions for the reason cited above. After correction for the appropriate base line, the CD signal was converted to the mean residue ellipticity, $[\theta]_\lambda$, at the wavelength, λ, at 2.5-nm intervals by using the concentration obtained from the absorbance spectrum.

The X-ray diffraction patterns were obtained by irradiating fibers in glass capillaries with Cu Kα radiation obtained from a Norelco fine focal spot X-ray tube. Diffraction patterns were recorded at room temperature (ca. 25 °C) in a Norelco microcamera modified to accommodate a specimen to a film distance of ca. 34 mm. Calibration of the exact distance as well as the procedures used for measuring distances in the films has been previously described (Zimmerman & Pheiffer, 1979). Humidity was controlled for the fiber pictures by continuously flushing the camera chamber containing the unsealed capillary with a stream of helium that had been passed through the appropriate saturated salt solution ($KClO_3$ for 98% rh, NH_4Cl for 79% rh, and $MgCl_2 \cdot 6H_2O$ for 33% rh) as well as by the presence of a dish of the salt solution within the camera. For a relative humidity of <5%, the helium stream was passed through a column of $CaSO_4$ (Drierite). The capillaries containing wet fibers, prepared as previously described (Zimmerman & Pheiffer, 1979), were sealed with wax on both ends and irradiated while the camera chamber was flushed with dry helium. Upon the completion of these experiments, the fibers were maintained at or below −15 °C until reexamination by CD spectroscopy.

For the latter measurements, the capillaries containing the fibers were crushed in 3 mL of 20 mM NaCl and 10 mM NaH_2PO_4/Na_2HPO_4, pH 7, and stirred at room temperature for ca. 6 h and overnight (ca. 12 h) in the cold. (In the case of the 3.5-h fibers from sample set 1, 3 days in the cold were required for solution.) The glass debris was removed prior to CD spectroscopy by spinning at top speed in an Eppendorf centrifuge for 10 min.

Results and Discussion

CD Spectroscopy. Figures 1 and 2 display the CD spectra of sample sets 1 and 2, respectively. The spectra in panel A of Figure 1 are those of solutions prepared with the gels of sample set 1 from which the X-ray fibers were drawn. The progressive decrease of the positive band above 260 nm as the exposure time in the reaction mixture increased is due to the increased amine content as described below. These spectral

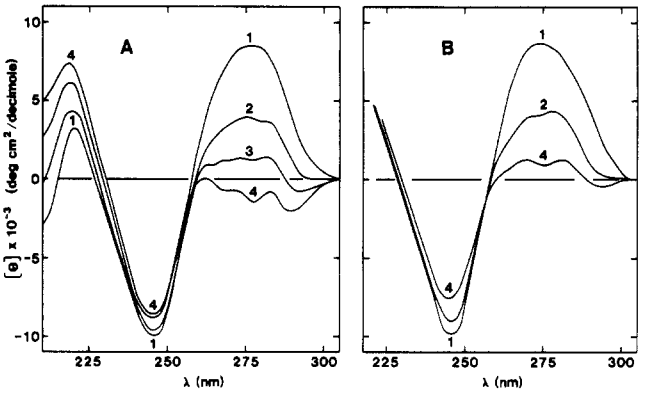

FIGURE 1: CD spectra of sample set 1 in 20 mM NaCl and 10 mM NaH_2PO_4/Na_2HPO_4 buffer, pH 7.0 at 27 °C. (A) Spectra were obtained of dilutions (0.2 mM) of the gel pellets from which the fibers examined in the X-ray diffraction experiments were drawn. Curve 1 is that for the control, and curves 2, 3, and 4 are the spectra of the 20-min, 55-min, and 3.5-h samples whose amine contents are 0.06, 0.10, and 0.13 mol of amine/mol of nucleotide, respectively. (B) Spectra of solutions prepared from the fibers examined in the X-ray diffraction experiments. Curve 1 is that of the control at 0.023 mM. Curves 2 and 4 are those of the fibers prepared from the 20-min and 3.5-h samples, respectively, at a nucleotide concentration of 0.05 mM. The amine contents of curves 2 and 4 are 0.06 and 0.10 mol of amine/mol of nucleotide, respectively.

