Nucleic Acids Research, 1993, Vol. 21, No. 6 1433–1438

Demonstration of Z-d(^{5Br}CGAT^{5Br}CG) and B-d(CGCGATCGCG) form crystal stuctures in DNA-cobalt hexammine complexes by Kr 647.1 nm excitation of Raman spectra*

J.M.Benevides, A.H.-J.Wang[1] and G.J.Thomas, Jr+
Division of Cell Biology and Biophysics, School of Biological Sciences, University of Missouri—Kansas City, Kansas City, MO 64110-2499 and [1]Biophysics Division and Department of Cell and Structural Biology, University of Illinois, Urbana–Champaign, Urbana, IL 61801, USA

Received December 7, 1992; Revised and Accepted February 5, 1993

ABSTRACT

Cobalt hexammine [$Co(NH_3)_6^{3+}$] is an efficient DNA complexing agent which significantly perturbs nucleic acid secondary structure. We have employed red excitation (647.1 nm) from a krypton laser to obtain Raman spectra of the highly colored complexes formed between cobalt hexammine and crystals of the DNA oligomers, d(^{5Br}CGAT^{5Br}CG) and d(CGCGATCGCG), both of which incorporate out-of-alternation pyrimidine/purine sequences. The $Co(NH_3)_6^{3+}$ complex of d(^{5Br}CGAT^{5Br}CG) exhibits a typical Z-form Raman signature, similar to that reported previously for the alternating d(CGCGCG) sequence. Comparison of the Raman bands of d(^{5Br}CGAT^{5Br}CG) with those of other oligonucleotide and polynucleotide structures suggests that C3′-endo/syn and C3′-endo/anti thymidines may exhibit distinctive nucleoside conformation markers, and tentative assignments are proposed. The Raman markers for C2′-endo/anti adenosine in this Z-DNA are consistent with those reported previously for B-DNA crystals containing C2′-endo/anti dA. Raman bands of the cobalt hexammine complex of d(CGCGATCGCG) are those of B-DNA, but with significant differences from the previously characterized B-DNA dodecamer, d(CGCAAATTTGCG). The observed differences suggest an unusual deoxyguanosine conformer, possibly related to a previously characterized structural intermediate in the B–Z transition. The present results show that crystallization of d(CGCGATCGCG) in the presence of cobalt hexammine is not alone sufficient to induce the left-handed Z-DNA conformation. This investigation represents the first application of off-resonance Raman spectroscopy for characterization of highly chromophoric DNA and illustrates the feasibility of the Raman method for investigating other structurally perturbed states of DNA-cobalt hexammine complexes.

INTRODUCTION

The barrier to interconversion of double-stranded DNA between left-handed and right-handed helical forms is dependent upon the base sequence, the presence of covalently modified nucleotides and various environmental factors, including the concentrations of metal ions and polyamines (1). The base sequence most favorable to the formation of left-handed Z-DNA consists of alternating pyrimidines and purines with anti and syn glycosyl conformations, respectively. However, the requirement for strict (anti)pyrimidine/(syn)purine alternation may be overcome by appropriate adjustment of environmental conditions. For example, trivalent cobalt hexammine [$Co(NH_3)_6^{3+}$] may induce or stabilize the left-handed Z form of DNA in nonalternating pyrimidine/purine sequences (2–4). Thus, the hexanucleotide, d(^{5Br}CGAT^{5Br}CG), which lacks strict pyrimidine/purine alternation, has been shown by X-ray crystallography to form a left-handed Z structure in the presence of cobalt hexammine (2). This unusual Z-DNA crystal structure is distinguished from that of d(CGCGCG), the prototypical Z-DNA oligomer, by the occurrence of syn thymines paired to anti adenines and by the 5-bromo substituent on each cytosine ring. Comparison of the crystal structure of d(^{5Br}CGAT^{5Br}CG) with other Z form oligonucleotide structures provides insights into surface characteristics of the left-handed double helix which may be essential to protein recognition and binding (2).

To extend the analysis of DNA polymorphism to longer base sequences, as well as to noncrystallizable oligonucleotides, structural methods complementary to X-ray crystallography are required. Laser Raman spectroscopy is one method which is

* Paper XLV in the series Raman Spectral Studies of Nucleic Acids

+ To whom correspondence should be addressed

applicable to both low and high molecular weight DNA and useful for comparing crystal and solution structures with one another (5). Raman spectra have been obtained on a large number of DNA oligonucleotide crystals and polynucleotide fibers in the B, A and Z forms (6–8). Bands distinctive of nucleotide sugar and phosphodiester conformations have been catalogued (5, 9). These studies provide an empirical database of conformation-sensitive Raman bands which can be used for characterization of an unknown DNA structure. A review of applications has been given recently (10). Further extension of the empirical Raman database to include structures such as cobalt hexammine complexes of DNA is desirable. The DNA structures previously characterized by Raman spectroscopy, as well as the model compounds upon which these structural analyses have been based, are transparent to visible radiation and therefore amenable to investigation using conventional argon laser (blue or green) excitation. Cobalt hexammine complexes of DNA, on the other hand, are highly absorbing of short wavelength visible radiation. Accordingly, the cobalt complexes are not compatible with argon-laser excitation of Raman spectra.

