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The high salt form of poly(dG-dC).poly(dG-dC) is left-handed Z-DNA: Raman spectra of crystals and solutions

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ABSTRACT

The laser-Raman spectra of crystalline d(CpGpCpGpCpG) and of aqueous poly(dG-dC)·poly(dG-dC) in high salt (4M NaCl) and low salt (0.1M NaCl) solutions have been measured and compared. The spectra of the crystal and the high-salt solution show a striking congruence, which indicates clearly that the high-salt form of the aqueous polymer has the left-handed Z-DNA structure of the crystalline oligomer. These two spectra differ substantially from that of the low-salt form of the polymer, which has been found previously to have spectral characteristics of the B-form of DNA. The high salt spectrum shows a unique line due to guanine residues at 625 cm^{-1} which should be useful for qualitative and possibly quantitative assessment of the amount of Z-structure present in a sample of DNA.

The DNA polymer containing alternating guanine and cytosine residues, poly(dG-dC), can exist in two different forms. In 1972 Pohl and Jovin showed that raising the salt concentration of a poly(dG-dC) solution to 4M NaCl produced a near-inversion of the circular dichroism spectrum (1). At low ionic strength the circular dichroism curve was similar to that of native DNA; when the ionic strength was raised, the curve virtually inverted with the mean position of the inversion found at about 2.7M NaCl. Raising the concentration of magnesium chloride also produced an inversion with the halfway point near 0.8M $MgCl_2$ (1). A number of studies of these two different conformational forms of the polymer have been carried out by Pohl and his associates (1-3). However, the physical nature of the converison was not understood. In 1979 the oligonucleotide d(CpGpCpGpCpG) [$d(CG)_3$] was crystallized in a form which diffracted to 0.9 Å resolution. The three-dimensional structure of the crystal was solved at atomic resolution (4,5) and it revealed an unusual conformation of the DNA

double helix in which the guanine and cytosine residues form Watson and Crick hydrogen bonded base pairs and the sugar phosphate chains are antiparallel. However, the form of the molecule is not that of the familiar right-handed B-DNA, but rather is in a left-handed helical form in which the sugar phosphate backbone pursues an irregular, zig-zag course. This is called Z-DNA. While right-handed B-DNA has two helical grooves, left-handed Z-DNA has only one groove with the base pairs forming the outer convex part of the molecule. What is most striking about comparing the B and Z forms of DNA is the fact that the phosphate groups from the two polynucleotide strands are considerably closer together in Z-DNA than in B-DNA. Other unique features include the fact that the guanine residues are all in the syn conformation in Z-DNA rather than in the anti conformation, as in B-DNA. Further, the ring pucker of the guanine sugar residues is different from that of the cytidine residues. Hydrated magnesium ions in the lattice were shown to complex with some guanine bases (5). The relationship between the polynucleotide chain and the bases is altered in that the bases are "flipped over" in Z-DNA relative to what they are in B-DNA.

A ^{31}P NMR study of the oligomer, $d(CG)_8$ revealed two ^{31}P resonance peaks in the high-salt solution, whereas only a single peak was found in the low-salt solution (6). Since the phosphate groups are found in two different conformations in the Z-DNA crystals, this result supports the idea that Z-DNA and the high-salt form of poly(dG-dC) are the same. Pohl and his colleagues have demonstrated that the laser Raman spectrum of poly(dG-dC) changes when the conformation changes from that seen in the low salt to the high salt solution (3).

Here we carry out a laser-Raman analysis of the crystals of the hexanucleotide $d(CG)_3$, which have the Z-DNA structure, and compare this to the laser Raman spectrum of poly(dG-dC) in both low-salt and high-salt solutions. From comparison of these spectra, we can establish the identity of Z-DNA with the high-salt form of poly(dG-dC).

MATERIALS AND METHODS

The crystals of $d(CG)_3$ used in this study were those on

which the X-ray studies of Wang et al. (4, 5) were carried out. For solution studies of the same material, the crystals were dissolved in both 0.1M NaCl and in 4M NaCl. The alternating copolymer poly(dG-dC) was purchased from P-L Biochemicals.

