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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., Pfeiffer, 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 preparations). 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 & Petricolas, 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.

trum, as well as settling the issue of whether the oriented state of the fiber imposes its own conformational requirements.

Experimental Procedures

The details of the purification of the calf thymus DNA (Calbiochem, lot 900007) by phenol extraction and the preparation of the *n*-butylamine complexes and their controls were identical with those described in the preceding paper (Chen et al., 1983). Except for a 3-day transit time, the gels at ca. 12 mg/mL of sample set 1 prepared for use in the X-ray experiments (Chen et al., 1983) were maintained in the frozen state until used in these current experiments. Their concentrations were suitable for the fiber preparations but were too low for Raman spectra of gels of DNA which required, minimally, 25 mg/mL. Accordingly, the day before the Raman spectra were to be obtained, aliquots of the thawed gels of sample set 1, sufficient to contain 0.5 mg of DNA, were redissolved in small amounts of 0.12 M NaCl and precipitated with 2 volumes of cold ethanol. The precipitates were collected by low-speed centrifugation in 1.5-mL Eppendorf tubes, washed exhaustively with 70% ethanol, and dried under N₂ for ca. 5 h. A 10- μ L aliquot of 20 mM NaCl and 16 mM sodium cacodylate/cacodylic acid, pH 7, was then added to each tube to yield DNA gels of ca. 40 mg/mL.

An additional sample set (sample set 3) was prepared for the Raman experiment. Aliquots for this sample set were removed at reaction times of 15 min, 45 min, and 3 h. Since these were to be used only in the Raman experiments, the complexes and their control were dialyzed consistently against 20 mM NaCl, pH 7, to remove reactants. They were then precipitated with ethanol, washed and dried as previously described (Chen et al., 1983), and stored at -10 °C, except for a 3-day transit time at 0 °C. The day before the Raman spectra were to be obtained, ca. 0.4–0.5 mg of each sample was redissolved in 10 μ L of 20 mM NaCl, pH 7, to produce a gel of 40–50 mg/mL.

Absorbance and CD spectra were obtained with the instrumentation and precaution reports in Chen et al. (1983). Concentrations of DNA were estimated by using the absorbance data and an extinction coefficient of 6700 M⁻¹ cm⁻¹ at 259 nm (Chen et al., 1981).

The Raman spectra displayed in the present work were obtained by using a Spex Model 1401 Ramalog spectrometer with an argon laser (Coherent Radiation, Model CR 2). Some of the fiber spectra discussed in the text were obtained with a Jarrel Ash double-grating Raman spectrometer interfaced to a Cromemco Z-2 computer and equipped with a Coherent Radiation Model CR 52 laser tube. All spectra were excited with 150–300 mW of 514.5-nm radiation. Spectra which are displayed in the figures were recorded in the analog mode at either 5- or 10-cm⁻¹ resolution.

DNA gels and fibers for Raman spectroscopy were contained in 1-mm diameter capillary tubes which were thermostated at 25 °C by means of a custom-made jacket previously described (Thomas & Baryloki, 1970). Precise frequencies of lines in the spectra of the gels of sample set 1 were established by incorporating 0.016 M sodium cacodylate/cacodylic acid, pH 7.0, buffer. This gives rise to a band with a maximum at 607 cm⁻¹. Intensities of DNA spectral lines were estimated by measuring the vertical distance from the peak to a linear base line drawn between two flanking troughs. These intensities were adjusted for differences in DNA concentrations and instrumental conditions by normalizing to the intensity of the PO₂⁻ stretch at 1094 cm⁻¹, estimated in a similar manner. The same trough positions were used consistently for a given peak.