In this paper we demonstrate the feasibility of krypton (647.1 nm) laser excitation of Raman spectra of DNA-cobalt hexammine complexes. We interpret the Raman spectra obtained from two oligonucleotide single crystals, d(^{5Br}CGAT^{5Br}CG) and d(CGCGATCGCG), both of which were crystallized in the presence of cobalt hexammine trichloride. The d(^{5Br}CGAT^{5Br}CG) crystal structure, which has been solved by X-ray crystallography (2), is the first Z-DNA incorporating C3′-endo/syn thymidine and C2′-endo/anti adenosine conformers to be characterized by Raman spectroscopy. Its out-of-alternation AT pairs yield a phosphodiester Raman marker which is distinct from that seen in the Z form d(CGCGCG) crystal (11). We also show that the crystal structure of d(CGCGATCGCG) is a right-handed B form DNA, but with notable differences from the previously characterized B-DNA dodecamer, d(CGCAAATTTGCG) (12). The present Raman spectra expand the existing database of Raman conformation markers of DNA, and also help to define limits on Z-DNA induction by cobalt hexammine.

EXPERIMENTAL PROCEDURES

Materials

The oligonucleotides d(^{5Br}CGAT^{5Br}CG) and d(CGCGATCGCG) were synthesized by the phosphate triester method and were purified to >95% by HPLC methods. 5-Bromodeoxycytosine nucleotides were used as the starting material in the synthesis of d(^{5Br}CGAT^{5Br}CG). The crystals of d(^{5Br}CGAT^{5Br}CG) were grown according to conditions described by Wang et al. (2). The deep yellow color of the crystals indicated that cobalt hexammine ions were incorporated within the crystal lattice. The X-ray structure analysis did not reveal any ordered $Co(NH_3)_6^{+3}$ ions, indicating that the ions were randomly distributed in the solvent channels of the crystals (2).

The decamer d(CGCGATCGCG) contains a sequence expected to favor the Z-DNA conformation, except for the central AT dinucleotide which disrupts the pyrimidine/purine alternation. For crystallization of this decamer, the $Co(NH_3)_6^{+3}$ ion was used with the expectation of inducing the Z structure. (As shown below, this structure did not crystallize in the Z form.) The crystallization mixture contained 2.3 mM oligonucleotide, 33 mM sodium cacodylate buffer (pH 7.0), 25 mM $MgCl_2$, 25 mM $Co(NH_3)_6^{+3}$, 25 mM $CaCl_2$ and 3% 2-methyl-2,4-pentanediol (2-MPD). Crystal formation was induced by vapor diffusion with 25% 2-MPD at room temperature. Orthorhombic crystals of intense yellow color were obtained. These crystals did not diffract X-rays to sufficient resolution for a three-dimensional structure analysis, but yielded Raman spectra of high quality and with the typically sharp bands indicative of structural homogeneity. Syntheses and crystallizations for d(CGCGCG) and d(CGCAAATTTGCG) were as described previously (12, 13).

Raman spectroscopy

Crystals of d(^{5Br}CGAT^{5Br}CG) and d(CGCGATCGCG) were each transferred with approximately 10 μL of the respective mother liquor to a glass capillary tube (Kimax #34507) employed as the Raman sample cell. The cell was jacketed with a constant temperature device (14) and maintained at 10°C in the sample illuminator of a Spex 1401 double spectrometer. Approximately 400 mW of 647.1 nm Kr radiation from a Coherent Innova mixed gas (argon-krypton) laser was focused on the crystals to excite the Raman spectrum. Data were collected with an 8 cm^{-1} spectral slit width at intervals of 1 cm^{-1} and with an integration time of 2s. Raman spectra of crystals of d(CGCGCG) and d(CGCAAATTTGCG) were obtained as described previously (11, 12).

Raman spectra shown in the figures represent the averages of several scans, each of 1.5 cm^{-1} or better repeatability. The sloping background and scattering by the mother liquor were removed using standard techniques (11). The spectrum of the mother liquor was always recorded with the same instrumental conditions employed for the crystals. When indicated in the legends, noise was reduced by a least squares fit of third-order polynomials to overlapping 15-point regions. This procedure did not alter the Raman band frequencies or intensities. All Raman frequencies are accurate to within ±2 cm^{-1}.

