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Solution Conformations of DNAs Containing Binding Sites of the Catabolite Gene Activator Protein and *lac* Repressor Protein: Characterization by Raman Spectroscopy†

Henry DeGrazia,‡ Daniel Brown,‡ Susannie Cheung,§ and Roger M. Wartell*,‡

Schools of Physics and Applied Biology, Georgia Institute of Technology, Atlanta, Georgia 30332, and Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Received January 19, 1988; Revised Manuscript Received April 25, 1988

ABSTRACT: Raman spectra from three subfragments of the *Escherichia coli* lactose promoter region were obtained in 0.1 M NaCl. The three DNAs are 21, 40, and 62 bp in length. The 21 and 62 bp DNAs contain the binding site for the catabolite gene activator protein (CAP). The 40 bp DNA contains the binding site for the *lac* repressor. A quantitative analysis of Raman band characteristics indicates an overall B-type conformation for these gene regulatory sites. Bands which correspond to A-family (807 cm^{-1}) and B-family (834 cm^{-1}) deoxyribose phosphate vibrations have the same intensities as bands found in heterogeneous DNAs. The spectra of the 21 bp CAP site have, however, a small band at 867 cm^{-1} and several other small differences similar to some characteristics observed in C-DNA spectra. Several dG nucleosides in the CAP site appear to be altered from the conventional C2′-endo/anti conformation. At 45 °C, well below the melting region of these DNAs, small changes occur in the spectra of the 40 bp *lac* repressor site which are not observed in the other DNAs. A weak band occurs at 705 cm^{-1}, and intensity changes are observed at 497, 682, and 792 cm^{-1}. The changes suggest that the conformations of several dG nucleosides are altered and that a small region may exist with characteristics of an A-family backbone. This conformational change at 45 °C coincides with previous NMR observations indicating an enhanced imino proton exchange rate at a GTG sequence within the *lac* operator site.

Crystal structures of DNA duplex oligomers (Wing et al., 1980; Kopka et al., 1983; Brown et al., 1986) and solution studies (Nilges et al., 1987; Prescott et al., 1985) have demonstrated that local structural variations can occur within a B-family DNA structure. These structural variations appear to depend upon base pair sequence. The ability of a DNA sequence to adopt specific conformations may play several roles in the selective interaction between a gene regulatory protein and its DNA site. The spatial location of chemical groups on DNA will affect the initial interaction between a protein and DNA site. DNA sequence may also influence a protein-induced alteration in DNA secondary structure (Hogan & Austin, 1987). How base pair sequence and environment influence DNA structure, particularly in solution, is not well understood (Dickerson, 1987).

We present in this report a Raman spectroscopic study on DNA molecules which contain protein binding sites within the *Escherichia coli* lactose promoter. Three DNA fragments, 62, 40, and 21 bp, were examined. The 21 and 62 bp DNAs contain the binding region of the catabolite gene activator protein, CAP (also known as cAMP receptor protein, CRP). The 40 bp DNA contains the binding site of the *lac* repressor. Previous ^1H NMR studies on the latter DNA indicated that

† This work was supported by NIH Grant GM 33543.
* Author to whom correspondence should be addressed.
‡ Georgia Institute of Technology.
§ University of Pennsylvania.

0006-2960/88/0427-6359$01.50/0

the imino proton exchange rate of the AT base pair within GTG sequences was considerably higher than for other AT base pairs (Lu et al., 1983).

Previous Raman studies have correlated a number of base and backbone Raman bands with specific conformational features of duplex DNA (Peticolas et al., 1986; Thomas, 1986; Nishimura & Tsuboi, 1986). We have employed these assignments together with a quantitative analysis of band characteristics (Wartell & Harrell, 1986) to examine the solution conformations of DNA sites for CAP and *lac* repressor. The Raman spectra of these DNAs clearly indicate that they are in the B-type conformation. On the basis of Raman bands at 834 and 807 cm^{-1}, the populations of B-family backbone conformers to A-family conformers are quantitatively the same as heterogeneous sequence DNAs with similar GC content. Several small features in the spectrum of the 21 bp fragment indicate, however, that a few nucleotides of this DNA may adopt C-DNA characteristics. Also, the 40 bp repressor site shows small changes in base and backbone modes at 45 °C not seen with the other DNAs. A band at 705 cm^{-1} characteristic of an A-type backbone is observed, and intensity changes at 497 and 682 cm^{-1} indicate conformational alterations in dG residues. These temperature changes appear to coincide with the ^1H NMR observations described above. The absence of similar changes in the 21 bp site, which also contains GTG sequences, indicates that this triplet alone is not responsible for the unusual temperature characteristics of the *lac* repressor site.