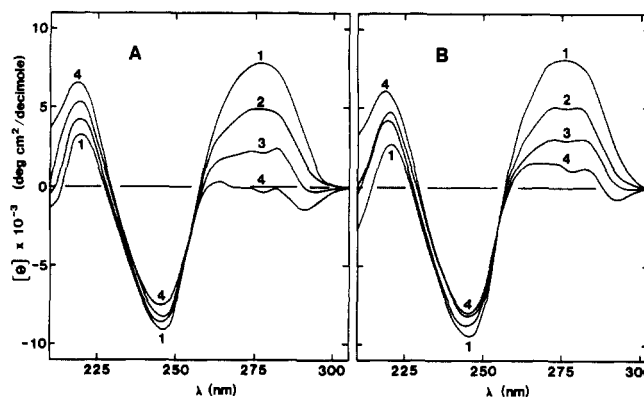


FIGURE 1: CD spectra of dilutions of the DNA samples examined by Raman spectroscopy. (A) Spectra 1, 2, 3, and 4 are those of sample set 1 at 0, 0.05, 0.09, and 0.12 mol of amine/mol of nucleotide, respectively, in 20 mM NaCl and 16 mM sodium cacodylate/cacodylic acid, pH 7. (B) Spectra 1, 2, 3, and 4 are those of sample set 3 at 0, 0.05, 0.08, and 0.10 mol of amine/mol of nucleotide, respectively, in 20 mM NaCl, pH 7 (unbuffered). The temperature was 27 °C, and the DNA concentrations were ca. 0.2 mM in all cases.

Fibers for Raman spectra were drawn in a specially designed fiber pulling box at 75% rh, over saturated NaCl. Sixty microliters of the appropriate gel of sample set 1 was placed on two movable glass rods mounted within the box. The box was sealed, and the two rods were gradually pulled apart over a 6–12-h period to produce, ultimately, a fiber of ca. 0.6 mm in diameter. After 12–24 h, the fiber was removed and transferred to a capillary tube where it was placed about 2 cm from the end. This end was then flame sealed. At the other end, 10–15 μ L of an appropriately saturated salt solution required to maintain the desired humidity within the tube was introduced, and this end was sealed. Relative humidity of 98% was maintained by CaSO₄·5H₂O, 92% rh by Na₂C₄H₄O₆, 79% rh by NH₄Cl, and 31% rh by CaCl₂·6H₂O. Fibers were allowed to equilibrate for a minimum of 8 days in these capillaries before spectra were recorded.

A very low salt fiber using the derivative containing 0.10 mol of amine/mol of nucleotide from sample set 3 was prepared by placing 0.5 mg of this preparation in a drop of water suspended between the two glass rods. The DNA sample was then pulled apart in the sealed chamber at 92% rh over a 5-h period to a length of 3 mm. After equilibrating overnight, it was removed and transferred to a capillary tube together with a small amount of saturated Na₂C₄H₄O₆ solution required to maintain 92% rh. This fiber was allowed to equilibrate only 2 days in the sealed capillary before its spectrum was obtained.

Results

CD Spectroscopy. The CD spectra of sample sets 1 and 3, recovered from the Raman cells after spectroscopy, are shown in panels A and B, respectively, of Figure 1. The recovered samples always exhibited a higher rotational strength of the positive band above 260 nm than the same sample prior to spectroscopy, reflecting amine loss during the process of solution preparation or spectroscopy. Nevertheless, the rotational strength and range of amine substitution are comparable to those for samples examined by X-ray diffraction. The amine content was estimated from the values of $[\theta]_{275}$ by using eq 1 of Chen et al. (1983). These values are reported in the legends for each figure as well as in Table I.

The amine loss as estimated by CD spectral properties from the redissolved fibers prepared from sample set 1 was more marked than had been observed for the redissolved X-ray fibers reported in the preceding paper. For instance, the value of

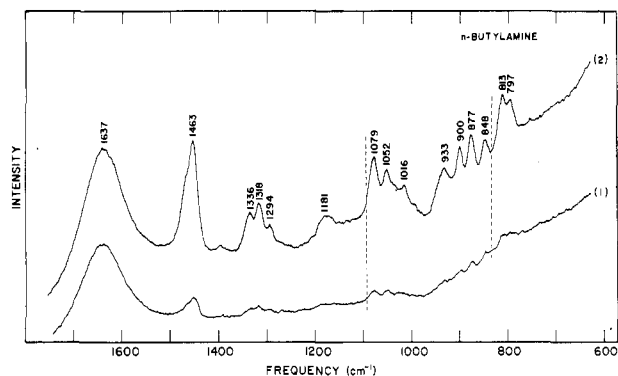


FIGURE 2: Raman spectra of *n*-butylamine hydrochloride in H₂O (pH 7 and 25 °C) at 0.072 M (curve 1) and 0.264 M (curve 2). Spectral conditions: laser power, 300 mW; slit width, 10 cm⁻¹.

