Ultraviolet Resonance Raman Spectroscopy of DNA with 200–266-nm Laser Excitation

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Abstract: Raman spectra are compared for the deoxynucleotides dAMP, dTMP, dGMP, and dCMP with those of their alternating copolymers, poly(dA–dT) and poly(dG–dC), and calf thymus DNA, using 266-, 240-, 218-, and 200-nm excitation from a H_2-Raman-shifted Nd:YAG laser. Characteristic frequency shifts are seen in the exocyclic modes of the purine and pyrimidine bases when incorporated into the duplex structure. Consistent with previous infrared results, up- and downshifts are seen for the G and C C=O stretches, due to vibrational coupling in the G–C base pair; a similar shift pattern is seen for the NH_2 scissors modes and is likewise ascribed to interbase coupling. In contrast, the NH_2 scissors frequency of A is unaltered in the poly(dA–dT) duplex, but the C_4=O frequency of T is shifted up significantly. The ring-mode frequencies are altered very little upon duplex formation, but the intensities are lowered upon stacking of the bases as has previously been observed for visible and near-UV excitation, due to the hypochromism of the resonant electronic transitions. Intensity values and hypochromic ratios (duplex/nucleotides) are reported for calf thymus DNA at all four wavelengths. Analysis of the poly(dG–dC) intensities at 240 and 200 nm shows a general correlation between the hypochromic ratio and the band intensity, consistent with a mechanism in which the strongest bands gain most of their intensity from the locally resonant hypochromic transition, whereas the weaker bands gain a larger fraction of their intensity from higher lying electronic transitions. The lowest hypochromic ratio at 240 nm is 0.36, in good agreement with the square of the absorption hypochromic ratio, 0.41, the value expected from simple scattering theory. With 200-nm excitation, the hypochromic ratios are much lower, reaching a value of 0.08. The lower than expected ratios are suggested to result from an energy shift of the cytosine electronic transition when G and C stack. The spectrum of Z-form poly(dG–dC), obtained by addition of salt, is reported for 240- and 200-nm excitation, in H_2O and D_2O. Frequency shifts for the G modes are similar to those previously observed with visible excitation but are seen in greater detail; a B → Z shift is also observed for a C ring mode. With 218-nm excitation, the well-known conformationally sensitive G mode is observed at 682 and 624 cm^{-1} for B and Z poly(dG–dC). An additional conformationally sensitive G mode is seen at 862 and 842 cm^{-1} for B and Z poly(dG–dC) and 854 cm^{-1} for dGMP. The spectrum of rGMP contains pairs of bands in this region, at 682/666 and 854/863 cm^{-1}, attributed to the equilibrium between C2′- and C3′-endo conformations of the ribose ring.

Resonance Raman (RR) spectroscopy with deep UV laser excitation offers considerable promise as a probe of purine and pyrimidine structure and environment in nucleic acids.[1-4] RR spectra of the nucleotides have recently been reported with excitation wavelengths from 266 to 200 nm, and the resonance enhancement patterns have been analyzed.[3,4] It is important to assess the effects on these spectra of incorporating the bases into nucleic acid duplex structures. Perturbations are expected from the electronic effects of base pairing and stacking and from alterations in vibrational coupling to modes of the ribose–phosphate backbone and possibly from interstrand coupling effects. In this study we compare nucleotide spectra at 266-, 240-, 218-, and 200-nm excitation, with spectra of the alternating copolymers poly(dA–dT) and poly(dG–dC), as well as the spectra of calf thymus DNA. A number of alterations in the purine and pyrimidine RR spectra are observed on duplex formation, which will be important for future UV RR studies of DNA structure.

The frequencies of exocyclic modes, C=O stretching and NH_2 bending, are perturbed by the base pairing and stacking interactions. For poly(dG–dC), up- and downshifts in both of these modes are observed and are attributable, following the pioneering infrared studies by Miles and co-workers[5] with ^{18}O-labeled G, to vibrational coupling within the G·C base pair of the nearly equivalent C=O and C–NH_2 bonds on the G and C partners. This pseudosymmetry does not exist for A·T base pairs (see Figure 1), and the NH_2 scissors band is at the same frequency for poly(dA–dT) as for dAMP. The C_4=O stretch of T, however, shifts up 14 cm^{-1} between dTMP and poly(dA–dT), possibly due to interactions between adjacent base pairs.