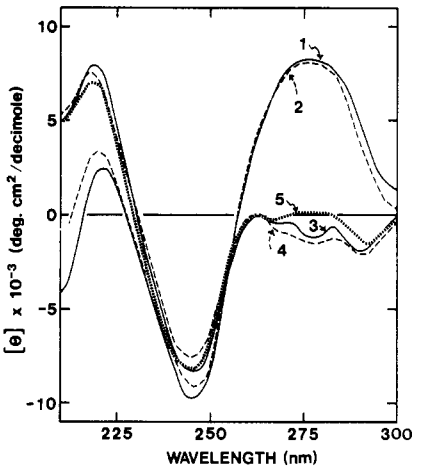

FIGURE 2: CD spectra of sample set 2 at 27 °C. Curves 1 (—) and 2 (- - -) are spectra of the control at 46 and 0.2 mM, respectively, and curves 3 (—) and 4 (- - -) are spectra of a DNA·butylamine complex with 0.14 mol of amine/mol of nucleotide at 42 and 0.2 mM, respectively. Curve 5 (···) is the spectrum at 0.02 mM of a redissolved fiber of the DNA·amine complex recovered from an X-ray diffraction experiment whose corresponding amine content is 0.12 mol of amine/mol of nucleotide. The solvent for spectra 2, 4, and 5 is 20 mM NaCl and 10 mM NaH_2PO_4/Na_2HPO_4 buffer at pH 7. The solvent for spectra 1 and 3 is 1 mM NaCl, pH 7.

changes in this wavelength region are very similar to those induced by increasing concentrations of simple electrolytes such as CsCl, LiCl, or NH_4Cl.

The spectral changes below 260 nm are not consistent with what is usually observed in electrolyte solutions, and we can offer no explanation at the present time as to their origin. The nature of the amine seems to affect the magnitude of the increase at 220 nm and the decrease at 245 nm: For the same value of $[\theta]_{275}$, the value of $[\theta]_{220}$ is lower for NH_4^+ than for either ethylamine or butylamine, which suggests that the changes are due to changes in the electronic properties of the chromophores as a result of the amine attachment rather than a reflection of conformational differences. This spectral region is known to be more sensitive to compositonal and sequence differences than is the region above 260 nm (Johnson et al.,

1981). In light of our previous studies (Chen et al., 1981), the one statement which we can make about these changes is that they are *not* a reflection of denaturation or the presence of a significant fraction of single-stranded or disorganized forms.

As we have previously noted (Chen et al., 1981), these amine adducts are reasonably stable at pH 7 although extensive dialysis, long times at room or elevated temperatures, and pHs significantly different from 7 will result in a variable amount of amine loss. We therefore took the precaution of examining the CD properties of solutions prepared from the fibers after the X-ray experiments were completed. These are shown in panel B of Figure 1 for the control, 20-min, and 3.5-h samples. (The fiber prepared from the 55-min sample was lost in an attempt to get a Raman spectrum of it.) The differences observed between these two sets of spectra in panels A and B reflect the loss of amine from the complexes due to a combination of the experimental measurements and manipulations coupled with the time required to redissolve the dehydrated fibers. Despite the partial reversal of the effect on the positive band above 260 nm due to amine loss, the CD specral properties above 260 nm exhibited by the combined sample sets 1 and 2 span a range of rotational strengths which would be observed if underivatized DNA were examined over a range of salt concentrations corresponding to 0–11 *m* LiCl or 0–9 *m* NH_4Cl, ranges in which Hanlon et al. (1975) had previously suggested that DNA might be moving along a conformational continuum from a B to a C state.

In Figure 2, we have displayed several spectra from sample set 2. The dashed lines represent the spectra of dilute solutions prepared from the gels of the control and the 2-h sample from which the fibers were pulled. The spectra shown are those for DNA concentrations of 0.2 mM in nucleotide residues, but identical spectra were also attained at 2 mM. The solid lines represent the spectra of the gels themselves measured in a 10-μm thin-path cell. These spectra at concentrations of 42–46 mM are essentially identical with those of the solutions at 0.2 mM. These results demonstrate the lack of concentration dependence of the CD spectral properties over a range of 0.2–46 mM.[2] Although this latter concentration is still significantly lower than that of DNA in the hydrated fibers (ca. 900 mM), it was only about a factor of 3 lower than that employed in the Raman experiments reported in the following paper (Fish et al., 1983).