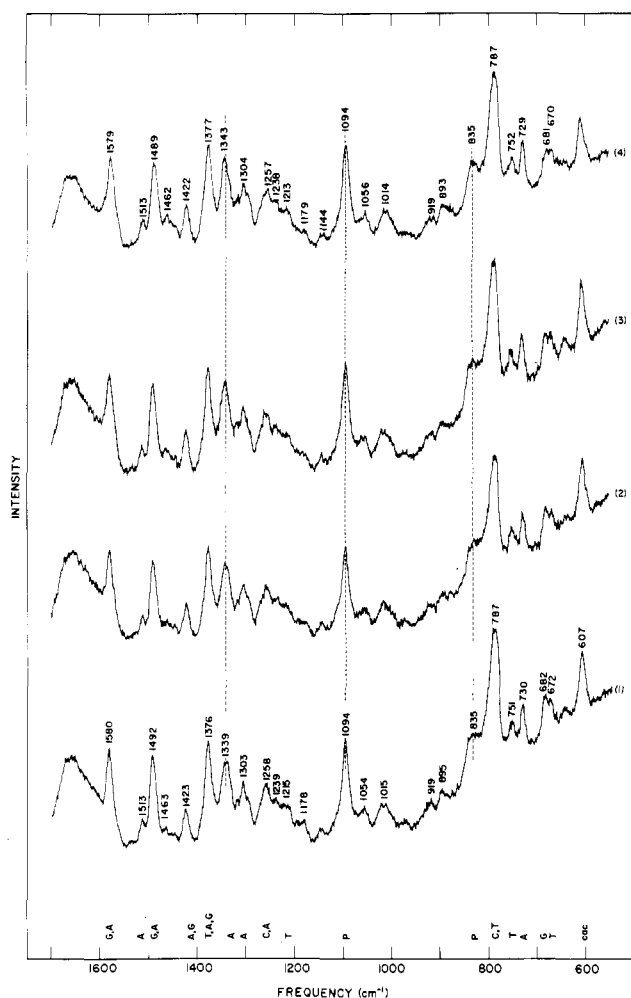


FIGURE 3: Raman spectra of gels of sample set 1 in 20 mM NaCl and 16 mM sodium cacodylate/cacodylic acid, pH 7.0 at 25 °C. Curve 1 is the spectrum of the control, and curves 2, 3, and 4 are the spectra of the amine complexes at 0.05, 0.09, and 0.12 mol of amine/mol of nucleotide, respectively. Sample concentrations are 4% (40 mg/mL or 120 mM) in all cases except curve 4 which was taken at 5.0% DNA (50 mg/mL or 151 mM). Spectral conditions: laser power, 300 mW; slit width, 5 cm⁻¹.

$[\theta]_{275}$ for the solution prepared from the 98% rh fiber of the 3.5-h sample was $2.0 \times 10^3 \text{ deg cm}^2/\text{dmol}$, corresponding to an amine content of 0.089 mol/mol of nucleotide. The value for the solution of the X-ray fiber prepared from the same sample was $0.9 \times 10^3 \text{ deg cm}^2/\text{dmol}$, corresponding to an amine content of 0.102 mol/mol of nucleotide. The fibers from Raman spectroscopy required an exceptionally long period to