RESULTS AND DISCUSSION

The interval 600–900 cm^{-1} of the Raman spectrum of DNA contains bands which are highly sensitive to the geometry of the base-furanose network of the DNA nucleotides (8, 9). These bands, termed nucleoside conformation markers, are useful for distinguishing between C3′-endo and C2′-endo furanose puckers and between syn and anti glycosyl bond torsions. The same spectral interval contains bands which are highly sensitive to phosphodiester bond torsions (7, 8). The latter, termed backbone conformation markers, are particularly useful for distinguishing among the A, B and Z families of double-stranded DNA (5). An abbreviated list of conformation marker bands is given in Table 1. A more comprehensive tabulation is given elsewhere (10).

1. d(^{5Br}CGAT^{5Br}CG)

Raman spectra of crystals of d(^{5Br}CGAT^{5Br}CG) and d(CGCGCG) in the region 600–900 cm^{-1} are shown in Fig. 1. Also shown is a difference spectrum, computed with d(^{5Br}CGAT^{5Br}CG) as minuend and d(CGCGCG) as subtrahend. The generally close correspondence between marker bands of d(^{5Br}CGAT^{5Br}CG) and d(CGCGCG), particularly for the bands near 625–627, 670, 745–748, 782–783, 810, 845 and 865 cm^{-1} of both spectra (Table 1), is confirmation that each structure is in the Z-DNA family. This is in accordance with

X-ray crystallography (2, 13). Specifically, the Raman bands of d(^{5Br}CGAT^{5Br}CG) at 627 cm^{-1} and d(CGCGCG) at 625 cm^{-1}, identify C3′-endo/syn conformers of dG in each structure (15). We note also that the spectra of Fig. 1 lack marker bands diagnostic of right-handed B-DNA and A-DNA structures. Notwithstanding the similar global structures, several difference bands are evident in the difference spectrum of Fig. 1. The positive difference bands are due to the presence of dA and dT residues in d(^{5Br}CGAT^{5Br}CG) and to the effects of 5-bromination of cytosine (11). The negative difference bands reflect the correspondingly greater percentages of dG and dC in d(CGCGCG). We next consider these spectral differences in more detail.

The Raman spectrum of the Z form of d(^{5Br}CGAT^{5Br}CG) deviates from spectra of other AT-containing Z-DNA oligomers (8, 11) by virtue of the fact that its AT segment disrupts (anti)pyrimidine/(syn)purine alternation. The x-ray structure of d(^{5Br}CGAT^{5Br}CG) shows that dA residues assume the C2′-endo/anti conformation. Accordingly, as in B-DNA (12), we assign the 729 cm^{-1} band of d(^{5Br}CGAT^{5Br}CG) to the C2′-endo/anti dA residues. Both the frequency and intensity of the 729 cm^{-1} band in d(^{5Br}CGAT^{5Br}CG) (Fig. 1) are comparable to those of the corresponding dA marker bands in d(CGCAAATTTGCG) (12) and d(CGCGATCGCG).

Assignment of specific marker bands to C3′-endo/syn dT conformers in d(^{5Br}CGAT^{5Br}CG) is more problematic. The

Table 1. Selected conformation markers in Raman spectra of A-, B- and Z-DNA[a]

Residue	B-DNA	A-DNA	Z-DNA
G	682±2	664±2	625±3
	1318±2	1333±3[b]	1316±2
A	663±2	644±4	624±3
	1339±2	1335±2	1310±5
C	782±2	780±2	784±2
	1255±5	1252±2	1265±2
T	748±2	745±2	
	790±3	777±2	
	1208±2	1239±2	
Backbone	790±5	705±2	745±3 (GC)
			711±4 (AT)[c]
	835±7[d]	807±3[e]	
	1092±1	1099±1	1095±2
	1422±2	1418±2	1425±2

[a]Frequencies in cm^{-1} units are determined from Raman spectra of DNA crystals and fibers of known structure unless otherwise noted (5).
[b]A weak companion band near 1316 cm^{-1} is also observed in B-DNA structures.
[c]This work.
[d]The position of this band is sensitive to base composition as discussed in the text.
[e]This band occurs at 813 cm^{-1} in A-RNA structures. In Z-DNA structures a much weaker band occurs near 810 cm^{-1}.