Raman spectra were excited by the 488 nm line of a Coherent Radiation CR3 Ar^+ laser, with power incident on the sample of about 100 mW. Spectra of radiation scattered at 90° to the incident beam were recorded by a Spex Ramalog 4 spectrometer with a spectral slit width of about 4 cm^{-1}. Photon counting was used and the spectra were recorded at a scan rate of 0.5 cm^{-1}/sec. Experimental procedures were usually similar to those described in reference 7. Peak frequencies are accurate to ± 2 cm^{-1} except for lines that are broad or badly overlapped. Intensities were measured as peak intensities above an estimated background. Reproducibility of peak intensities is good to better than 10% but because of the rather arbitrary nature of the estimated background, comparison of intensities between different spectra may be less accurate than this. Temperature of the sample is estimated as 28 ± 2°C.

RESULTS

Figures 1 and 2 (top) show the spectrum of a polycrystalline sample of $d(CG)_3$. Also shown in Figure 2 are the spectra of a solution of sodium poly(dG-dC)·poly(dG-dC) in 4M NaCl (middle) and sodium poly(dG-dC)·poly(dG-dC) in 0.1M NaCl (bottom). Figure 3A shows the spectrum of the crystal. The crystals of $d(CG)_3$ were dissolved and its spectrum was measured in 4M NaCl (Figure 3B) and in 0.1M NaCl (Figure 3C). For comparison with Figure 2, Figure 3D gives the spectrum of poly(dG-dC)·poly(dG-dC) in 3M $MgCl_2$ solution. The numerical values of the peak frequencies and intensities from these spectra are listed in Table I. Intensities in Table IC are referred to that of the 983 line of 0.1M $SO_4^=$ taken as 1.00; remaining intensities in Table I are referred to the 785 cytosine line taken as 0.72.

Figure 4 compares the spectra of monomeric deoxyguanosine in low-salt (0.1M NaCl) (top), high-salt (4M NaCl) (second), and 3M $MgCl_2$ solution (third), together with the latter solution without deoxyguanosine (bottom).

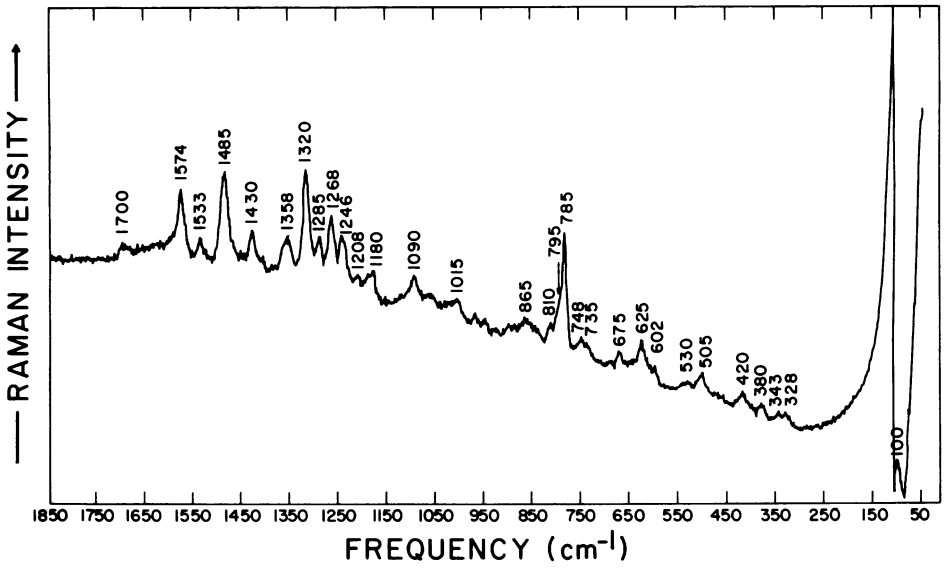

Figure 1. Raman spectrum of crystals of d(CpGpCpGpCpG) [$d(CG)_3 \cdot d(CG)_3$] (direct tracing of original recording). Conditions are given in the text. The frequencies of the major peaks are indicated.

The spectrum of $d(CG)_3 \cdot d(CG)_3$ in 3M $MgCl_2$ (not shown) was also determined and found to be substantially the same as that in 4M NaCl, Figure 2 (middle).