Materials and Methods

DNAs. The 144 bp DNA containing the *lac* promoter region was isolated from the plasmid pRMW27 (Wartell & Reznikoff, 1981). Isolation procedures have been previously described by DeGrazia et al. (1985). The 144 bp fragment was separated from the plasmid vector by digestion with *Eco*RI restriction enzyme followed by selective poly(ethylene glycol) precipitation and RPC-5 chromatography. The 62 bp DNA was obtained by cleaving some of the 144 bp DNA into two subfragments with *Msp*I. The 62 bp *Eco*RI–*Msp*I DNA was separated from the other subfragment (80 bp) by RPC-5 chromatography. The 62 bp DNA was also obtained from plasmid pCRM30 containing three copies of the 62 bp sequence cloned into the *Eco*RI site of the pUC9 plasmid. Although these two samples have slightly different ends, no differences were noted in their spectra. The 40 bp *lac* operator DNA was also isolated from a plasmid preparation as described (Nick et al., 1982). This DNA contains 36 paired bases and *Eco*RI ends (Figure 1). At the concentrations employed for the Raman experiments, we assume the *Eco*RI ends are base paired to form linear multimers. The 21 bp CAP site was synthesized by the cyanophosphoramidite method using an Applied Biosystems DNA synthesizer. After deblocking and lyophilization, the oligomer strands were purified by extraction with ether, RPC-5 chromatography, and dialysis. Electrophoresis through a 20% denaturing gel indicated a purity of >95%. Equimolar amounts of the strands were mixed and heated to 80 °C in 0.05 M NaCl and allowed to cool slowly to room temperature. The duplex product was verified on a nondenaturing gel. DNA samples were concentrated to about 1 mg/mL with a Centricon centrifuge filter (Amicon Inc.). They were then ethanol precipitated or lyophilized, washed with 80% ethanol to remove excess salts, and then lyophilized again to dryness. Samples were resuspended in 0.1 M NaCl to a concentration of 50 mg/mL.

Raman Measurements. Spectra were obtained by using the 5145-Å emission line from an argon ion laser which delivered

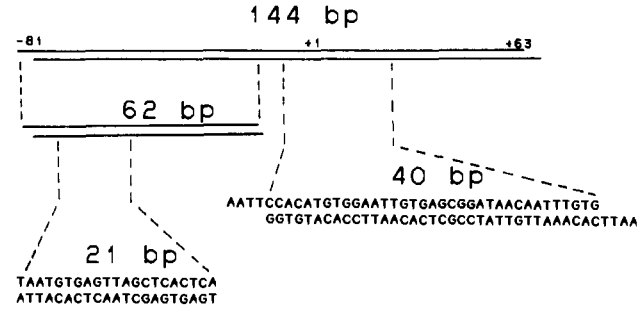

FIGURE 1: Four DNA fragments used in the Raman study. The 144 bp DNA contains the *lac* promoter region. The 62 bp DNA spans the region from −81 to −20, and the 21 bp DNA goes from −72 to −52. The 40 bp DNA includes base pairs from −10 to +26 and is assumed to form base pairs at the *Eco*RI ends under the conditions of the experiment. Each molecule is thus considered to have 40 bp.

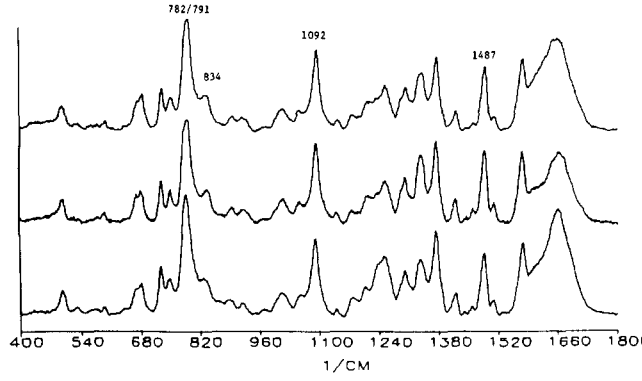

FIGURE 2: Raman spectra from 400 to 1800 cm^{-1} of the three DNAs at 25 °C in 0.1 M NaCl. (Top) 62 bp; (middle) 40 bp; (bottom) 21 bp.

200 mW to the samples. Each spectrum is the sum of four consecutive scans from 400 to 1900 cm^{-1}. Individual scans were recorded at 1 cm^{-1} intervals with a 2-s integration time. DNA samples were placed in 2-mm i.d. capillary cells which were held within a thermally regulated block. Further details of the system have been previously given (Martin & Wartell, 1982). Raman band parameters were determined by a nonlinear least-squares fitting procedure. Lorentzian functions convoluted with the instrument function represented the Raman bands, and a polynomial curve approximated the background. Peak heights evaluated from repeated analysis were within 5% of the mean for medium and large peaks. The full width at half-height (fwhh) had greater uncertainty but in general did not vary by more than 15%. Peak heights were employed for the intensity measurements since the fwhh of a band was more susceptible to small spurious variations due to differences in the background curves. Any significant variations in fwhh of a band are noted. Further details concerning the fitting procedure are given in Wartell and Harrell (1986).