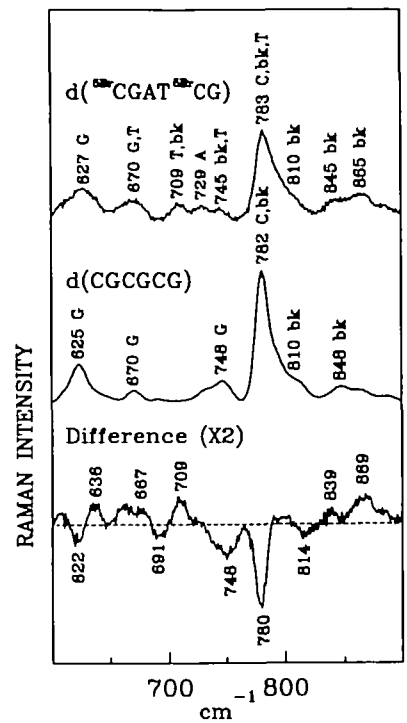

Figure 1. Raman spectra in the region 600−900 cm^{-1} of crystals of Z form DNA. Top: cobalt hexammine complex of d(^{5Br}CGAT^{5Br}CG). Middle: d(CGCGCG). Bottom: Twofold amplification of the d(^{5Br}CGAT^{5Br}CG)-minus-d(CGCGCG) difference spectrum. Frequencies of the principal bands are given in cm^{-1} units and relevant assignments are indicated by the abbreviations, A (deoxyadenosine), C (5-bromodeoxycytidine), G (deoxyguanosine), T (thymidine), bk (backbone). Detailed assignments are given in Table 2.

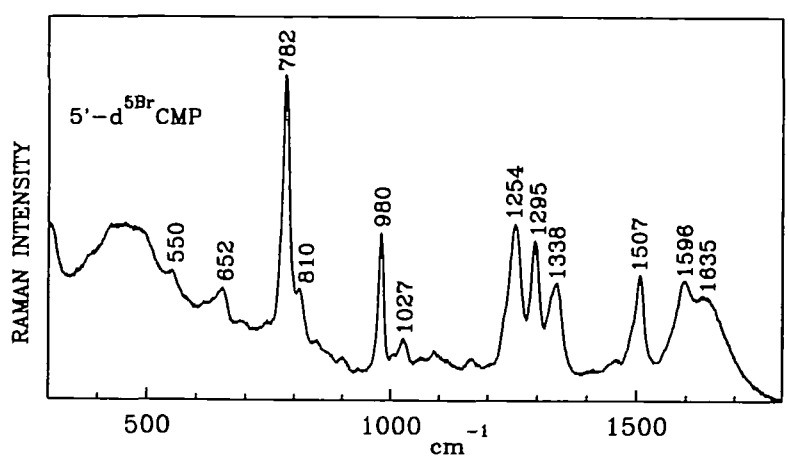

Figure 2. Raman spectrum in the region 300−1800 cm^{-1} of a 0.2 M solution of 5-bromo-2′-deoxycytidine-5′-monophosphate in H_2O (pH 7).

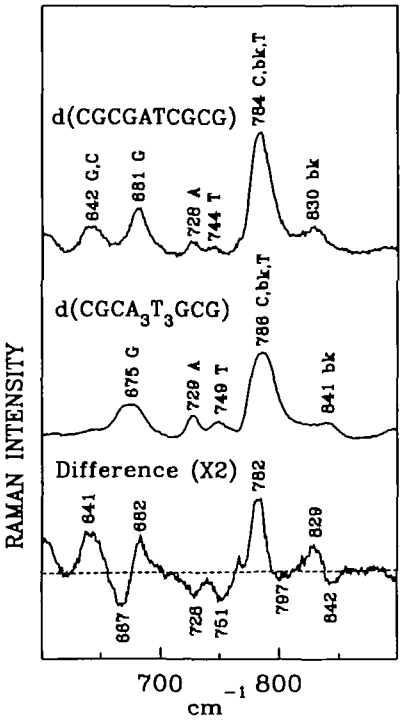

Figure 3. Raman spectra in the region 600–900 cm^{-1} of crystals of B form DNA. Top: cobalt hexammine complex of d(CGCGATCGCG). Middle: d(CGCAAATTTGCG), from reference 12. Bottom: Twofold amplification of the d(CGCGATCGCG)-minus-d(CGCAAATTTGCG) difference spectrum. Other notation is defined in the legend to Figure 1.

obvious candidate is the 709 cm^{-1} band, which is completely absent from d(CGCGCG). In support of this assignment, we note that the integrated intensity of the 709 cm^{-1} band is comparable to that of the 729 cm^{-1} dA marker. We tentatively assign the band near 709 cm^{-1}, in part (see below), to C3′-endo/syn dT. Since the spectrum of d(^{5Br}CGAT^{5Br}CG) displays broader bands than that of d(CGCGCG) at ca. 670 and 783 cm^{-1}, it is also possible that contributions from dT may occur at these frequencies. Raman spectra of other dT conformers also reveal bands near 660 and 790 cm^{-1} (5).