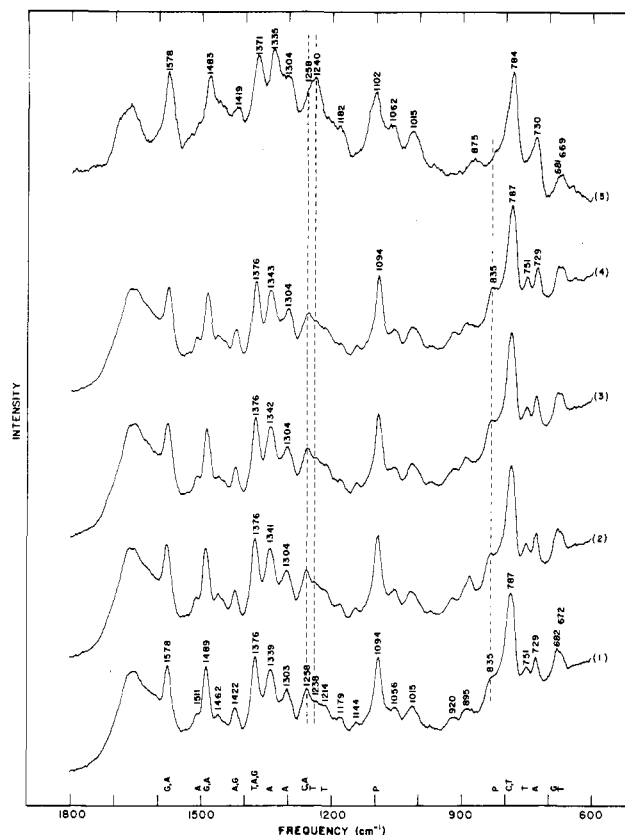


FIGURE 4: Raman spectra of sample set 3. Curves 1–4 are spectra of gels of 4% DNA in 20 mM NaCl, pH 7 at 25 °C. Curve 1 is the control, and curves 2, 3, and 4 are the DNA-amine complexes at 0.05, 0.08, and 0.10 mol of amine/mol of nucleotide, respectively. Curve 5 is a low salt fiber of the 0.10 mol of amine/mol of nucleotide sample at 92% rh. Slit width was 10 cm⁻¹ in all spectra. Laser power was 150 mW in spectrum 5 and 300 mW in spectra 1–4.

achieve solution (ca. 1 week of continuous stirring at 7 °C). This coupled with the higher temperatures to which the fibers were exposed while in the laser beam of the Raman spectrometer probably accounts for the greater loss of amine. Even if all of this loss occurred in the Raman cell, however, the mean residue ellipticities of solutions prepared from the recovered fibers are still in the same range as those from samples examined by X-ray diffraction.

Raman Spectroscopy. In preparation for interpreting the Raman spectra of the DNA samples containing various amounts of *n*-butylamine, we obtained Raman spectra of *n*-butylamine at pH 7. These are shown in Figure 2. Spectrum 1 of Figure 2 is the lowest concentration (0.072 M) at which we could obtain a satisfactory spectrum with the instrumental conditions employed. This concentration is about 5-fold higher than that expected in the gels of the most heavily derivatized samples.

A better estimation of the positions in the amine spectrum at which significant intensities are exhibited is obtained from spectrum 2 at 0.264 M. The vertical dashed lines in Figure 2 indicate positions in the spectrum where no Raman lines of butylamine occur but where conformationally sensitive lines of DNA will be monitored. In fact, the only spectral region in which the amine should give rise to significant interference with DNA is $1460\text{--}1470\text{ cm}^{-1}$.

The Raman spectra of gels of sample sets 1 and 3 are shown in Figures 3 (spectra 1-4) and 4 (spectra 1-4), respectively. The number to the right of each Raman spectrum in these figures corresponds to the sample designation of Figure 1. The positions of the Raman bands and their relative intensities