Results

Raman Spectra of lac Promoter DNA Fragments. The four DNAs examined are shown in Figure 1. The 144 bp DNA contains the entire transcription control region for the lactose operon, spanning base pairs −81 to +63 relative to the transcription start point at +1. The operator site is centered within the 40 bp DNA. The CAP binding site is located within the 62 bp DNA, and its central sequence is the 21 bp DNA. Raman spectra of the 62, 40, and 21 bp DNAs from 400 to 1800 cm^{-1} are shown in Figure 2. The 144 bp spectrum which is not shown was essentially identical with that of the 62 bp DNA. The spectra were normalized to the 1092 ± 1 cm^{-1}

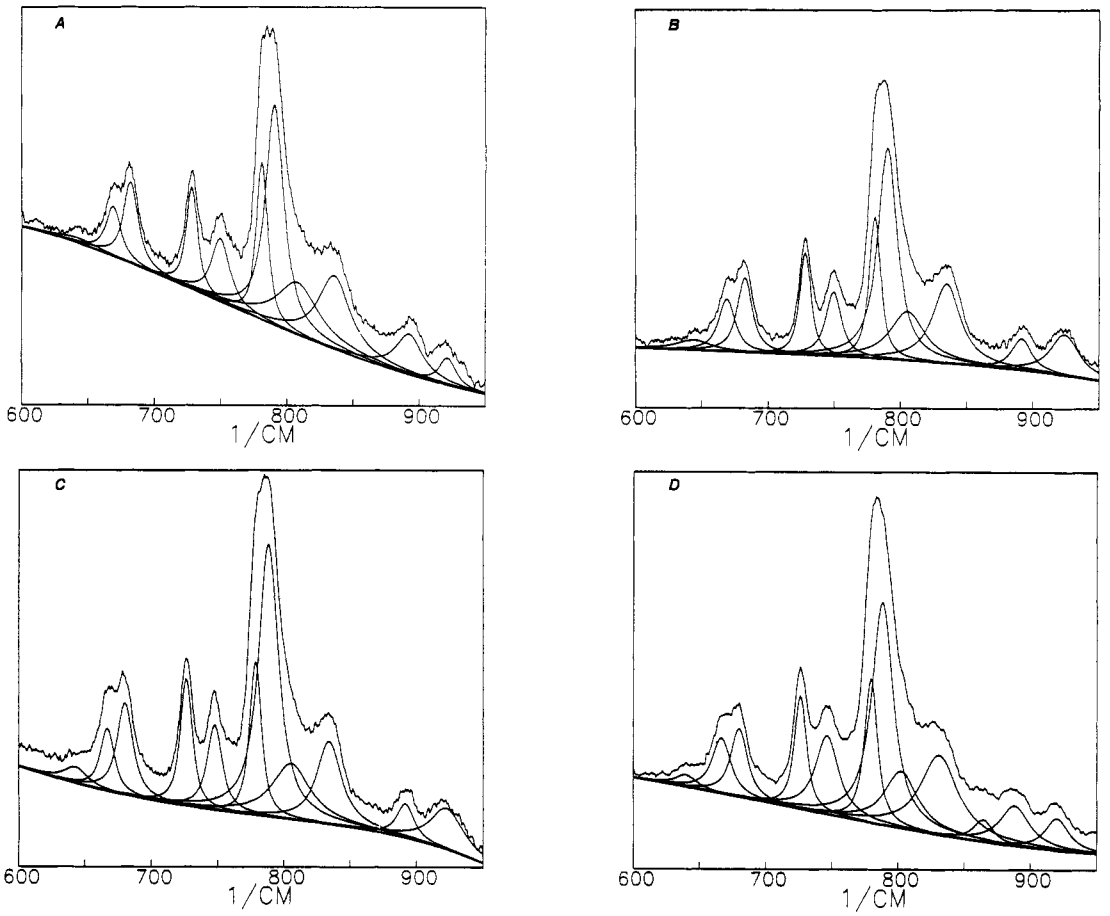

FIGURE 3: Raman spectra for four *lac* DNAs from 600 to 950 cm^{-1} are shown. The background curves and Raman bands were determined from the analysis procedure: (A) 144 bp DNA; (B) 62 bp DNA; (C) 40 bp DNA; (D) 21 bp DNA.

PO_2^- symmetric stretch vibration after subtracting polynomial-fitted background curves as described under Materials and Methods. The characteristics of all the spectra are generally similar, and typical of B-type DNA. The spectra from 600 to 960 cm^{-1} have the features observed in B-DNA fibers with 40–50% GC content (Erfurth et al., 1972). All spectra have a broad band (fwhh = 25–31 cm^{-1}) centered at 834 ± 1 cm^{-1} which follows a large peak near 785 cm^{-1}. The 834 cm^{-1} band is a deoxyribose phosphate vibration characteristic of DNA with a C2′-endo family furanose ring and B-type backbone torsion angles. Studies on dinucleotide crystals suggest that the intensity of the 834 cm^{-1} band is sensitive to the number of furanose rings in the C2′-endo conformation (Thomas & Peticolas, 1983). The 785 cm^{-1} peak consists of at least two bands, a 782 cm^{-1} cytosine vibration and a 791 cm^{-1} phosphate mode. A band at 809 ± 5 cm^{-1}, required by curve resolution analysis, is also present in the DNA spectra of Figure 2 (see below). This band is assigned to a furanose–phosphate vibration with A-type furanose characteristics. Since the relationship between furanose–phosphate torsion angles and Raman band intensities at 809 and 834 cm^{-1} is uncertain, we refer to these bands as A-family and B-family bands, respectively. Frequencies and intensities of Raman bands from Figure 2 are listed in Table I along with assignments based on previous work from several labs (Thomas, 1986; Peticolas et al., 1986; Wartell & Harrell, 1986). Although the spectra of the 21 bp DNA are quite similar to the longer fragments in Figure 2, some small differences are visible to the right of the 834 cm^{-1} band and in the 1200–1260 cm^{-1} region.