Characteristic Raman bands are also anticipated from the 5-bromocytosine ring. The Raman spectrum of 5-bromo-2′-deoxycytidine-5′-monophosphate, shown in Fig. 2, suggests that reasonable assignments for the d^{5Br}C residues of d(^{5Br}CGAT^{5Br}CG) occur at 783 cm^{-1} (intense) and on the high frequency side of the dG marker band at 627 cm^{-1} (weak shoulder). The latter is apparent in the difference spectrum as a positive peak ca. 636 cm^{-1}.

A distinguishing feature of Z-DNA is inequivalent phosphate conformations at CpG and GpC steps. In d(CGCGCG) there are three CpG and two GpC steps. However, d(^{5Br}CGAT^{5Br}CG) has two CpG and no GpC steps. This distinction is expected to yield non-identical Raman markers in the 700–900 cm^{-1} region. Since the 745 cm^{-1} marker band of d(^{5Br}CGAT^{5Br}CG) is weaker and slightly shifted from the corresponding band of d(CGCGCG) at 748 cm^{-1}, we may tentatively assign this difference to the inequivalent phosphate steps. The unique GpA, ApT and Tp^{5Br}C steps in d(^{5Br}CGAT^{5Br}CG), on the other hand, probably contribute to the positive difference bands ca. 840–870 cm^{-1} in Fig. 1. We cannot exclude the possibility that the different backbones of d(^{5Br}CGAT^{5Br}CG) and d(CGCGCG) also contribute to the apparent difference band near 709 cm^{-1}, assigned above in part to C3′-endo/syn dT. Raman spectra of other AT-containing Z-DNA crystals likewise reveal a very weak band in the 705–715 cm^{-1} interval (12, 16, 17). The available data suggest that if a putative Raman marker of the ApT (or TpC) step occurs in Z-DNA ca. 705–715 cm^{-1}, it is significantly weaker than the principal Z backbone marker near 745–750 cm^{-1}.

Table 2. Raman frequencies and assignments of d[(^{5Br}CGAT^{5Br}CG)]$_2$ and d[(CGCGATCGCG)]$_2$[a]

d[(^{5Br}CGAT^{5Br}CG)]$_2$	d[(CGCGATCGCG)]$_2$	Assignment
563 (2.4)		?
	580 (1.3)	T, G
	592 (3.0)	G, C
	607 (1.1)	?
627 (4.1)		G [C3′endo/syn]
	642 (1.9)	G [C3′exo/syn], C?
670 (1.7)		G [C2′endo/syn]
		T [C3′endo/syn]
		T [C2′endo/anti]
	681 (3.2)	G [C2′endo/anti]
709		T [C3′endo/syn], bk [OPO]
729	728	A
745		bk [OPO], T
	744	T
783 (10.0)	784 (10.0)	C*, C, T, bk [OPO]
810 (3.5)		bk [OPO]
	830 (1.9)	bk [OPO]
845 (2.7)		bk [OPO]
865 (3.4)		bk [OPO]
888 (2.1)		bk
939 (1.4)		bk
	1003 (1.2)	bk
1025 (2.0)		bk
1059 (2.3)	1057 (1.3)	bk [CO]
1095 (3.3)	1092 (2.6)	bk [PO_2^-]
1190 (2.2)	1179 (1.4)	T, C*, C
	1216 (2.4)	T
1224 (2.7)		T
1238 (3.1)	1237 (2.9)	T
	1256 (3.5)	C, A
1261 (4.4)		C*
	1297 (2.2)	C
1318 (6.2)		G [C3′endo/syn]
1321 (3.2)		G [C2′endo/anti]
1335 (5.1)	1335 (3.7)	A
1353 (3.2)		G (?)
	1371 (2.9)	T, A, G
1383 (0.7)		T
1427 (1.9)	1418 (1.7)	bk [CH_2]
1481 (3.7)	1485 (4.3)	G, A
	1529 (0.5)	C
1576 (3.2)	1576 (3.5)	G, A
1658 (1.6)		G, C* [C=O]
1669 (1.7)	1664 (1.2)	G, C*, C, T [C=O]
	1695 (0.7)	G [C=O]

[a]Raman frequencies are given in cm^{-1} units and intensities (in parentheses) are on a scale of 0–10, with 10 assigned arbitrarily to the most intense band in each spectrum. B-DNA and Z-DNA assignments are taken from from references 11 and 22, respectively, and citations therein. Abbreviations for residue assignments are: A = deoxyadenosine, C = deoxycytidine, C* = 5-bromodeoxycytidine, G = deoxyguanosine, T = thymidine and bk = backbone. Probable vibrational assignments are given in square brackets following residue assignments.