Aside from these changes, purine and pyrimidine ring modes are largely unaltered in frequency by duplex formation, but they do decrease in intensity, in some cases by large factors. This Raman hypochromism is associated with the decrease in UV absorption strength that accompanies the stacking interactions in DNA duplexes;[6] it is seen, in attenuated form, even with visible laser excitation, via a preresonance Raman mechanism.[7] In addition, evidence is presented that the electronic transitions near 200 nm are actually blue-shifted in the duplex, leading to an especially large intensity reduction in this region. Hypochromic ratios have been determined for calf thymus DNA, which we expect to be useful in future studies of DNA unstacking. The B → Z conformational transition of poly(dG–dC) has also been studied. The vibrational frequency changes, which have previously been examined with visible[8-10] and near-UV[11] Raman excitation, have been extended and augmented with the UV measurements. In particular, a new guanine mode near 860 cm^{-1} is seen with 218-nm excitation, and its frequency is sensitive to conformation.

Experimental Section

5′-Deoxyribonucleotides were the highest grade available (Sigma) and were used without further purification. Poly(deoxyribonucleotides) were obtained from PL Pharmacia Biochemicals and prepared for Raman

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Table I. Scattering Coefficients, j, and Hypochromic Ratios, r, for Calf Thymus DNA UVRR Bands at Various Excitation Wavelengths

266 nm			240 nm			218 nm			200 nm		
ν[a]	j[b]	r[c]	ν	j	r	ν	j	r	ν	j	r
1655	215	0.47	1655	36	0.38	1649	107	0.28	1650	150	0.41
1577	115	0.47	1577	99	0.38	1577	267	0.22	1577	227	0.43
1484	344	0.55	1528	63	0.35	1528	138	0.23	1485	237	0.24
1421	172	0.28	1486	198	0.36	1482	138	0.23	1423	128	0.28
1371	318	0.37	1425	36	0.15	1420	61	0.18	1364	65	0.32
1338	600	0.29	1369	48	0.46	1364	69	0.22	1334	92	0.31
1302	259	0.30	1335	135	0.27	1338	77	0.27	1294	205	0.21
1250	233	0.30				728	123	0.27	782	73	0.36

[a] ν is the band frequency in inverse centimeters. [b] $j = I_\nu/I_{SO_4} \times C_{SO_4}/C_N$, where I_ν and I_{SO_4} are the Raman intensities for the nucleic acid band and the 981-cm^{-1} band of SO_4^{2-}, present as an internal standard. C_{SO_4} is the molar concentration of sulfate and C_N is the sum of the molar concentrations of the nucleotide bases present in the calf thymus DNA. [c] r is the intensity ratio for a given band of calf thymus DNA and a solution having the same concentrations of the individual nucleotides.

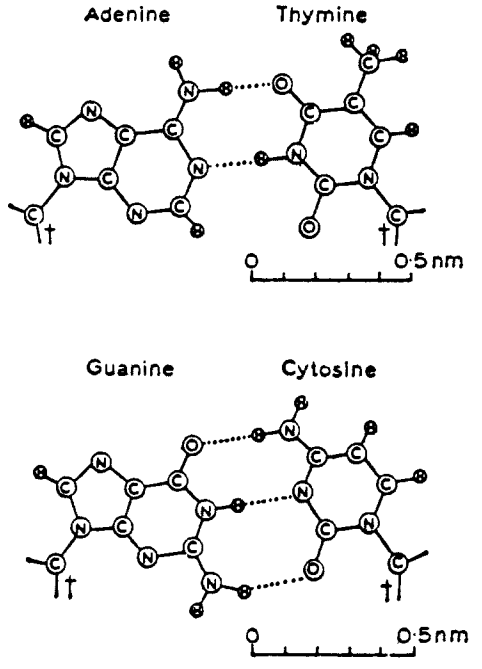

Figure 1. Structural diagram for A–T and G–C Watson–Crick base pairs. Adapted from ref 23.