A more detailed examination of the Raman spectra of the above DNAs was made by quantitatively comparing their band characteristics. Curve-fitting analysis was applied to evaluate the relative peak heights, bandwidths, and positions of Raman bands. Figure 3 shows the results of the curve-fitting analysis for the *lac* promoter DNAs in the 600–950 cm^{-1} region. The number of bands employed was the least number required to fit each spectrum. The 21 bp DNA required a small band centered at 867 cm^{-1} not observed in the other spectra. A band around 860–880 cm^{-1} is one of the characteristics of low-humidity DNA fibers with a C-conformation backbone (Erfurth et al., 1972; Fish et al., 1983; Nishimura & Tsuboi, 1986). This band has been correlated with pairs of backbone torsional angles by Nishimura and Tsuboi (1986) and Thomas (1986).

The data from Figure 3 were employed to plot band intensities vs percent GC for the 21, 40, and 62 bp DNAs. Figure 4 plots the relative intensities of four bands associated with base ring vibrations. Previous data from three genomic DNAs (Wartell & Harrell, 1986) are shown as closed symbols. The 596 cm^{-1} band was empirically assigned to a cytosine vibration (Wartell & Harrell, 1986). Theoretical normal mode analysis also finds cytosine ring and cytosine-coupled backbone modes at or near this frequency (Prasad & Prohofsky, 1984). The relative intensities of the three *lac* DNAs at 596 cm^{-1} are similar and fall close to the values expected from the genomic DNA data. The 669 cm^{-1} thymine band and 682 cm^{-1} guanine band are markers for C2′-endo family/*anti*-dT and C2′-endo family/*anti*-dG conformers, respectively (Thomas, 1986; Peticolas et al., 1986). The "C2′-endo family" includes C1′-exo and C3′-exo puckering. The intensities of these bands in the 62 and 40 bp DNA spectra are close to the values expected from their GC content. For the 21 bp DNA, however, the intensity at 682 cm^{-1} is lower than expected. The 669 cm^{-1} band has a slightly higher intensity than expected for a DNA

Table I: Frequencies, Intensities, and Assignments of Raman Bands of *lac* Promoter DNAs

frequency[a] (cm^{-1})	rel intensities[b] 62 bp	40 bp	21 bp	assignment[c]
497	0.25	0.26	0.30	G/T
577	0.06	0.07	0.09	G, C
596	0.14	0.13	0.14	C
643	0.05	0.05	0.06	A, C
669	0.28	0.29	0.28	T; C2′-endo/anti
682	0.40	0.40	0.35	G; C2′-endo/anti
728	0.58	0.60	0.62	A
749	0.37	0.38	0.38	T, u
782	0.81	0.79	0.81	C, u
791	1.10	1.15	1.07	bk
807	0.26	0.27	0.27	bk; C3′-endo, A-bk
834	0.38	0.37	0.38	bk; C2′-endo, B-bk
867			0.08	bk; C-bk
891	0.17	0.18	0.17	bk
922	0.18	0.19	0.15	bk
998	0.08	0.10	0.12	
1014	0.29	0.25	0.24	A/T
1052	0.18	0.18	0.21	bk
1092	1.0	1.0	1.0	bk; PO^{2-}
1113	0.12	0.10	0.11	
1141	0.15	0.16	0.12	T
1175	0.15	0.14	0.17	G/C
1186	0.09	0.09	0.16	
1211	0.32	0.30	0.29	mm
1234	0.28	0.25	0.36	mm
1255	0.52	0.46	0.51	C, A
1263	0.14	0.16	0.28	
1290	0.24	0.26	0.19	
1300	0.54	0.56	0.53	A
1316	0.17	0.16	0.14	G
1331	0.48	0.54	0.43	mm
1340	0.57	0.59	0.46	A
1361	0.15	0.09	0.14	G/C
1374	1.05	1.08	1.07	A, G, T
1420	0.37	0.37	0.36	R
1443	0.08	0.07	0.07	
1460	0.18	0.16	0.18	
1487	1.06	1.06	1.00	G, A

[a] Frequencies are within ±2 cm^{-1}. [b] Relative intensities are the ratio of the peak height of the band to the band at 1092 cm^{-1}. [c] Abbreviations: A, adenine; C, cytosine; G, guanine; T, thymine; bk, backbone; u, unknown smaller contribution; R, deoxyribose; slant means *and/or*; comma implies *and*; mm implies multiple modes contribute.

with 48% GC content and is also somewhat broader, 15 cm^{-1} at fwhh compared to 11 cm^{-1} for other DNAs. Studies on model compounds show that the 682 cm^{-1} dG band is sensitive to conformational changes in the guanine nucleoside (Benevides et al., 1986; Nishimura et al., 1986). When the sugar pucker of dG is altered from the C2′-endo family toward the C3′-endo range, the band shifts down to about 666 cm^{-1}. The band shifts to 625 cm^{-1} in the B to Z conformational transition when the dG glycosidic angle becomes syn (Thamann et al., 1981). The 728 cm^{-1} thymine band of the 21 bp DNA also shows a slightly higher than expected intensity. These differences are small but reproducible. In contrast, deoxyribose phosphate bands at 791, 807, and 834 cm^{-1} have similar intensities for all three DNAs (Table I). These intensities are essentially the same as those observed in genomic DNAs (Wartell & Harrell, 1986). Overall, this conformationally sensitive region shows that the typical B-type conformation is dominant in all three DNAs but that some variations are observed in the 21 bp CAP site. It is undoubtedly easier to detect small structural variations in shorter DNAs than in longer ones.