Table I: Raman Spectral Characteristics of the DNA-Butylamine Complexes, Set 1

band positions $\pm 1 \text{ cm}^{-1}$ for mol of amine/mol of nucleotide of				band assignment ^a	normalized intensities ($I/I_{1094 \text{ cm}^{-1}} \pm 0.02$) for mol of amine/mol of nucleotide of				ΔI
0	0.05	0.09	0.12		0	0.05	0.08	0.10	
672	671	670	670	T	0.28	0.30	0.31	0.32	↑
682	682	681	681	G	0.38	0.37	0.34	0.32	↓
730	729	729	729	A	0.47	0.47	0.47	0.48	NC ^b
751	751	751	751	T	0.40	0.40	0.42	0.40	NC
787	787	786	787	C, T	1.64	1.63	1.59	1.46	↓
~835	~835	~834	~835	deoxy-Rib-P	0.58	0.61	0.60	0.60	↑
895	895	895	894	deoxy-Rib	0.14	0.14	0.15	0.18	↑
919	920	920	919	deoxy-Rib	0.13	0.12	0.13	0.12	NC
1015	1016	1015	1014	C-O	0.24	0.27	0.25	0.24	NC
1054	1055	1054	1055	C-O	0.19	0.27	0.21	0.23	NC
1094	1094	1094	1094	PO ₂					
1144	1144	1143	1143						
1178	1179	1179	1179	C-N	0.18	0.11	0.13	0.13	↓
1215	1214	1215	1214	T	0.31	0.31	0.30	0.28	NC
1239	1238	1238	1238	T	0.34	0.33	0.33	0.34	NC
1258	1259	1259	1258	C, A	0.48	0.46	0.43	0.42	↓
1303	1304	1304	1304	A	0.47	0.48	0.46	0.45	NC
1339	1341	1341	1343	A	0.83	0.84	0.82	0.81	NC
1377	1377	1376	1377	T, A, G	1.05	1.05	1.01	0.98	↓
1423	1423	1422	1422	A, G	0.30	0.32	0.33	0.31	NC
1463	1463	1462	1462	deoxy-Rib	0.14	0.17	0.19	0.22	↑ (57%)
1491	1491	1490	1488	G, A	0.95	0.87	0.87	0.84	↓
1513	1513	1512	1512	A	0.24	0.22	0.23	0.22	NC
1580	1579	1579	1578	G, A	0.70	0.70	0.70	0.72	NC

^a Taken from Erfurth et al. (1972) and Thomas & Kyogoku (1977). ^b NC, no change.

(normalized to that of the 1094-cm⁻¹ PO₂⁻ band in each spectrum) are given in Table I for sample set 1. Spectrum 4 of Figure 3 and the intensity data for the 0.12 mol of amine/mol of nucleotide sample of sample set 1 are those taken from the spectra of a solution which was ca. 50 mg/mL (5%). Spectra on this same sample at a lower concentration of 30 mg/mL (3%) were identical in shape and line positions, although small differences in the normalized line intensities were noted.

A visual inspection of the two figures shows little change in going from the control to the most heavily substituted samples, and this is confirmed by the data given in Table I for sample set 1. A similar analysis of the gel spectra of sample set 3 gave the same results. The characteristics of these spectra displayed are those assigned to the B form of DNA by Erfurth et al. (1972). The earmarks of this latter spectrum include the shoulder or peak at 835 cm⁻¹ attributed to the C-O-P-O-C backbone vibration, the PO₂⁻ stretch at 1094 cm⁻¹, and a characteristic profile of base vibrational modes at 1376, 1339, 1258, 1239, 751, 729, 682, and 672 cm⁻¹. These Raman features clearly distinguish B-DNA from A-DNA, C-DNA, and Z-DNA structures (Erfurth et al., 1972; Thamann et al., 1981; Prescott et al., 1983) as well as the denatured random-coil form (Erfurth & Peticolas, 1975).

The main differences between the B-DNA Raman spectra and a typical C-DNA Raman spectrum are demonstrated by a comparison of spectra 4 and 5 in Figure 4. Spectrum 5 of Figure 4 was obtained on a very low salt fiber prepared at 92% rh from the same derivative used for the preparation of the gel whose spectrum is shown in spectrum 4 of Figure 4. In spectrum 5, which is a C-type spectrum, the 835-cm⁻¹ band has been markedly reduced in intensity while a new weak band has appeared at 875 cm⁻¹, obscuring weak deoxyribose lines at 895 and 919 cm⁻¹. The PO₂⁻ line has shifted to 1102 cm⁻¹. The 1339- and 1376-cm⁻¹ lines have shifted by about 5 cm⁻¹ to lower frequency, and their intensities relative to each other have changed substantially. The relative intensities of the 1259- and 1238-cm⁻¹ lines have also reversed, while the in-

tensities of the 681- and 670-cm⁻¹ lines have become more nearly equal with the B → C transition. The intensity of the 729-cm⁻¹ line has increased substantially, obscuring the weaker 751-cm⁻¹ band. The only difference between spectrum 5 seen in Figure 4 and that assigned to the C form by Erfurth et al. (1972) is the position of the band which falls at 875 cm⁻¹ in our spectrum and at 884 cm⁻¹ in that of Erfurth et al. (1972). Others (Goodwin & Brahms, 1978) have reported a variable position for this band also. This is not surprising in view of the breadth of the band and the difficulty in assigning its center to within $\pm 5 \text{ cm}^{-1}$.