2. d(CGCGATCGCG)

Raman spectra of crystals of d(CGCGATCGCG) and d(CGCAAATTTGCG) in the region 600–900 cm^{-1} are shown in Fig. 3. Also shown is a difference spectrum, computed with

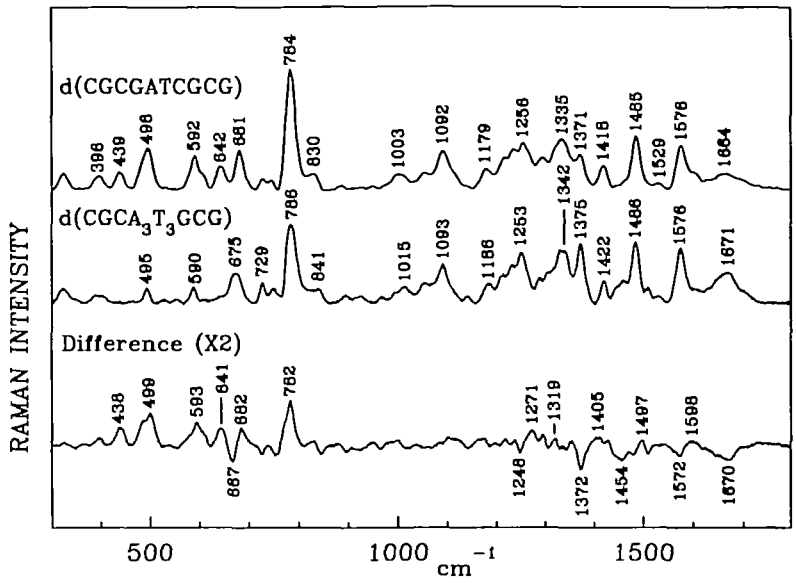

Figure 4. Raman spectra in the region 300−1800 cm^{-1} of crystals of B form DNA. Top: cobalt hexammine complex of d(CGCGATCGCG). Middle: d(CGCAAATTTGCG), from reference 12. Bottom: Twofold amplification of the d(CGCGATCGCG)-minus-d(CGCAAATTTGCG) difference spectrum.

d(CGCGATCGCG) as minuend and d(CGCAAATTTGCG) as subtrahend. The latter is an authenticated B-DNA crystal structure (12). Although the structure of d(CGCGATCGCG) has not been solved by X-ray crystallography, the generally close correspondence between marker bands (Table 1) in the two crystals confirms that d(CGCGATCGCG) is also a structure within the B-DNA family. Specifically, the moderately intense band at 681 cm^{-1} in the spectrum of d(CGCGATCGCG) indicates that most dG residues in this crystal structure exhibit the C2′-endo/anti conformation. Comparison of Fig. 3 with Fig. 1 confirms the major differences which exist between Raman signatures of B-DNA and Z-DNA.

Interestingly, the principal backbone conformation marker of d(CGCGATCGCG) occurs at 830 cm^{-1}, while that of d(CGCAAATTTGCG) occurs at 841 cm^{-1}. Although both bands are within the range diagnostic of B form DNA, they indicate significant differences in detailed backbone geometry. Analysis of Raman spectra of a large number of model B-DNA structures shows generally that the 841 cm^{-1} band is indicative of the relatively narrow minor groove associated with AT-rich sequences in B-DNA, and the 830 cm^{-1} marker is indicative of the more typical, wider minor groove occurring in GC-rich sequences (8, 12). Accordingly, the somewhat narrower minor groove associated with the AT-rich central domain of d(CGCAAATTTGCG) presumably dominates the structure of this dodecamer accounting for its 841 cm^{-1} band. Conversely, we conclude that the secondary structure of d(CGCGATCGCG) is characterized by a rather typical, wider minor groove, consistent with its GC-rich composition. Several DNA decamers with related sequences, crystallizing in the orthorhombic $P2_12_12_1$ space group, all exhibit a B-DNA conformation with a more typical, wide minor groove (18−20). It would not be surprising that the decamer d(CGCGATCGCG) has a similar conformation, irrespective of the presence of cobalt hexammine in the lattice.

Other spectral differences between d(CGCGATCGCG) and d(CGCAAATTTGCG) in the 600−900 cm^{-1} interval can be attributed largely to differences in primary structure. For example, d(CGCGATCGCG) is richer in dC and dG, yielding positive difference bands at 641 and 782 cm^{-1}, while d(CGCAAATTTGCG) is richer in dT and dA, yielding negative difference bands at 667, 728 and 751 cm^{-1}. Nonetheless, it is not possible to reconcile the surprisingly high intensity of the 642 cm^{-1} band of d(CGCGATCGCG) entirely with its base composition. We believe the intense 642 cm^{-1} band of d(CGCGATCGCG), and the vanishingly weak intensity of the corresponding band in d(CGCAAATTTGCG), reflect a significant structural difference between these oligomers. A plausible interpretation of the 642 cm^{-1} band is the following.