Spectral differences between the 21 bp DNA and the 40 bp DNA in the 1150–1450 cm^{-1} region are shown in Figure 5. The computed difference spectrum shows that the 21 bp

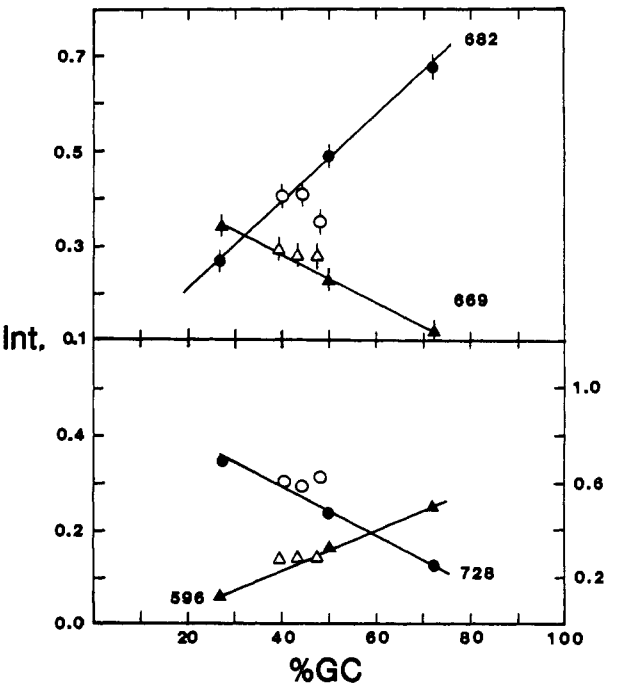

FIGURE 4: Raman intensities vs percent GC are plotted for bands at 596, 669, 682, and 728 cm^{-1}. Closed symbols correspond to data from genomic DNAs (Wartell & Harrell, 1986). Open symbols refer to the 21, 40, and 62 bp DNAs which are 48%, 40%, and 44% GC, respectively.

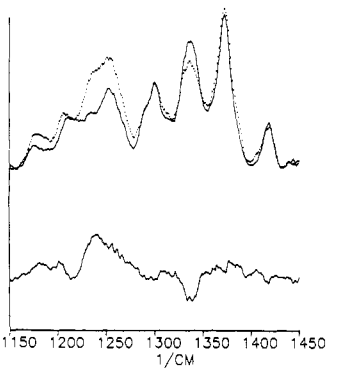

FIGURE 5: Top two curves are Raman spectra of the 40 bp (—) and 21 bp DNAs (···) from 1150 to 1450 cm^{-1}. Both are individually normalized to their 1092 cm^{-1} bands. The difference spectrum of the 21 bp DNA minus the 40 bp DNA is shown in the lower part of the figure.

DNA has increased intensity around 1230–1260 cm^{-1} and decreased intensity at 1340 cm^{-1}. Some decrease in intensity at 1340 cm^{-1} is expected since an adenine ring mode occurs at this frequency, and the 21 bp DNA has a higher AT content. This explanation cannot be applied to the 1230–1260 cm^{-1} region. Previous studies show that the intensity of bands in this region does not significantly change with GC content (Wartell & Harrell, 1986).

The intensity vs GC content plots of bands at 1234, 1265, 1332, and 1340 cm^{-1} reinforce the above conclusion. Figure 6 displays the data for these four bands. For this region, analysis was first carried out from 950 to 1450 cm^{-1} to help establish accurate background curves. Additional analyses were then made from 1150 to 1450 cm^{-1}. The accuracy of the band characteristics in this region suffers from the uncertainty created by many overlapping bands. The curve-fitting program nonetheless yields reproducible results for mild perturbations in the initially assumed band positions (±3 cm^{-1}) and widths (±20%). Figure 6 indicates that the 1234 and 1265

Table II: Raman Band Characteristics at 25 and 45 °C

	intensity/fwhh[a]					
	21 bp		40 bp		62 bp	
frequency (cm^{-1})	25 °C	45 °C	25 °C	45 °C	25 °C	45 °C
497	0.31/11	0.30/13	0.26/16	0.39/19	0.25/10	0.26/13
669	0.28/15	0.28/16	0.29/12	0.29/16	0.27/13	0.26/12
682	0.35/13	0.31/14	0.40/13	0.32/11	0.40/11	0.38/11
705				0.12/25		
728	0.62/9	0.61/9	0.60/8	0.62/8	0.59/8	0.61/9
749	0.38/20	0.36/20	0.38/12	0.37/16	0.37/13	0.37/15
782	0.81/10	0.83/11	0.79/10	0.81/8	0.81/8	0.79/8
791	1.07/18	1.05/18	1.15/16	1.4/17	1.1/16	1.1/18
807	0.28/29	0.28/29	0.27/32	0.28/32	0.25/30	0.24/27
834	0.37/32	0.34/33	0.37/25	0.32/27	0.38/26	0.37/25

[a] Intensity is relative intensity using the 1092 cm^{-1} band as internal standard; fwhh is full width at half-height; these are average values based on three or more analyses. Intensity values are ±0.01 for intensity <0.5 to ±0.03 for higher intensity values. fwhh values are ±15%.