As the entries in Table I show, there are small but significant differences in the spectral characteristics of the gels as the level of amine attachment increases. These changes were also observed reproducibly in the spectra of sample set 3. Since some of the lines do not suffer a significant change in their normalized intensities, these differences cannot be attributed to a change in intensity of the 1094-cm⁻¹ band as the level of amine substitution increases. The intensity increase of 54% in the otherwise weak DNA line at 1465 cm⁻¹ is clearly due to the contribution of butylamine at this frequency (see Figure 2). Other changes cannot be due to direct contributions by the amine but more probably reflect subtle conformational effects of the derivatization and/or effects on base vibrations due to covalent attachment. With the exception of the 835-cm⁻¹ line, which shows an increase in intensity, most of the significant intensity changes ($\geq 7\%$) are decreases seen at 682, 787, 1179, 1258, 1377, and 1491 cm⁻¹. Some of these lines are known to be conformationally sensitive, but the pattern of changes is not interpretable in terms of a small conformational movement along a B → disordered form, B → A or B → C continuum. The intensity decreases do parallel the decreases observed in the rotational strength of the positive band of the CD spectra of the derivatives. Since the latter effect correlates with increases in the winding angle of the DNA base pairs (Baase & Johnson, 1979; Chan et al., 1979), the observed intensity changes may reflect changes in this conformational parameter. We have recently demonstrated

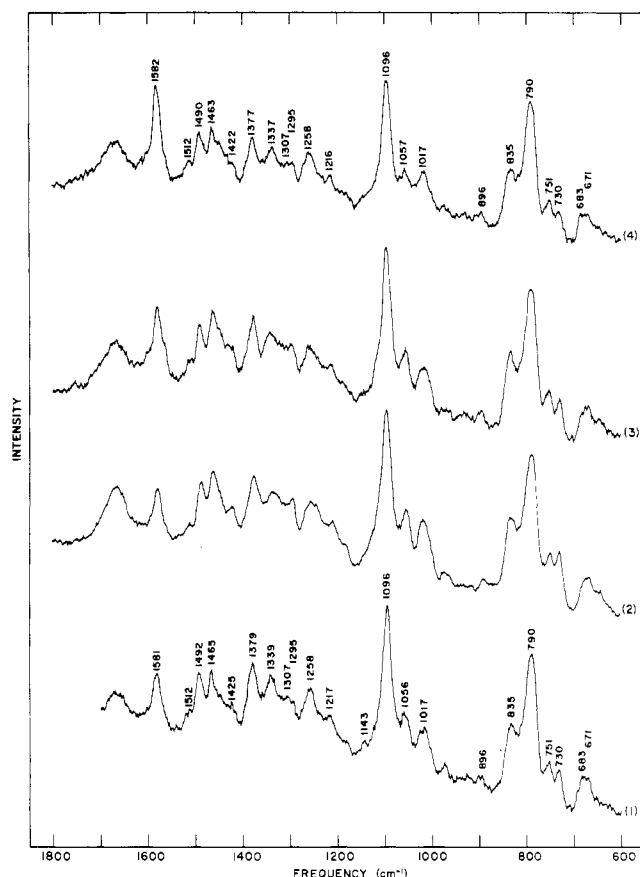


FIGURE 5: Raman spectra of fibers of sample set 1 at 98% rh. These fibers were prepared from the same gel pellets used in the X-ray diffraction experiments. Curve 1 represents the control, and curves 2, 3, and 4 are the spectra of the DNA-amine complexes designated as the 20-min, 55-min, and 3.5-h samples, respectively. The amine content of these complexes was originally 0.06, 0.10, and 0.13 mol of amine/mol of nucleotide. After redissolving, amine contents were found to be 0.06 and 0.09 mol of amine/mol of nucleotide for the 55-min and the 3.5-h sample, respectively. Laser power was 100 mW, and slit width was 10 cm^{-1} .

that similar winding angle and rotational strength changes also result when *n*-butylamine is coupled to covalently closed PM 2 DNA (Kilkuskie, 1982; R. E. Kilkuskie, N. Wood, and S. Hanlon, unpublished results).