spectroscopy by sonication at 4 °C for 3–5 min at 10-s bursts in a Branson Model 200 sonifier. Electrophoresis on 2% agarose gels gave an apparent length between 100 and 300 base pairs. After centrifugation to remove titanium particles dislodged from the probe during sonication, the solutions were extensively dialyzed against buffer (20 mM pH 7.2 phosphate, 50 mM NaCl) and lyophilized. The synthetic duplex was resuspended in buffer (10 mM pH 7.2 phosphate, 50 mM NaCl) at a final concentration of 20 260-nm-o.d. units per milliliter. Calf thymus DNA (Sigma) samples were prepared by the same protocol, with an additional phenol/chloroform extraction step to remove any contaminating proteins, which might have interfered with the UV Raman experiment. Subsequent experiments showed the omission of the phenol/chloroform extraction to have negligible effects.

For measurements of Raman hypochromism, mixtures of the mononucleotides and samples of calf thymus DNA were dissolved in 0.3 M sodium sulfate at identical concentrations (based upon an extinction coefficient of 6.3×10^3/(mol cm) for calf thymus DNA[13]), allowing for direct comparison of spectral intensities with the 981-cm^{-1} sulfate band as the intensity standard. Typical solution volumes of 2.5 mL were required for the Raman experiment.

The laser excitation was the 266-nm fourth harmonic of a Quanta Ray DCR1A Nd:YAG laser or the H_2-shifted anti-Stokes output of the 266-nm line at 240, 218, or 200 nm. Incident power was estimated at 1–5 mW. The samples were circulated via a peristaltic pump and jet assembly, as described in ref 3. The spectral resolution was 8 cm^{-1}; conditions of data collection are given in the figure captions.

UV absorption spectra were obtained with a N_2-purged Cary 118 scanning absorption spectrophotometer.

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Table II. Molar Scattering Coefficients, j, and Hypochromic Ratios, r, for Poly(dG–dC)[a]

240 nm			200 nm		
ν	j	r	ν	j	r
1652 (C)[b]	121	0.93	1679 (G)[b]	297	0.65
1364 (G)	147	0.84	1652 (C)[b]	454	0.43
1489 (G)	534	0.66	682 (G)	167	0.36
782 (C)	75	0.62	1579 (G)	523	0.30
1579 (G)	271	0.54	1528 (C)	327	0.28
1326 (G)[c]	210	0.46	1364 (G)	368	0.23
1528 (C)	404	0.36	782 (C)	442	0.14
			1294 (C)	942	0.08

[a] See Table I for symbol definitions; j values are reported for individual mononucleotides. [b] Values for exocyclic modes are complicated by overlap of C=O and δ_{NH_2} as described in text. [c] Splits into two modes in duplex.

Results

To gauge the effect of duplex formation, solutions of synthetic DNA containing alternating self-complementary purine–pyrimidine sequences, A–T and G–C, were compared with equimolar mixtures of the corresponding deoxyribonucleotides and with calf thymus DNA (56% A–T, 44% G–C). Figures 2–5 show RR spectra for these solutions with excitations at the four UV wavelengths 266, 240, 218, and 200 nm. Because of differential enhancement via the various UV electronic transitions, different base modes are emphasized at the four different wavelengths, as analyzed in ref 3. The dominant purine and pyrimidine contributors to the various bands are indicated by the appropriate letters in the figures.

The most notable changes seen upon duplex formation are in the band intensities. There is a general loss of intensity in the DNA spectra when compared with the nucleotide spectra, and the effect tends to be greatest for the strongest bands. Thus the relative intensity variation among the bands is less for the duplexes than for the nucleotides. The intensity loss, seen also at longer wavelengths, in the preresonance region,[7] is related to the absorption hypochromism of the resonant electronic transitions which is associated with the stacking interactions of the bases in the duplex structures.[6] The intensity loss was quantitated for calf thymus DNA, using sodium sulfate as internal standard, and the molar scattering factors and hypochromic ratios, relative to a solution containing the same concentration of the nucleotides, are listed in Table I. Intensities were also measured for poly(dG–dC) and for the nucleotides with 240- and 200-nm excitation, and the results are given in Table II. The implications of these measurements are discussed below.