DISCUSSION

Some years ago the effect of ionic strength on the conformation of poly(dG-dC)·poly(dG-dC) was examined by Pohl and coworkers using various techniques, including Raman spectroscopy (3). They showed that at high salt concentration (e.g., 4.4M NaCl), the Raman spectrum of poly(dG-dC)·poly(dG-dC) changes considerably from that of the low-salt form (1M NaCl or less). From a study of the phosphate group frequencies, they concluded that the low-salt form was a double helix with Watson-Crick pairing, similar to the B-form of native DNA.

The crystalline form of the low-molecular weight oligomer $d(CG)_3$ was solved by X-ray crystallography (4, 5) and was found to have a left-handed double-helical structure quite different

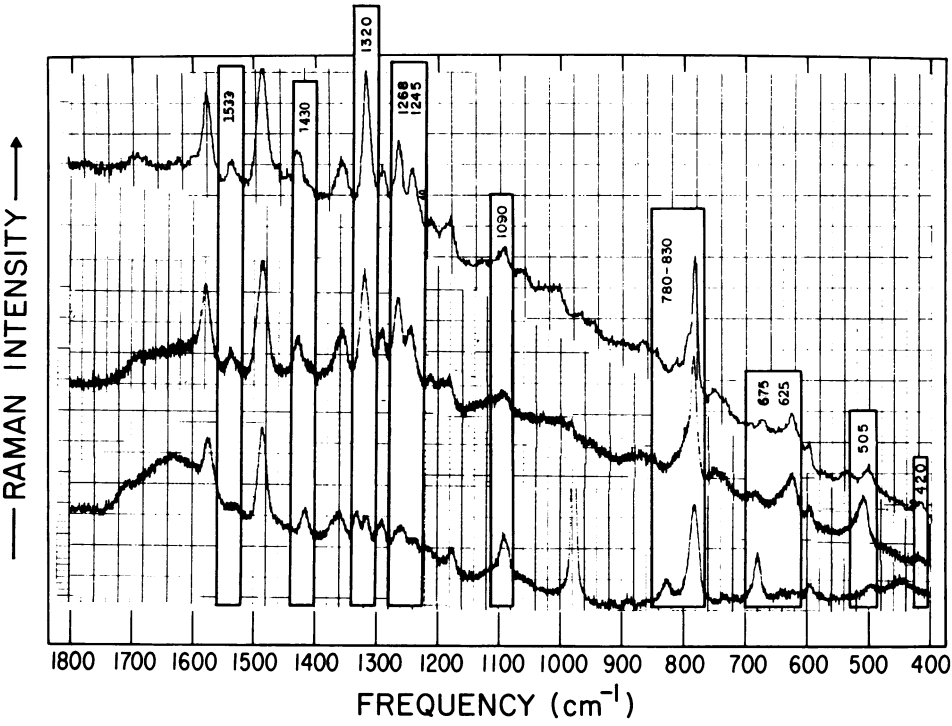

Figure 2. Comparison of Raman spectra of crystals of $d(CG)_3 \cdot d(CG)_3$ (top), aqueous poly(dG-dC)·poly(dG-dC) in 4M NaCl (middle), same in 0.1M NaCl (bottom). All spectra are original recordings under same spectroscopic conditions as listed in the text. Boxes emphasize points of difference between high salt and low salt solutions. The bottom spectrum contains 0.1M $SO_4^{=}$ ion which produces the sharp peak at 983 cm^{-1}.

from that of B-DNA. The relation between the conformation in the crystal and the high-salt conformation of the polymer is of great interest. Comparison of the Raman spectra of these materials is given in Figure 2 and Table I. These reveal a remarkable congruence between the spectrum of the crystal and that of the high-salt form of the polymer. A peak-by-peak survey shows only minor differences in frequency and intensity between the two, apart from the expected difference in the background due to the aqueous solvent. An intensity difference which does seem significant is the ratio of the intensity I_{675} of the weak G-line at 675 cm^{-1} to