The only significant change in band position with derivatization occurs for the adenine line at 1339 cm^{-1} , which undergoes a 4- cm^{-1} shift to higher frequency. As shown in Figure 3, the flanking bands at 1303 (an A line) and 1376 cm^{-1} (a T, A, G band) do not shift, and hence, this cannot be attributed to direct contributions of butylamine, which has a triplet in this spectral region and which would be expected to affect these positions as well. This shift suggests that adenine may be a major attachment site for the amine.

Raman spectra were also obtained on fibers prepared from the gels of sample set 1. The spectra obtained at 98% rh are shown in Figure 5. Both the control and the derivatized samples show typical B-type patterns, as would be expected on the basis of the X-ray results, but samples at lower relative humidity gave somewhat discrepant results. The control samples at 79% rh exhibited A-type patterns. The spectra of the derivatized samples showed some earmarks of a C-type Raman spectrum at 79% rh, insofar as the intensity of the 730- cm^{-1} line was enhanced and the ratio of the 1238- and 1258- cm^{-1} lines was more appropriate for the C form. In the most highly derivatized sample, the weak band at 880 cm^{-1} was also observed. The 835- cm^{-1} line in the spectra of all

derivatives obtained at 79% rh was clearly apparent, however, although reduced in intensity relative to the 787- cm^{-1} line. This reduction amounted to ca. 25% for the most derivatized sample compared to its normalized intensity at 98% rh. The appearance of this line indicates a significant fraction of B character in the conformation present at 79% rh in the Raman cell. The observed pattern was not definitive for C structure until 31% rh. In the spectrum at 31% rh, the small broad peak in 800- cm^{-1} region was again centered at 877 cm^{-1} , a position consistent with spectrum 5 of Figure 4.

This discrepancy between the Raman and X-ray results on fibers of the derivatized samples at 79% rh is puzzling. The salt content is a determining factor for the stabilization of a given form at a specific relative humidity, and hence, it is not surprising that a fiber prepared with one of the DNA-amine complexes (sample set 3) at very low salt concentrations should exhibit the C spectrum shown in spectrum 5 of Figure 4 at relative humidities as high as 92%. Unlike this example, however, the fibers of sample set 1 for the X-ray and the Raman experiments were drawn from the same gel sample. Although the two sets of fibers were of different lengths and diameters, the salt content of the two should, nevertheless, have been identical and equal to ca. 0.6% NaCl, in terms of total dry weight of salt plus DNA.

The temperatures of the fibers in the two types of measurements, however, may not have been the same. The fibers in the laser beam in the Raman experiments cannot be effectively thermostated since the fibers do not make good thermal contact with the capillary walls which contain them. Consequently, the temperature of the fiber is likely to be higher than room temperature. Temperature differences will certainly lead to differences in hydration and affect the position of conformational equilibria. We have, in fact, observed subtle transformations of the spectra of low salt fibers of the complexes at 92% rh in the 700–1100- cm^{-1} region over a limited range of laser power (75–125 mW). These temperature effects do not apparently affect the B \rightarrow A transition, as the controls at 79% rh show the A form in both sets of experiments. The B \rightarrow C transition may be more temperature sensitive.

Conclusions

The Raman results unequivocally establish that the butylamine derivatives assume the B structure at higher degrees of hydration (98% rh and in gels), and there is no evidence from these experiments for the presence of significant amounts of C, A, Z, or disordered forms at any level of substitution examined. This confirms the conclusion based on the X-ray experiments and supports the contention that the orientation of DNA molecules in fibers does not influence the conformational state assumed by DNA, at least at high degrees of hydration. The previous assignment of the nonconservative spectrum of DNA (with approximately zero rotational strength above 260 nm) seen in concentrated electrolyte solutions (Hanlon et al., 1975) and in core particles of chromatin to a C-type structure (Tunis-Schneider & Maestre, 1970; Hanlon et al., 1972) is not substantiated by these Raman and X-ray data. The conformation of DNA giving rise to this CD spectrum has a higher winding angle than that present at more modest concentrations of electrolyte (Baase & Johnson, 1979; Chan et al., 1979) but clearly retains the backbone geometry of the B form.