Table III lists the vibrational frequencies, determined at the optimum wavelength for the band in question, for the nucleotides and the alternating copolymers. Most of the bands arise from purine and pyrimidine ring modes, and these frequencies are for the most part unaffected by duplex formation. This is as expected, since the ground-state structures of the chromophores are not significantly perturbed by the relatively weak stacking interactions in the duplex. There are, however, measurable frequency lowerings of the adenine ring modes at 1581 (→1577) and 1309 (→1302)

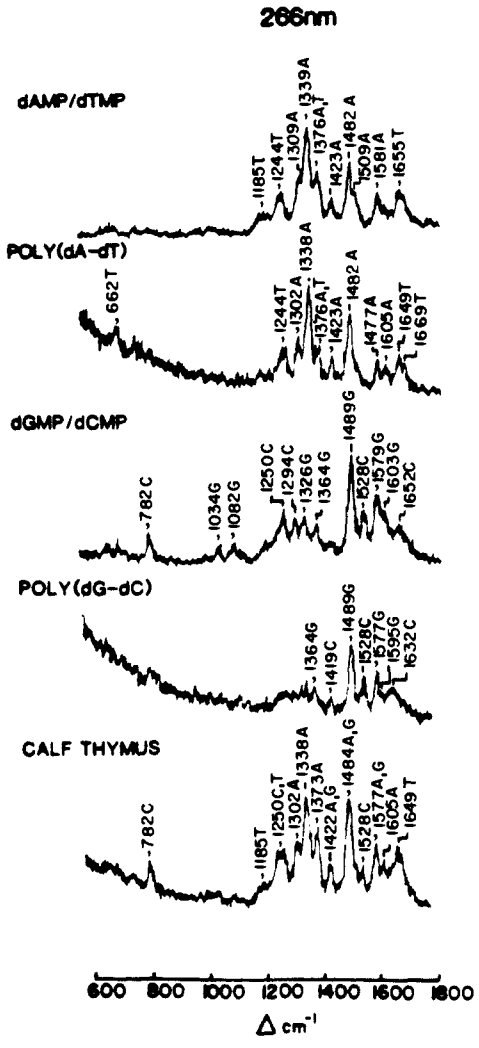

Figure 2. Raman spectra, with 266-nm excitation, for calf thymus DNA (20 absorbance units/mL at 260 nm), poly(dG–dC)·poly(dG–dC) (same absorbance, 1.4 mM in base pairs), poly(dA–dT)·poly(dA–dT) (same absorbance, ~1 mM in base pairs), equimolar dGMP + dCMP (1 mM each) and dAMP + dTMP (1 mM each). The dominant purine and pyrimidine contributors to the Raman bands are indicated by appropriate letters. The intensities of the different spectra are not scaled. Spectral acquisition: 1–3 s per point for mononucleotide spectra, 7–10 s per point for polynucleotide spectra.

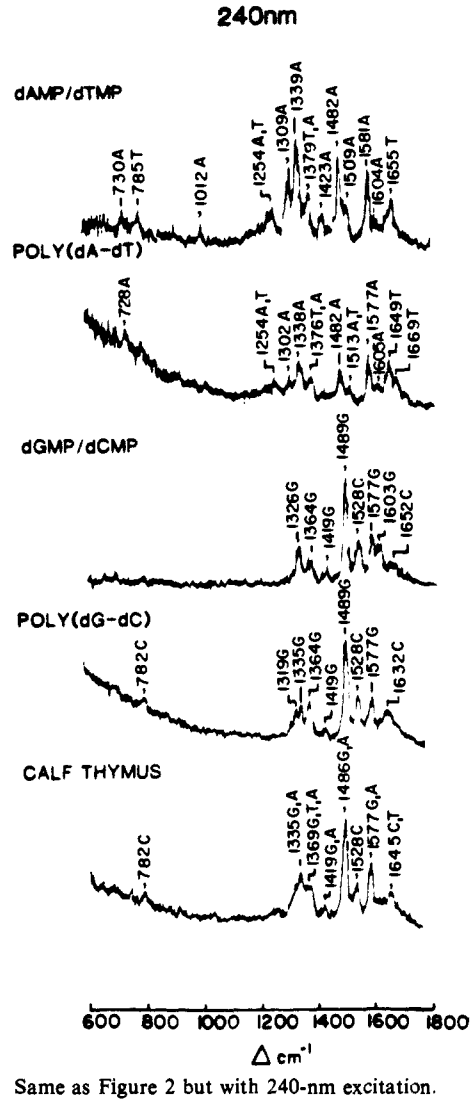

Figure 3. Same as Figure 2 but with 240-nm excitation.