A band at 642 cm^{-1} has been identified by Pohl et al. (21) with a structural precursor to the Z form of poly(dG-dC).poly(dG-dC). The band is evident at salt concentrations below those required to fully stabilize the authentic Z form. Urpi et al. (6) also noted the presence of a band ca. 640 cm^{-1} in the Raman spectrum of the d(CCGCGG) crystal and assigned it to an intermediate in the B-Z transition. Prescott et al. (22) proposed the assignment of a Raman band near 645 cm^{-1} in the spectrum of the Pf3 virion to an unusual, perhaps syn, dG conformer in packaged Pf3 DNA. Examination of Raman spectra of mononucleotide single crystals (9) suggests C3′-exo/syn dG as a possible assignment. Whatever nucleoside conformer may give rise to the 642 cm^{-1} marker, it is clearly of lesser population than the C2′-endo/anti dG population (681 cm^{-1} marker) and does not drive the backbone to a conformation outside the B form family. A more definitive assignment must await solution of the X-ray structure of the d(CGCGATCGCG) crystal.

Spectral dissimilarities between d(CGCGATCGCG) and d(CGCGAAATTTGCG) are not confined to the 600−900 cm^{-1} interval, but occur throughout the vibrational spectrum, as shown in Fig. 4. The multitude of peaks and troughs in the Fig. 4 difference spectrum are considered to reflect the combined effects of (i) differences in primary structure, (ii) variations in secondary structure within the B genus, and (iii) coordination of cobalt hexammine to the DNA decamer.

SUMMARY AND CONCLUSIONS

Raman frequencies and assignments for the cobalt hexammine complexes of d(^{5Br}CGAT^{5Br}CG) and d(CGCGATCGCG) are summarized in Table 2. Assignments for the hexamer are based largely upon the X-ray determined crystal structure (2), and are in accord with Z-DNA structures investigated previously (5). Assignments for the decamer are deduced by analogy with spectra of the authenticated B-DNA crystal, d(CGCAAATTTGCG) (12), although significant differences exist between spectra of the two B-DNA oligomers. These spectral distinctions are attributed to differences in structural detail between d(CGCGATCGCG) and d(CGCAAATTTGCG), and are believed due to the combined effects of their different sequences and the presence of cobalt hexammine ions in the lattice of the decamer.

We have noted that the Raman signature of d(CGCGATCGCG) exhibits an unexpected band (642 cm^{-1}), which may be due to an unusual dG conformer. A similar spectral feature has been attributed elsewhere to a nucleoside conformation arising in a putative 'intermediate' in the B→Z transition (6, 21). Further studies will be required to reach a more definitive assignment. Although we cannot identify unequivocally the alternative conformation(s) responsible for the unusual Raman marker, the present results clearly confirm that cobalt hexammine is a promoter of substantial structural change in DNA. We are continuing the investigation by Raman spectroscopy of novel DNA conformations, including cobalt hexammine complexes in crystals and solutions.

We make one additional point regarding the presence of cobalt hexammine in DNA crystal lattices. In the case of d(CGCGATCGCG), crystallization of a Z-DNA structure had been anticipated, because of both the near perfect pyrimidine/purine alternation in its sequence and the Z induction effect attributed to $Co(NH_3)_6^{3+}$ (4). The fact that a B form structure occurs in the decamer crystal presumably reflects relatively weak interaction between $Co(NH_3)_6^{3+}$ and DNA sites. One may envision the cobalt hexammine ions as migrating rather freely among potential binding sites in solvent channels of the crystal. This is consistent with the absence also of strong interactions between $Co(NH_3)_6^{3+}$ and d(^{5Br}CGAT^{5Br}CG), which despite its Z form structure, reveals no ordered $Co(NH_3)_6^{3+}$ groups in the electron density map (2). This is somewhat surprising in view of the well-coordinated $Co(NH_3)_6^{3+}$ and $Ru(NH_3)_6^{3+}$ ions located in the corresponding crystal structures of d(CGCGCG) (3, 23). The driving force for the Z form structure of d(^{5Br}CGAT^{5Br}CG) evidently arises not only from the presence of $Co(NH_3)_6^{3+}$ but also from 5-bromination of deoxycytidine residues.

The Raman spectra obtained from crystals of cobalt hexammine complexes of d(^{5Br}CGAT^{5Br}CG) and d(CGCGATCGCG) confirm a pattern recognized previously (11): The B form structure of DNA in the crystal exhibits greater conformational heterogeneity than structures of the Z (or A) form. This suggests that for B-DNA, the crystallization process has a pronounced effect on intramolecular architecture. That this is not generally the case for either A or Z family structures, may be due to greater compatibility between the low water activity required for Z and A structures and the environment of the crystallization medium.