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Specific Receptor Sites for 1-*O*-Alkyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine (Platelet Activating Factor) on Rabbit Platelet and Guinea Pig Smooth Muscle Membranes[†]

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ABSTRACT: By using tritiated 1-*O*-alkyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine (³H-PAF), we have directly identified its specific binding sites on rabbit platelet plasma membranes. The equilibrium dissociation constant for ³H-PAF is $1.36 (\pm 0.05) \times 10^{-9}$ M at 0 °C. The number of binding sites is $1.61 (\pm 0.34) \times 10^{12}$ /mg of membrane, which corresponds to approximately 150–300 receptors/platelet (depending on membrane vesicle orientation). Binding of ³H-PAF to rabbit platelet plasma membrane is rapid ($t_{1/2} < 5$ min at 0 °C) and reversible. For a series of PAF analogues, their

affinity for the receptor sites parallels with their relative potency to induce platelet aggregation. PAF can cause contraction of smooth muscle of heart, parenchymal strip, trachea, and ileum. Specific PAF receptor binding was demonstrated with purified plasma membrane from several smooth muscles and from polymorphonuclear leukocytes but not from presumably PAF nonresponsive cells such as erythrocytes and alveolar macrophages. It is likely that the interaction of PAF with these binding sites initiates the specific responses of platelets, polymorphonuclear leukocytes, and smooth muscles.

The platelet activating factor (PAF)¹ derived from rabbit basophils has recently been identified as 1-*O*-alkyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine (Demopoulos et al., 1979; Benveniste et al., 1979). It is a potent mediator of the physiologic alteration of IgE anaphylaxis. It induces shape change, aggregation, and the release of granular contents of the platelet (Demopoulos et al., 1979; Benveniste et al., 1979; Hanahan et al., 1980). It also causes polymorphonuclear leukocyte (PMN) chemotaxis and enhances PMN adhesiveness, aggregation, and degranulation (Shaw et al., 1981a; O'Flaherty et al., 1981). The contraction of guinea pig ileum smooth muscle (Findlay et al., 1981) and hypotension induced by PAF (Blank et al., 1979; Muirhead et al., 1981) have also been reported. Investigations of synthetic phospholipid analogues (Tense et al., 1981; Tokumura et al., 1981; Satouchi et al., 1981; Hanahan et al., 1981) showed that the structural features necessary for biological actions are highly specific. The binding of PAF to rabbit platelets has also been reported (Shaw & Henson, 1980; Camussi et al., 1980; Brown & Thuy,

1981; Valone et al., 1982), and it was suggested that PAF action on platelets may be a receptor-mediated process. In our laboratory, we have studied the first step in the process of platelet activation and identified specific binding sites for PAF on rabbit platelet plasma membranes by using a radio-labeled PAF. Our results suggest that these binding sites are involved in the initiation of the platelet response to PAF.

In vivo administration of synthetic or natural PAF intravenously to normal rabbits induces all of the respiratory and cardiovascular alterations of IgE anaphylaxis (Halonon et al., 1976). Since bronchoconstriction and thrombocytopenia can be suppressed either by infusion of prostacyclin (Vargaftig et al., 1980) or in an immune platelet depressed system (Halonon et al., 1981), platelets were considered to be the primary sites for the PAF-induced respiratory alterations. On the other hand, bradycardia, right ventricular systolic hy-

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¹ Abbreviations: PAF, platelet activating factor or 1-*O*-alkyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine; DPPC, dipalmitoylphosphatidylcholine; PMN, polymorphonuclear leukocyte; IgE, immunoglobulin E; TLC, thin-layer chromatography; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; DSC, differential scanning calorimetry.