cm^{-1}. In addition, the 1326-cm^{-1} band of dGMP is replaced by two bands, at 1335 and 1319 cm^{-1}, in poly(dG–dC)·poly(dG–dC). Tsuboi and co-workers[13] attribute a similar effect seen in visible-excitation Raman spectra to sugar conformational variation, on the basis of an analysis of ribose-sensitive modes of guanine.

The RR spectra also contain bands due to exocyclic vibrations, i.e., the C=O stretching and NH_2 scissors modes.[3] These groups are directly involved in the base pairing interactions, and their frequencies are significantly affected by duplex formation, as seen in Table IV. The assignments for poly(dG–dC)·poly(dG–dC), discussed below, were complicated by band overlaps and required the examination of H_2O–D_2O difference spectra, with 240- and 200-nm excitation. These are shown in Figures 6 and 7.

The spectra of poly(dG–dC)·poly(dG–dC) in the Z DNA structure were studied by adding 4 M NaCl and are shown in figures 8 and 9. The frequencies are listed in Table III and show numerous shifts, relative to the normal B DNA, due largely to the different guanine (syn instead of anti) and deoxyribose (3′ endo instead of 2′ endo) conformations. Several of these shifts have already been noted in visible-excitation Raman spectra, including the large downshift of the 682 (→624)-cm^{-1} band assigned to a guanine ring mode that is coupled to the deoxyribose. The UVRR spectra reveal an additional conformationally sensitive band at 862 (→842) cm^{-1}. This band, which has not previously been detected, is enhanced selectively with 218-nm excitation. Its frequency also differs between B DNA (862 cm^{-1}) and dGMP (854 cm^{-1}), and Figure 10 reveals additional conformational sensitivity for the ribonucleotide rGMP, which exists in an equilibrium between C2′ and C3′-endo sugar conformations.[14]

Discussion

A. Hypochromism. The loss in strength for the ~260-nm absorption bands of nucleic acid bases upon duplex formation is well-known and has been analyzed by Tinoco and co-workers.[6] While the theory for the effect is complex, it can be viewed essentially as an induced polarization of the stacked bases, which depresses the transition moment. Absorption strength is transferred from the lowest electronic excitation to higher lying areas, although the expected *hyper*chromism of far-UV nucleic acid absorption bands has not been documented. A number of workers have noted losses in purine and pyrimidine Raman band intensity upon duplex formation, even with excitation in the visible or near-UV region.[7,15,16] This effect has been attributed to a preresonance enhancement mechanism. If the Raman bands in question gain some of their intensity via preresonance with the ~260-nm electronic transitions, then weakening of the latter should produce a decrease in Raman intensity.

The availability of deep UV excitation lines allows this resonance effect to be investigated directly. Raman intensity is

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Table III. Frequency (cm^{-1}) Comparison for UVRR Bands of the Nucleotides and the Alternating Purine–Pyrimidine Copolymer Duplexes[a]

					poly(dG–dC)·poly(dG–dC)			
					B[b]		Z[b]	
dAMP	dTMP	poly(dA–dT)·poly(dA–dT)	dGMP	dCMP	H_2O	D_2O	H_2O	D_2O
	1700[c]	*d*	1679[c]		1710[c]	1682		(1660)
	1655[c]	1669[c]		1652[c]	1632[c]	1642		1642
1648	1648	1649	1603[e]		~1620[e]		~1620	
1604[e]		1605[e]						
1581		1577		1605[c]	~1595[e]			
	1517	1514[f]	1579		1577	1578	1579	1576
1509	1484	1482[f]	1539			1554		1552
1482		1479[f]		1528	1528	1525	1528	1517
1423	1414	1423						
	1379			1500		1503		1503
1376		1376	1489		1489	1481	1485	1480
1339		1338	1419		1419	1410	1406	
1309		1302		1412				
	1276	1254[g]		1376				
1254	1244	1244[g]	1364		1364	1355	1355	1343
					1335			
1220			1326	1294	1319	1316	1318	1316
	1185		1253	1250	1294	1299		1292
1173				1237				
	1141		1217	1210				
1012		1010	1179		1179	1176	1179	
	785			1144				
730		728	1082		1086		1074	
			1034		1033			
				988				
			854		862	852	842	835
				782	782	771	781	771
			682		682	681	624	616