The present work demonstrates the capability of obtaining Raman spectra of excellent signal-to-noise quality from highly colored cobalt hexammine complexes of DNA by means of krypton 647.1 nm laser excitation. Raman data were obtained from two structurally dissimilar forms of cobalt hexammine DNA, a right-handed B form and a left-handed Z form, thus indicating the feasibility of Raman spectroscopy as a probe of polymorphism in cobalt hexammine complexes of double-stranded DNA. The present study also suggests a role for Raman spectroscopy as a probe of other DNA-cobalt hexammine interactions, including condensation reactions induced in high molecular weight DNA by cobalt ligand coordination.

ACKNOWLEDGMENTS

This research was supported by NIH Grants AI18758 (GJT) and GM41612 (AHJW). We also thank Coherent, Inc. and Mr. Arthur L.Swygard for loan of the prototype Coherent Spectrum mixed-gas laser used in this investigation.

REFERENCES

1. Rich, A., Nordheim, A. and Wang, A.H.-J. (1984) *Ann. Rev. Biochem.*, **53**, 791–846.
2. Wang, A.H.-J., Gessner, R.V., van der Marel, G.A., van Boom, J.H. and Rich, A. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 3611–3615.
3. Gessner, R.V., Quigley, G.J., Wang, A.H.-J., van der Marel, G.A., van Boom, J.H. and Rich, A. (1985) *Biochemistry*, **24**, 237–240.
4. Behe, M. and Felsenfeld, G. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 1619–1623.
5. Thomas, G.J., Jr. and Wang, A.H.-J. (1988) *Nucleic Acids and Molecular Biology*, **2**, 1–30.
6. Urpi, L., Ridoux, J.P., Liquier, J., Verdaguer, N., Fita, I., Subirana, J.A., Iglesias, F., Huynh-Dinh, T., Igolen, J. and Taillandier, E. (1989) *Nucleic Acids Res.*, **17**, 6669–6680.
7. Erfurth, S.C., Kiser, E.J. and Peticolas, W.L. (1972) *Proc. Natl. Acad. Sci. USA*, **69**, 938–941.
8. Thomas, G.J., Jr., Benevides, J.M. and Prescott, B. (1986) *Biomol. Stereodynamics*, **4**, 227–254.
9. Nishimura, Y, Tsuboi, M., Sato, T. and Aoki, K. (1986) *J. Mol. Struct.*, **146**, 123–153.
10. Thomas, G.J., Jr. and Tsuboi, M. (1993) *Adv. Biophys. Chem.*, **3** (in press).
11. Benevides, J.M., Wang, A.H.-J., van der Marel, G.A., van Boom, J.H., Rich, A. and Thomas, G.J., Jr. (1984) *Nucleic Acids Res.*, **12**, 5913–5925.
12. Benevides, J.M., Wang, A.H.-J., van der Marel, G.A., van Boom, J.H. and Thomas, G.J., Jr. (1988) *Biochemistry*, **27**, 931–938
13. Wang, A.H.-J., Quigley, G.J., Kolpak, F.J. and Rich, A. (1981) *Science*, **211**, 171–176.
14. Thomas, G.J., Jr. and Barylski, J.R. (1970) *Appl. Spectrosc.*, **24**, 463–464.
15. Thamann, T., Lord, R.C., Wang, A.H.-J. and Rich, A. (1981) *Nucleic Acids Res.*, **9**, 5443–5457.
16. Ridoux, J.P., Liquier, J. and Taillandier, E. (1988) *Biochemistry*, **27**, 3874–3878.
17. Wang, Y., Thomas, G.A. and Peticolas, W.L. (1987) *J. Biomol. Struct. Dyn.*, **5**, 249–274.
18. Grzeskowiak, K., Yanagi, K., Privé, G.G. and Dickerson, R.E. (1991) *J. Biol. Chem.*, **266**, 8861–8883.
19. Quintana, J.R., Grzeskowiak, K., Yanagi, K. and Dickerson, R.E. (1992) *J. Mol. Biol.*, **225**, 379–395.
20. Yuan, H., Quintana, J. and Dickerson, R. E. (1992) *Biochemistry*, **31**, 8009–8021.
21. Pohl, F.M., Ranade, A. and Stockburger, M. (1973) *Biochem. Biophys. Acta*, **335**, 85–92.
22. Prescott, B., Steinmetz, W. and Thomas, G.J., Jr. (1984) *Biopolymers*, **23**, 235–256.
23. Ho, P.S., Frederick, C.A., Saal, D., Wang, A.H.-J. and Rich, A. (1987) *J. Biomol. Struct. Dyn.*, **4**, 521–534.