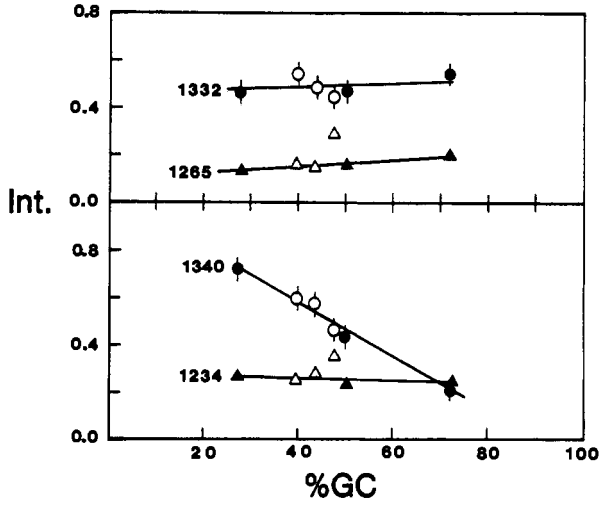

FIGURE 6: Intensity vs percent GC for four Raman bands; see Figure 4 for description of symbols.

cm^{-1} bands of the 21 bp DNA are both higher in intensity than expected while the 1330 and 1342 cm^{-1} bands follow the GC content dependence observed for the other DNAs. It is worth noting that independent of the band resolution of this region an increase in the intensity of the 1230–1250 cm^{-1} region is a characteristic of C-DNA Raman spectra when compared to B-DNA spectra (Erfurth et al., 1972; Fish et al., 1983).

Effect of Temperature on DNA Raman Spectra. The influence of temperature on DNA conformation was examined by comparing the 25 °C spectra of the 62, 40, and 21 bp DNAs to spectra obtained at 35 and 45 °C. Since these temperatures are at least 10 °C below the T_m obtained at 2000-fold lower DNA concentrations by UV melting curves, the DNAs are still predominantly duplex. Figure 7 compares the spectra of the 40 and 21 bp DNAs in the 450–950 cm^{-1} region at 25 and 45 °C. The 35 °C spectra were essentially the same as at 25 °C. Table II lists relative intensities of several conformationally sensitive Raman bands for the three DNAs. Previous studies indicate that the 1092 cm^{-1} band used as an internal standard does not change significantly between 25 and 45 °C (Erfurth et al., 1972; Brown & Wartell, 1986).

Several reversible temperature-induced changes occur for the 40 bp *lac* operator site. The peak height at 497 cm^{-1} increases by about 50% (Figure 7). This band has been assigned to both guanine and thymine vibrations (Wartell & Harrell, 1986; Prescott et al., 1984). Our initial assumption was that this increase reflected the melting of the four AT base pairs at the overlapping *Eco*RI ends. Several results, however, do not support this interpretation. They suggest that at least part of the 497 cm^{-1} increase reflects conformational changes

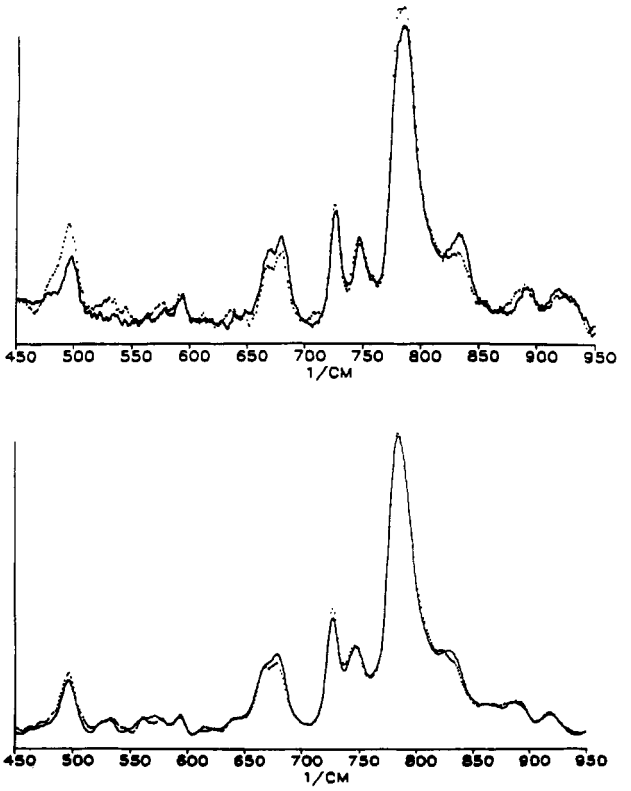

FIGURE 7: Raman spectra of 21 bp DNA (bottom) and 40 bp DNA (top) at 25 °C (—) and 45 °C (···). Spectra of the 21 bp DNA were smoothed by a third-order polynomial over 13-point intervals.