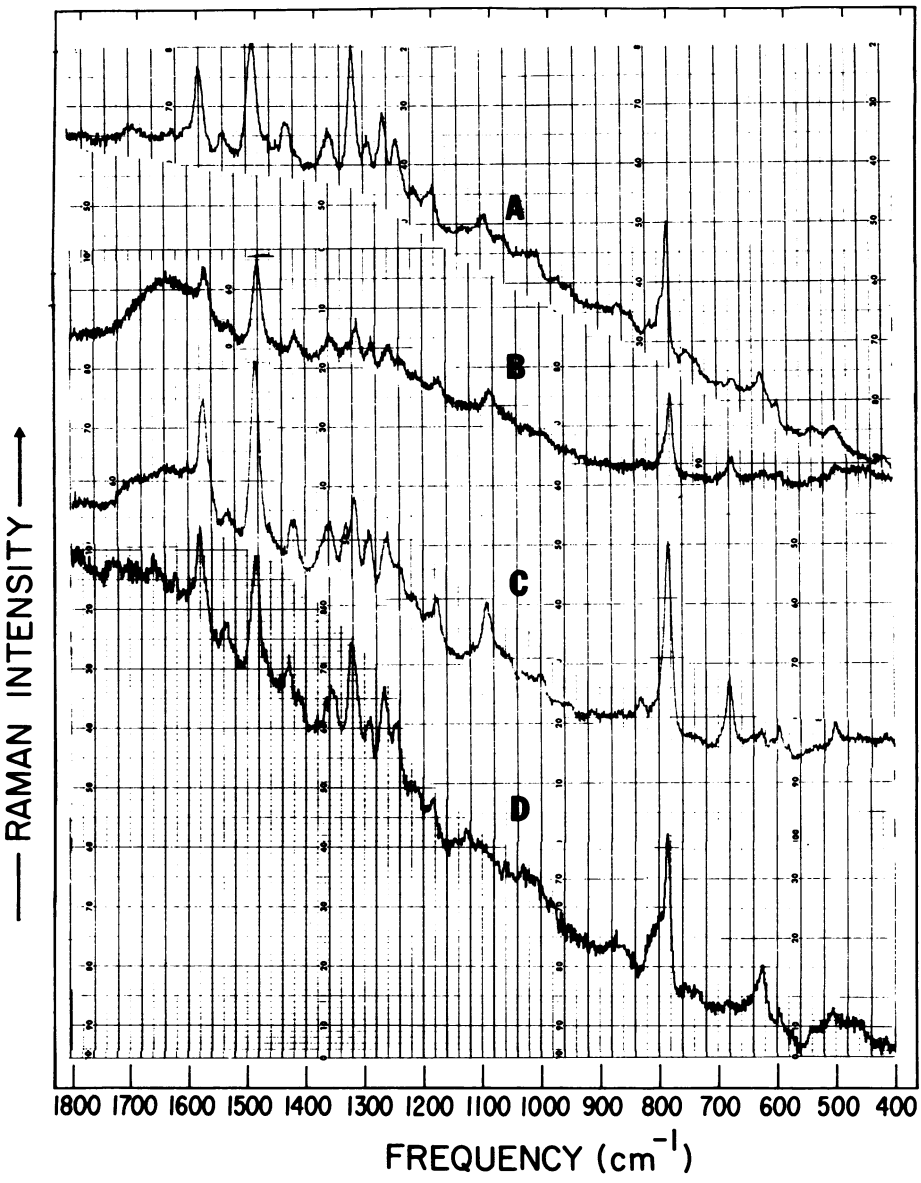

Figure 3. Comparison of Raman spectra of: (A) crystals of $d(CG)_3 \cdot d(CG)_3$; (B) aqueous $d(CG)_3 \cdot d(CG)_3$ in 4M NaCl; (C) aqueous $d(CG)_3 \cdot d(CG)_3$ in 0.1M NaCl; (D) aqueous poly(dG-dC)·poly(dG-dC) in 3M $MgCl_2$. Original recordings are shown.

that of the stronger line, I_{625}, at 625 cm^{-1}. In the spectrum of the crystal this ratio is about 0.4, whereas in the poly(dG-dC) spectrum it is smaller and difficult to measure (0.2 or less). The 625 frequency is presumed by us to arise from the guanine residues in Z-DNA as a result of the special conformation and environment of guanine in that structure. In Z-DNA, the guanine residues are stacked on cytosine on one side, and are stacked on the O_1' atom of an adjacent sugar residue on the other side. It seems possible that the "normal" G-frequency at 675 cm^{-1} in monomeric guanine systems and in B-DNA shifts in Z-DNA to 625 cm^{-1}, at the same time perhaps lending some additional Raman intensity to the weak guanine frequency at 505 cm^{-1}.