Using ^{14}C-labeled CH_2O or ethylamine to measure the extent of amine attachment to DNA, we have found a linear correlation between the rotational strength of the positive band of the CD spectrum and the ratio of moles of amine per mole of nucleotide (R) (Chen et al., 1981; D. Maibenco and S. Hanlon, unpublished results). This provides a convenient means of estimating the amount of amine bound to the various samples examined in these experiments. Using 1-^{14}C-labeled *n*-butylamine, we prepared a set of derivatives whose CD spectra and ^{14}C content were determined as described under Experimental Procedures. The relationship between the extent of covalently bound amine and the magnitude of the maximum of the positive band of the CD spectrum of its solution, $[\theta]_{275}$, is displayed in Figure 3. The characteristics of the linear regression analysis of the data shown are

$$R = \frac{\text{mol of } n\text{-butylamine}}{\text{mol of nucleotide}} = (-1.34 \times 10^{-5})[\theta]_{275} + 0.116 \quad (1)$$

[2] This was the highest concentration for these molecular weight samples which we could employ without generating significant orientation effects in the thin path cell which we used.

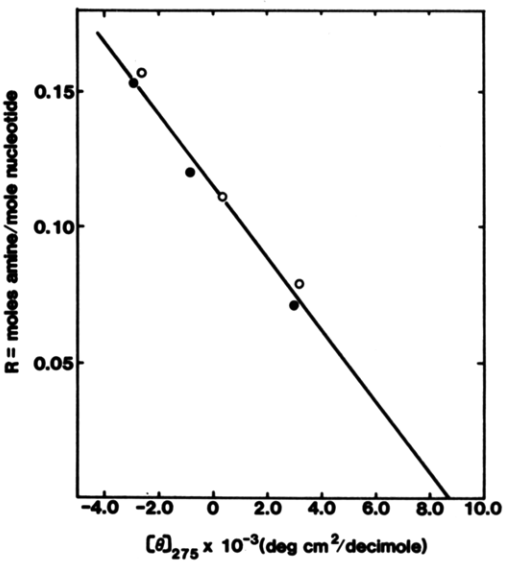

FIGURE 3: Relationship between the extent of amine bound and the maximum ellipticity of the positive band of the CD spectrum. The moles of *n*-butylamine (estimated by ^{14}C labeling) per mol of nucleotide are plotted on the ordinate against the value of the mean residue ellipticity at 275 nm, $[\theta]_{275}$, on the abscissa. The open and closed circles represent the results from two separate experiments. The line represents the results of the linear regression analysis whose slope and intercept are given in eq 1 in the text.

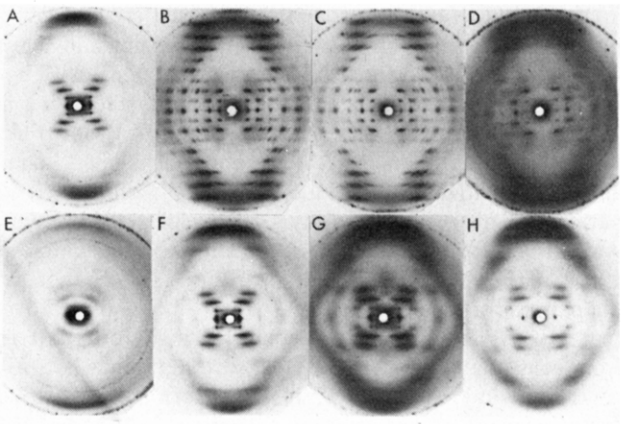

FIGURE 4: X-ray diffraction patterns of fibers formed from DNA·butylamine complexes and control DNA. The fibers are all from sample set 1 (see text). Patterns A, B, C, and D are from the control preparation at 98%, 79%, 33%, and <5% rh, respectively. Patterns E–H are from fibers pulled from the 3.5-h derivatized sample whose amine content is between 0.13 and 0.10 mol of amine/mol of nucleotide. Pattern E is a wet fiber preparation (water as solvent). Patterns F, G, and H are at 98%, 79%, and 33% rh, respectively.

This relationship was used to evaluate the amount of *n*-butylamine bound to DNA for all of the samples which we describe in this and the following paper.