in dG nucleotides. Thermal melting of calf thymus DNA from 25 to 95 °C showed that the 497 cm^{-1} band increased by only 13% relative to the 1092 cm^{-1} band (DeGrazia and Wartell, unpublished results). The Raman spectra of $d(A_6T_6)$ as a duplex at 25 °C, and as single strands at 55 °C, showed only a 8–10% increase at 497 cm^{-1} (DeGrazia, Wilson, and Wartell, unpublished results). The latter value was measured relative to either a 980 cm^{-1} sodium sulfate intensity standard or the 1092 cm^{-1} band. If the intensity increase at 497 cm^{-1} for the 40 bp operator DNA was due to end melting, a change much smaller than 50% is expected. An increase of intensity in this band by 250% has been observed in the B to Z transition of poly(dG-dC)·poly(dG-dC) (Thamann et al., 1981; Wartell et al., 1983). By contrast, the band decreases in the B to A transition of calf thymus DNA (Martin & Wartell, 1982; Prescott et al., 1984).

These observations indicate that the change at 497 cm^{-1} of the 40 bp DNA reflects a conformational rearrangement of several dG nucleosides and possibly dT nucleosides. Consistent

with the involvement of dG is the decrease in intensity observed at the 682 cm^{-1} guanine C2′-endo/anti mode (Table II). Two thymine bands in this region show only minor changes. The width of the adjoining 669 cm^{-1} thymine band increases slightly (11 to 15 cm^{-1}), as does the 748 cm^{-1} thymine vibration. Both bands undergo large intensity alterations in the B to A transition (Martin & Wartell, 1982; Prescott et al., 1984). No change is observed in the adenine vibrations at 728 cm^{-1}. The curve resolution of the 45 °C spectra indicates a small band at 705 cm^{-1} which is not observed at lower temperatures (Figure 7). This band has been previously assigned to an A-type DNA ribose phosphate vibration (Martin & Wartell, 1982; Prescott et al., 1984). Two other changes indicating backbone alterations are a decrease of intensity of the 834 cm^{-1} B backbone mode and an increase in intensity of the 791 cm^{-1} band. The 45 °C spectra of the 62 and 21 bp DNAs do not show the same changes as the 40 bp DNA. Smaller intensity decreases are observed in the 682 and 834 cm^{-1} bands of the 21 bp CAP site; however, no change is observed at 497, 705, and 791 cm^{-1}, or other bands from 400 to 950 cm^{-1}. No changes above 5% are observed in the 62 bp spectra. Examination of the 1150–1450 cm^{-1} region at 45 °C shows some small changes from 25 °C; however, the distinctions between the DNAs remain the same as at 25 °C.

Discussion

The Raman spectra of the 40 bp operator fragment and the 62 and 21 bp CAP sites indicate that at 25 °C these sequences are predominantly in a B-type conformation in solution. The intensities and frequencies of the 834 and 807 cm^{-1} bands indicate that the ratio of B-family conformers to A-family conformers is the same as in genomic DNAs. This outcome differs somewhat from a Raman study of the 17 bp O_L1 operator site (Prescott et al., 1985). The intensity of the 807 cm^{-1} band in the latter DNA suggested that six to eight nucleotides of the O_L1 site assume an A-form geometry. Increased intensity at 807 cm^{-1} is not observed for the *lac* promoter DNAs. Some local conformational variations within the CAP site are indicated, however, by small differences between the spectra of the 21 bp sequence and the other DNAs. These differences are consistent with several features of the Raman spectra of C-DNA fibers (Fish et al., 1983; Erfurth et al., 1972; Goodwin & Brahms, 1978; Nishimura & Tsuboi, 1986). There is a new broad band around 870 cm^{-1}, an increase in intensity at 1235–1240 cm^{-1} relative to 1258 cm^{-1}, and a decrease of intensity at 682 cm^{-1}. We note, however, that the CAP site Raman spectrum does not show some changes which are observed with C-DNA fibers. For example, there is no decrease in intensity of the 834 cm^{-1} band or shift in the relative intensities of the 1340 cm^{-1} adenine vibration and the 1371 cm^{-1} vibration assigned to thymine, adenine, and guanine. This discrepancy may be understood if the unobserved spectral changes are related to DNA hydration or require that the entire DNA assume a C-type conformation. Further studies are required to examine this point. The relative intensity of the 682 cm^{-1} dG band suggests that two or three of the dG residues are not in a conventional C2′-endo/anti conformation in the CAP site. It should be possible to amplify and localize these Raman signatures to a specific sequence by examining smaller portions of the 21 bp DNA. Structural variations in the *lac* repressor site may also occur at 25 °C. If they do, they apparently cannot be observed in the 40 bp DNA. It is interesting to note that Nishimura et al. (1983) have observed a band at 870 cm^{-1} in a DNA duplex, d(GGAATTCC). There are two GpA dinucleotides in this oligomer and three in the 21 bp CAP site.

The effect of temperature on the Raman spectrum of the 40 bp DNA suggests that a portion of this sequence undergoes a structural rearrangement. The Raman changes occur in the same temperature range (42–45 °C) as the enhanced base pair opening observed in this DNA by ^1H NMR (Lu et al., 1983). It is tempting to attribute these two observations to the same conformational change. The intensity increase at 497 cm^{-1} is the largest change. It did not occur with the 21 or 62 bp DNAs. Examination of this band's response to temperature and conformation in other DNAs indicates that the change involves dG residues. Decreased intensity at the 682 cm^{-1} dG mode supports this assignment. The presence of the small 705 cm^{-1} vibration is consistent with the occurence of A-like backbone characteristics for a few base pairs. The most likely single location for a structural change consistent with the above characteristics is the GTGGAA region where enhanced base pair opening is first observed.