In the crystal there are 2 terminal and 4 interior guanine residues per dimer unit $d(CG)_3 \cdot d(CG)_3$, so that one is tempted to ascribe the 675 line in the crystal to the terminal guanines and the 625 line to the internal guanines. On a purely numerical basis (with no intensity change resulting from stacking differences or from the alteration in the normal mode that causes the frequency shift 675-625), one would then expect $I_{675}:I_{625} = 0.5$. In the ideal Z-form of the polymer $d(CG)_n \cdot d(GC)_n$, however, there should be a negligibly small amount of terminal guanine and the ratio I_{675}/I_{625} should be near zero. The non-zero I_{675} seen in the polymer spectrum may result from end effects or the presence of small amounts of B-form in the polymer.

Apart from this relatively minor intensity difference, the spectra of the crystal and the high-salt form are so close that we confidently ascribe the Z-DNA structure to the high-salt form of aqueous poly(dG-dC). We should add that our spectra of the high- salt form of the polymer are in excellent agreement with that of Pohl *et al.* (3).

Support for the foregoing interpretation is provided by a comparison of the spectrum of the high-salt form with that of the low-salt form of the polymer (Figure 2 and Table I). In addition to the 675-625 shift, these spectra show major differences in the phosphodiester lines at 795, 810 (high-salt) versus 830 (low-salt), in the PO_2^- line at 1090 cm^{-1} (weak in high-salt, stronger in the low-salt), and in the guanine lines at 1320 (high-salt) (a doublet at 1315, 1335 in the low-salt form), 1430 and 1533 cm^{-1}.

Table I. Raman Frequencies and Normalized Intensities for: (A) Crystalline $d(CG)_3 \cdot d(CG)_3$; (B) Solution of poly(dG-dC)·poly(dG-dC) in 4.0M NaCl;[3] (C) Solution of poly(dG-dC)·poly(dG-dC) in 0.1M NaCl; (D) Solution of $d(CG)_3 \cdot d(CG)_3$ in 4.0M NaCl; and (E) Solution of $d(CG)_3 \cdot d(CG)_3$ in 0.1M NaCl.

Origin	A		B		C		D		E	
	cm^{-1}	I	cm^{-1}	I	cm^{-1}	I	cm^{-1}	I	cm^{-1}	I
	100	.30								
	328	.06								
	343	.06								
	380	.08	380 sh	.11	380 sh	.06			405	.02
	420	.11	410 sh	.18					415	.04
	440 sh	.05								
	470 sh	.06								
G	505	.21	505	.24	500	.08	500	.18	502	.06
	530	.08								
	564	.05								
					579	.05			580	.04
	602	.06	597	.10	595	.11	592	.06	596	.09
									610	.03
G	625	.19	625	.27			623	.03	627	.05
			642 sh		642	.08			640	.02
G	675	.08	685	.05	681	.28	675	.19	681	.26
	695 sh				695	.07				
	735	.08	735	.03	730 br	.02				
	748	.14	750	.05						
C	785	.72	782	.72	782	.72	780	.72	782	.72
	795 sh	.10	792 sh	.07						
phospho-diester symmetric stretch	810	.02	810 sh	.01	829	.19	828	.06	830	.08
	850 sh	.08								
	865	.12								
	900	.09								
			930	.17	927	.28				
	940	.07							952	.02
	962	.07								
					983*	1.00				
							991	.06	1000	.08
	1015 br	.08							1015	.04
									1030	.04
									1050 sh	.08

sh = shoulder; br = broad; vb = very broad; * = sulfate standard;
I = Normalized Intensity

Table I (cont'd)