When these data are compared to the results from similar experiments using $^{14}CH_2O$, we find that R values for CH_2O are consistently displaced along the R axis by +0.025 mol of $^{14}CH_2O$/mol of nucleotide for the same values of $[\theta]_{275}$ and a reaction time of 3–4 h. (Shorter reaction times result in smaller differences.) This displacement probably represents the result of intra- and interstrand cross-linking of DNA bases by CH_2O previously reported by others (Collins & Guild, 1968; Feldman, 1973). In view of the fact that the CD spectrum of the CH_2O-treated controls is identical with that of an untreated sample of DNA at the same ionic strength, it is unlikely that this low degree of cross-linking (2.5% of the nucleotide population) has had significant conformational effects on the DNA structure.

Table I: Helical Parameters of DNA·Butylamine Complexes at Various Relative Humidities

sample set	reaction time	R[a] max	R[a] min	rel humidity	DNA conformation	pitch (Å)	axial repeat	residues per turn	equatorial reflection (Å)
1		0		33	partially crystallized A[b]	27.0			11.0[c]
2		0		33	A[b]	27.4			11.0[c]
1		0		79	A	28.1			11.4[c]
2		0		79	A	28.1			11.4[c]
1		0		98	B	33.9	3.31	10.2	22.9
2		0		98	B	33.7	3.30	10.2	22.9
1	20 min	0.06	0.06	33	C	28	3.3	8.5	17.3
1				79	C	30.1	3.31	9.1	18.9
1				98	B	32.5	3.31	9.8	20.8
1	55 min	0.10		33	C	28.6	3.27	8.8	18.0
1				79	C	28.7	3.29	8.7	18.2
1				98	B	32.5	3.32	9.8	20.4
1	3.5 h	0.13	0.10	33	C	28.8	3.28	8.8	17.1
1				79	C	30.1	3.31	9.1	18.6
1				98	B	32.6	3.32	9.8	22.5
2	2 h	0.14	0.12	33	C	27.8	3.28	8.5	17.2
2				79	C	29.4	3.29	8.9	18.7
2				98	B	32.8	3.30	9.9	22.4
2				wet fiber	B	33.0	3.3		33.0

[a] R = moles of *n*-butylamine per mole of nucleotide. [b] Control fibers slowly change at 33% or <5% rh to a less crystalline A-like form. [c] The reported reflection is that of the most intense equatorial reflection and is not that corresponding to the smallest equatorial spacing.

X-ray Diffraction. Representative X-ray diffraction patterns obtained from fibers from sample set 1 are shown in Figure 4, and the results of more extensive measurements with both sample sets are summarized in Table I. The diffraction results with both sample sets 1 and 2 were indistinguishable. At high water contents (98% rh or wet fiber preparations), both control and all derivatized samples gave typical B-form diffraction patterns. This is illustrated by a comparison of Figure 4A, which is a diffraction pattern of the control from sample set 1 at 98% rh, with panels E and F of Figure 4, which represent the patterns of the 3.5-h sample as a wet fiber and at 98% rh, respectively. The helical periodicity of the B-form patterns from control and derivatized fibers differed somewhat, with controls giving values of 10.2 residues/turn and derivatized samples giving values between 9.8 and 9.9 residues/turn. Given the expected errors in the determination of the periodicity (standard deviation of ca. 0.15 residue/turn in more extensive series of measurements; Zimmerman & Pheiffer, 1979), this difference may not be significant. It is worth noting, however, that the control DNA samples which were exposed to EDTA and CH_2O gave values close to the average of 10.1 residues/turn and a pitch of 33.7 Å obtained from an extensive series of measurements of DNA fibers which were exposed to high levels of EDTA (Zimmerman & Pheiffer, 1983). In view of the correlation between the reduced rotational strength of the positive band at 275 nm and the increased winding angle noted for DNA (Baase & Johnson, 1979; Chan et al., 1979) and for amine derivatives prepared with covalently closed PM 2 DNA (Kilkuskie, 1982), it would seem likely that the observed change in helical periodicity is in the right direction, although the magnitude itself cannot be evaluated precisely from these results.

At the lower relative humidity of 79%, the controls assumed a characteristic A form, as expected for sodium salts (Figure 4B). At even lower relative humidities, 33% and <5% (panels C and D, respectively, of Figure 4), there was a slow transformation to a less crystalline A-like form.