Acknowledgments

We thank Poncy Lu for providing the facilities employed in the isolation of the 40 bp DNA and for his advice, assistance, and interest in the work. We also thank David Dripps, Charles McCampbell, and Jin Kim for their help in the preparation of the 62 and 21 bp DNA fragments.

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Cleavage of dT_8 and dT_8 Phosphorothioyl Analogues by *Escherichia coli* DNA Topoisomerase I: Product and Rate Analysis

Paul L. Domanico‡ and Yuk-Ching Tse-Dinh*

Central Research and Development Department, E. I. du Pont de Nemours and Co., Wilmington, Delaware 19898

Received March 14, 1988; Revised Manuscript Received May 10, 1988

ABSTRACT: *Escherichia coli* DNA topoisomerase I catalyzes the cleavage of short, single-stranded oligodeoxynucleotides with dT_8 as the shortest cleavable oligo(thymidylic acid). The 5′-^{32}P-labeled products formed from the cleavage of [5′-^{32}P]dT_8 are dT_5, dT_4, and dT_3 with over 70% of the substrate cleaved to dT_4. Mg(II) ions affect this product distribution by increasing the percentage of dT_4 formed. The substitution of a sulfur atom for a nonbridging oxygen atom in a phosphodiester linkage yields oligodeoxynucleotide phosphorothioyl (PS) analogues. The epimers of the analogues were separated, and the position and stereochemistry of the phosphorothiodiester bond were determined. Topoisomerase I is stereospecific in its reactivity toward these analogues. With the oligodeoxynucleotide PS analogue substrates, the rate of cleavage, the stereospecificity, and the product distribution depend upon the position and the stereochemistry of the phosphorothiodiester linkage.

Escherichia coli DNA topoisomerase I catalyzes the relaxation of negatively supercoiled DNA. After one relaxation event, the linking number of the supercoiled DNA substrate has increased by 1 (Brown & Cozzarelli, 1981). Evidence suggests that the enzyme breaks one strand of the DNA duplex in a single-stranded region (Wang, 1971), actively or passively passes the other intact strand through the break, and finally rejoins the broken strand (Gellert, 1981).

A two-domain model depicting the interaction of the bacterial enzyme with DNA has been proposed (Brown & Cozzarelli, 1981; Kirkegaard et al., 1984; Tse-Dinh, 1986). Evidence for a covalent complex via a protein tyrosine residue–DNA covalent linkage suggests a covalent binding domain (Tse et al., 1980). In this model then, a covalent binding domain contains a nucleophilic tyrosine residue at its active site which can displace the 5′ region of the DNA through a transesterification reaction during the strand breakage step. A covalent bond between the tyrosine and the 5′-phosphate of the 3′ region is formed. The model holds that a noncovalent binding domain interact with the broken DNA strand, if not both DNA strands, during strand breakage and passage. This domain may also facilitate re-formation of the DNA phosphodiester bond after strand passage by positioning the 3′-OH group of the 5′ region of DNA during the rejoining step.

The mechanism of *Escherichia coli* topoisomerase I catalyzed cleavage of oligodeoxynucleotides, or oligomers, is believed to mimic the covalent complexation between the protein's covalent binding domain and negatively supercoiled DNA. However, with a small, single-stranded oligomer substrate, the strand 5′ to the point of transesterification is released after cleavage (Tse-Dinh, 1986; Tse-Dinh et al., 1983). The dissociation of the substrate's 5′ region is probably due to its short length which does not allow for sufficient interaction with the enzyme's noncovalent binding domain. When long single-stranded DNA polymers are present, interaction with the noncovalent domain may come into play (Tse-Dinh, 1986). One major difference between the enzyme's reaction with supercoiled DNA and that with the oligodeoxynucleotides is that enzyme turnover with the latter is defined by the hydrolysis of the covalent complex (Tse-Dinh, 1986).

E. coli topoisomerase I cleaves dT_8 oligomers predominately between the fourth and fifth nucleotide (Tse-Dinh et al., 1983). The enzyme probably has substrate recognition sites both 5′ and 3′ to the actual site of cleavage. Toward understanding the enzyme's recognition criteria and the relative importance of these recognition sites, we analyzed the products formed and the rates of product formation from the enzymatic cleavage of dT_8 and phosphorothioyl analogues in both the presence and absence of divalent Mg ions. Three internucleotidyl phosphorothioyl substitutions were made—directly 5′ to the point of cleavage, at the major cleavage site, and directly 3′ to the point of cleavage. The dT_8 phosphorothioyl analogues or PS oligomers were purified to stereochemical homogeneity, and the position and chirality of the phosphorothiodiester linkage were verified. This study reports on the enzyme's ability to differentiate between the chiral phosphorothioyl epimers and the effects these nonbridging sulfur

‡Present address: Department of Biochemistry, Glaxo Research Laboratory, Five Moore Drive, Research Triangle Park, NC 27709.

0006-2960/88/0427-6365$01.50/0