Origin	A		B		C		D		E	
	cm^{-1}	I	cm^{-1}	I	cm^{-1}	I	cm^{-1}	I	cm^{-1}	I
O=P-O$^-$ symmetric stretch	1090	.13	1095	.11	1090	.30	1088	.21	1091	.24
			1115 sh	.15	1115 sh	.17				
	1130 br	.05								
	1180	.13	1180	.08	1177	.14	1171	.18	1178	.16
	1208	.08	1192	.07					1190 sh	.04
	1220	.09	1213	.06	1217	.11	1210	.13	1215	.12
C	1246	.30	1245	.27	1240	.14	1240	.24	1240 sh	.21
C	1268	.44	1265	.42	1262	.21	1258	.29	1262	.28
C	1285	.23	1287	.25	1293	.28	1284	.23	1290	.26
					1315	.22	1312	.40	1316	.37
G	1320	.63	1320	.57			1326	.18		
G					1335	.27			1331	.24
G	1358	.30	1355	.30	1362	.22	1358	.20	1360	.21
G	1386 sh	.14	1385	.08	1380 sh	.17				
G	1415 sh	.09	1413 sh	.22						
G	1430	.20	1430	.31	1422	.24	1427	.16	1421	.21
G	1485	.71	1482	.68	1487	.75	1481	.75	1485	.69
C	1533	.12	1535	.12	1530	.04	1530	.11	1533	.04
G	1574	.39	1580	.37	1580	.35	1572	.33	1574	.46
C=O stretch	1612 br	.17	1645	.46	1630 vb	.13	1633 vb	.52	1638 vb	.16
	1700 br	.16			1713	.16				

sh = shoulder; br = broad; vb = very broad; * = sulfate standard;
I = Normalized Intensity

These differences were pointed out by Pohl et al. (3), who noted that the spectrum of the low-salt form is similar to that expected for a polymer having the B-form of DNA. Additional intensity changes were found by us in the guanine line at 505 cm^{-1} (moderate in high-salt, weak in low-salt) and for the cytosine line near 1265 cm^{-1}, which is twice as strong (compared to the cytosine line at 1287) in the high-salt form as it is in the low-salt form. This latter intensity change is the only substantial evidence in our spectra of the effect on the cytosine base of the high-salt structural change. It appears to be of importance since it is also observed in the spectrum of the crystal.

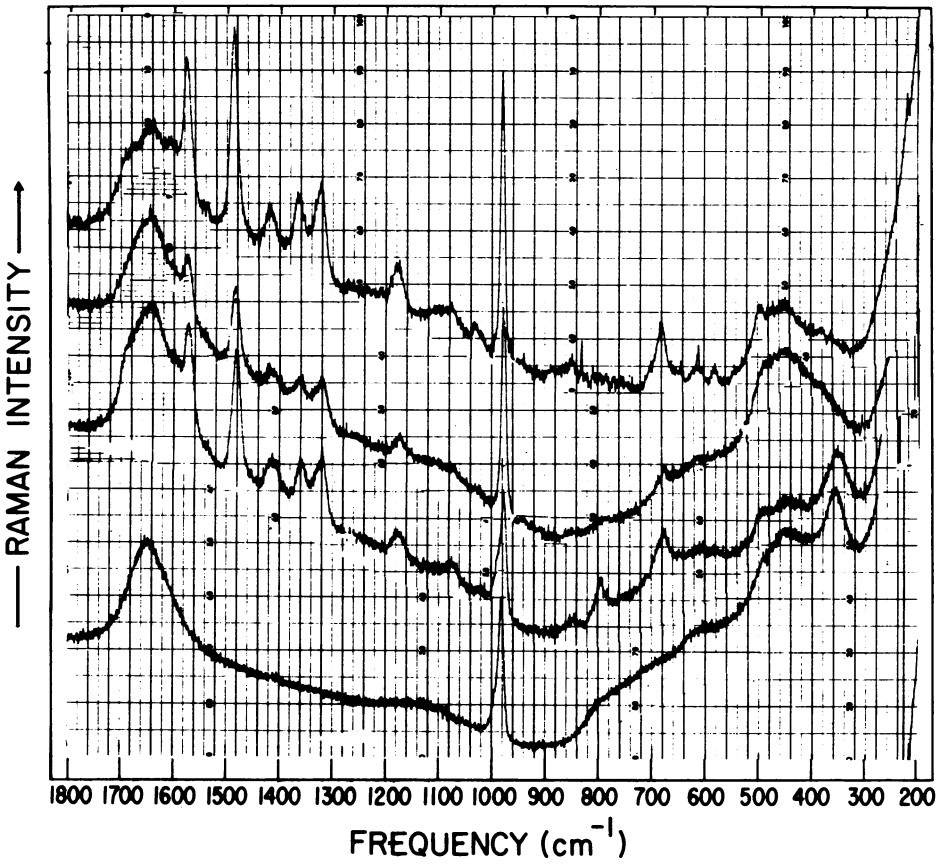

Figure 4. Raman spectra of monomeric deoxyguanosine in aqueous 0.1M NaCl (top), 4M NaCl (second) and 3M $MgCl_2$ (third), plus Raman spectrum of aqueous 3M $MgCl_2$ (bottom). (Original recordings). The line at 983 cm^{-1} is due to 0.1M $SO_4^=$ in the solutions. Apparent intensity variations between spectra are due to slightly different concentrations.