In contrast to the behavior of the controls, the samples containing various amounts of butylamine exhibit a stable C pattern at 79% rh which persists at 33% rh. This is illustrated by panels G and H of Figure 4 which were patterns obtained on the 3.5-h sample at 79% and 33% rh, respectively. As was previously observed for the C patterns obtained on fibers immersed in 12 M LiCl (Zimmerman & Pheiffer, 1980), these C structures have fewer base pairs per turn than those reported for LiDNA by Marvin et al. (1961).

Conclusions

It is interesting that fibers of the DNA·butylamine complexes prefer the C form upon dehydration, adopting this conformation at lower water contents which yield the A form for underivatized DNA. This would suggest that the conformational perturbation produced by the amine at higher hydration is moving the polynucleotide structure in the direction of the C form.

It is clear, however, that both the controls and the amine complexes which we have examined in this study exhibit typical B patterns and B helical characteristics in the highly hydrated state. Unless special constraints are imposed by the orientation of DNA in the fiber, it would seem reasonable to conclude that the DNA conformation giving rise to the CD spectrum with essentially zero rotational strength above 260 nm is a variant of the B structure and not the C form as has been previously assumed.

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Conformational Characteristics of Deoxyribonucleic Acid–Butylamine Complexes with C-Type Circular Dichroism Spectra. 2. A Raman Spectroscopic Study†

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ABSTRACT: The derivatives of calf thymus DNA in which *n*-butylamine is covalently attached as described in the preceding paper in this series [Chen, C. Y., Pheiffer, B. H., Zimmerman, S. B., & Hanlon, S. (1983) *Biochemistry* (preceding paper in this issue)] were examined by Raman spectroscopy. As previously mentioned, these complexes exhibit profoundly decreased rotational strengths of the positive band of the circular dichroism (CD) spectrum above 260 nm, with the most heavily substituted (ca. 0.12 mol of amine/mol of nucleotide) resembling that of DNA in 11 *m* LiCl. Raman spectra of all complexes and their controls in the form of either fibers at 98% relative humidity or gels at 40 mg/mL in 20 mM NaCl, pH 7, show typical B-type spectra with no evidence of significant amounts of C, A, Z, or disordered forms. We have thus concluded that the assignment of the nonconservative CD spectrum of DNA typically observed in concentrated electrolyte solutions to a C form is in error. Both these Raman data and the X-ray results reported in the previous paper indicate that the structure giving rise to the C CD spectrum has B-form backbone geometry.

In the preceding paper [see Chen et al. (1983)], we demonstrated that samples with circular dichroism (CD)[1] spectra which were similar to those previously assigned to a C conformation or could be interpreted as possessing significant C character gave B-form X-ray patterns when the fiber was highly hydrated (98% rh and wet fiber preprations). Although this would suggest that the C assignment to CD spectra with approximately zero rotational strength above 260 nm is in error, there is always the possibility that the fiber characteristics and necessity for orientation impose constraints on the conformation which are absent in solution. Thus, the conformation present in the highly hydrated fiber might not be equivalent to that found in the unconstrained solution state or isotropic gel form.

In order to evaluate this possibility, we have undertaken a Raman study of complexes and their controls similar to those employed in the X-ray diffraction study. Raman spectroscopy can be conducted with fibers as well as solutions of DNA, and the CD properties of the latter can be examined at concentrations almost as high as that required for the Raman experiments. Indeed, we have shown in the preceding paper (Chen et al., 1983) that the CD spectra of DNA and the butylamine complexes at concentrations only about one-half to one-third less than that required for Raman spectroscopy differ insignificantly from those obtained at the usual concentrations employed in CD spectroscopy. Firm spectral assignments exist for the A, B, C, and disordered forms of random-sequence DNA (Erfurth et al., 1972; Erfurth & Peticolas, 1975; Thomas & Kyogoku, 1977; Prescott et al., 1983) as well as a Z form for poly(dG-dC) for both solutions and the fiber state (Thamann et al., 1981). Raman spectroscopy is thus an ideal technique for identifying the conformational state of DNA which exhibits a C-type CD spec-

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[1] Abbreviations: CD, circular dichroism; EDTA, ethylenediaminetetraacetate; rh, relative humidity.

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