Before considering the utilization of these various differences between the spectra of the B-form and Z-form of DNA, we discuss two additional systems for which we have obtained spectra.

Comparison of the spectra of crystalline $d(CG)_3$ and its high-salt and low-salt solution spectra:

The spectra of $d(CG)_3$ are presented in the crystalline form

and in high and low-salt solutions in Figure 3, and intensities are listed in Table I. It can readily be seen that the differences between the two solution spectra are quantitative only, and that the relative peak intensities in the two spectra are about the same for all lines. This quantitative difference is presumed to be due to different concentrations in the two samples and not to structural changes. From an analysis of the high-salt spectrum, we conclude that the effect of high ionic strength on the hexamer is not sufficient to convert all of it into the Z-form. All of the characteristics of the low-salt form of poly(dG-dC) as identified in the previous section can be found in the high-salt solution of the hexamer, and it appears that most of the hexamer (>75%) has the "low-salt" structure.

However, there is also evidence for a small amount of the Z-form in the low-salt solution. These results are in agreement with the investigations by Pohl and Jovan (1), who show that there is a considerable effect due to chain length. The shorter chain lengths were found to have less cooperativity and take a longer time to convert to the Z-form when placed in a 4M NaCl solution. The present evidence suggests that at about 28°C and 4M NaCl, the hexamer is a mixture of roughly 25% Z and 75% B.

These results are also in approximate agreement with the recent investigation by Quadrifoglio et al. (8), who have measured the circular dichroism of $d(CG)_3$ as a function of temperature in a 5M NaCl solution. Their results indicate that at 0°C in this solution, the hexamer can form a full Z-conformation as judged by the inversion of the circular dichroism. However, at more elevated temperatures the circular dichroism is not completely reversed, and this was judged to indicate that the hexamer is a mixture of Z and B states. Their data at 30°C suggest about 40% B conformation, differing somewhat from the above values from the Raman spectrum of the 4M solution. It is not easy to make an exact comparison, however, because of the difference in ionic strengths and the fact that the hexamer $d(CG)_3$ used by Quadrifoglio et al. (8) was a hexanucleoside hexaphosphate, whereas our material was a hexanucleoside pentaphosphate. There is one phosphate less in our oligomer.

It should also be pointed out, however, that intensities in

the Raman spectra can be used as a quantitative measure of the volume concentration of the different species, since the contribution of each species to its Raman spectral intensity is linearly proportional to its concentration. The same cannot be said for the circular dichroism, which has end effects for oligomers, the nature of which is not fully understood. Indeed, because there is not an adequate theory fully accounting for the origin of circular dichroism spectra, one cannot use these spectra in a strictly quantitative way to determine the relative amounts of different species. Any quantitative interpretation of DNA containing both the B and Z components based on circular dichroism must be regarded as tentative until it is confirmed by data such as can be obtained, for example, from Raman analysis.

Examination of the effect of high Na^+ and Mg^{2+} concentration on the spectrum of the guanine ring:

To find out if the changes in Figures 2 and 3 might be related to the binding of Na^+ or Mg^{2+} ions to the guanine ring, we have determined the Raman spectrum of deoxyguanosine in 0.1M NaCl, 4M NaCl, and 3M $MgCl_2$. These spectra, which also contain the 983-line of 0.1M sulfate ion as an internal standard, are shown in Figure 4. Comparison of the low-salt, high-salt and high-Mg^{2+} spectra reveals that the effects of high concentrations of Na^+ and Mg^{2+} on the characteristic Raman frequencies and intensities of the guanine ring system are minor. In particular, there is no indication that the intensity ratio I_{675}/I_{625} changes from $>>1$ at low-salt to $<<1$ at high Na^+ or Mg^{2+}. From this we conclude that the presence of the 625 line and absence of the 675 line in the spectrum of the high-salt form of the polymer is not a result of association between the guanine ring and the cations, but arises from some other cause.

Raman spectroscopic assessment of Z-content in DNA

We are now in a position to consider the quantitative differences between the Raman spectrum of the high-salt form of poly(dG-dC), which is that of the Z-form in crystalline $d(CG)_3$, and the spectrum of the low-salt form, presumably a suitable model for the B-form of DNA. Our objective is to determine whether the Raman spectrum could be used to measure the amount of Z-form present in a sample of DNA, and if so, to estimate the

minimum detectable amount.

As we saw above (Figure 2 and text relating thereto), there are some ten places in the Raman spectrum of poly(dG-dC) where substantial differences are found between the high-salt and low-salt forms. Five of these are G-lines (1533, 1430, 1320, 625 and 505 cm^{-1}), two are phosphate frequencies (1090 and ~800) and three are due to C (1265, 1245, 785). Unfortunately, all the G lines with the exception of 625 occur in coincidence with or near to A-lines and are thus unlikely prospects for quantitative measurement of the Z-form in DNA, where substantial amounts of A would be present. The phosphate line at 1090 due to PO_2^- differs in intensity but not position and would be difficult to use for the detection, and a fortiori for the quantitative determination, of the Z-form. The phosphodiester frequencies near 800 cm^{-1}, though different for the high- and low-salt forms of the polymer, occur very close to the strong C- and T- line at 785 and would likewise be difficult to exploit for detection and measurement of minority amounts of the Z-form. The C-lines at 1265 and 1245 fall close to C- and T-lines in B-DNA near 1245 and an A-line near 1255, and therefore are not promising as an analytical frequency, and the line at 785, while noticeably sharper in the high-salt form, would probably not be usable for analytical purposes.

We are thus left with the guanine line at 625 cm^{-1} as the most likely prospect. It is well known that the G- line at 675 in B-DNA is unusual in its structural sensitivity and hyperchromism (9). Thus, its displacement to 625 in the Z-form is not surprising in the light of earlier studies. Moreover, there are no A frequencies in this neighborhood and the only serious interference would appear to arise from the line due to T at about 670 cm^{-1}, sufficiently far removed from 625 cm^{-1} to permit some optimism about the utility of the latter. There are some very weak lines in DNA spectra in the range 600-650, but the extent to which they would interfere does not appear to be large.

It seems premature to discuss here the various problems of using the 625 line for quantitative purposes. The total amount of G present should be related to the sum of I_{625} and I_{675} as corrected for hyperchromism and background intensity. The cor-

rected ratio I_{625}/I_{675} should then give the relative amounts of G bases in Z-form and B-form. Qualitatively, however, the appearance of a clearcut line at 625 should mean the presence of the Z-form and crudely one may estimate that the relative amount of the two kinds of G is given by the above ratio. At present it seems unlikely that a ratio smaller than 0.01 would be reliably detected, while 0.1 should be readily seen.

Finally, we note that our only marker for the Z-form thus far is a G-line. If A-T sequences should also give rise to the Z-form, there might be more of it present than is measured by I_{625}.

Raman Spectra of Crystals and Solutions

The present type of study is becoming more important as conformational changes in macromolecules are increasingly the object of biophysical studies. Here data were available from a single-crystal x-ray diffraction analysis which showed the detailed conformation of a molecule, and data were also available from solution studies which demonstrated that conformational changes of the same material can occur when the environment is altered. The general question is how one can make an unequivocal demonstration that the conformation of the molecule in the crystalline state is the same as one of the alternative conformations in solution. It should be noted that Raman spectra offer a major opportunity to effect an identification of this type, since they arise from fundamental characteristics of the molecule -- the normal modes of vibration -- which are themselves dependent upon conformation. Thus, if the conformation changes with solution concentration, ionic strength, crystallization, or other factors, the Raman spectrum is sensitive to these changes and can be used as the basis of identification. Further, the physical origins of the spectra are well understood, as mentioned earlier, and so the technique has the additional utility that it can be used in a quantitative manner to measure the amounts of different conformations present in a mixture.

ACKNOWLEDGEMENT

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