[a] See ref 3 for suggested assignments. [b] B form in low salt; Z form in 4 M NaCl. [c] Exocyclic C=O stretches. [d] Too weak or overlapped to be observed. [e] Exocyclic NH_2 scissors modes. [f] These T and A modes are distinguished at 266 and 218 nm; A is strong while T is weak at the former wavelength and vice versa for the latter. [g] These T and A modes are distinguished at 266 and 240 nm; T is strong while A is weak at the former wavelength and vice versa for the latter. [h] Obscured by overlap with the 1642-cm^{-1} C mode. The frequency is taken from Benevides and Thomas.[10]

Table IV. Exocyclic Mode Frequencies (cm^{-1}) for the Deoxynucleotides and Their Alternating Copolymers (B Form)

	dGMP		poly(dG–dC)		dCMP	
	freq	assignt	freq	assignt	freq	assignt
H_2O	1679	$\nu_{C_6=O}$(G)	1632	$\nu_{C_2=O}$(C)	1652	$\nu_{C_2=O}$(C)
			1710	$\nu_{C_6=O}$(G)		
	1603	δ_{NH_2}(G)	~1595	δ_{NH_2}(C)	1605	δ_{NH_2}(C)
			1620	δ_{NH_2}(G)		
D_2O	1661	$\nu_{C_6=O}$(G)	1642	$\nu_{C_2=O}$(C)	1649	$\nu_{C_2=O}$(C)
	(1664)[a]		(1647)[b]		(1656)[c]	
			1682	$\nu_{C_6=O}$(G)		
			(1688)[b]			
	dTMP		poly(dA–dT)		dAMP	
	freq	assignt	freq	assignt	freq	assignt
H_2O	1655	$\nu_{C_4=O}$(T)	1605	δ_{NH_2}(A)	1606	δ_{NH_2}(A)
			1669	$\nu_{C_4=O}$(T)		

[a] Numbers in parentheses are from IR spectra.[5] [b] These values are for polyG·polyC.[5] [c] This value is for polyC.

proportional to the square of the induced polarizability, which, at resonance, is given by[17]

$$\alpha' = A + B$$

where

$$A = \mu_e^2 \sum_v F_v/(\Delta\nu_v + i\Gamma_v); \qquad F_v = \langle j|v\rangle\langle v|i\rangle$$

$$B = \mu_e \mu_s h \sum_v F_v'/(\Delta\nu_v + i\Gamma_v)$$

$$h = \langle s/\delta H/\delta Q/e\rangle/(\nu_s - \nu_e)$$

$$F_v' = \langle j|Q|v\rangle\langle v|i\rangle + \langle j|v\rangle\langle v|Q|i\rangle$$

μ_e is the transition dipole moment to the resonant excited state, *e*, of which *v* is a particular vibrational level, $\Delta\nu_v$ is the difference between the frequency of level *v*, of bandwidth Γ_v, and the laser excitation frequency, F_v is the product of Franck–Condon integrals between the intermediate level *v* and the initial and final levels *i* and *j*, μ_s is the transition dipole to another excited state, and *h* is an electronic integral that allows *e* to mix with *s* along the normal coordinate *Q*; the vibrational overlap function F_v' contains *Q*-dependent integrals for the *B* term.

For resonance with dipole-allowed transitions the *A*, or Franck–Condon, term is dominant, but for weak transitions that gain intensity via vibronic mixing, the *B* term can be important. (An additional term, *C*, which involves mixing with the ground state, can be significant for dipole-forbidden transitions.[17,18]) If a Raman band gains all of its intensity via *A*-term resonance with state *e*, then a loss in absorptivity should produce a geometric loss in Raman intensity, since Raman intensity is proportional to μ_e^4

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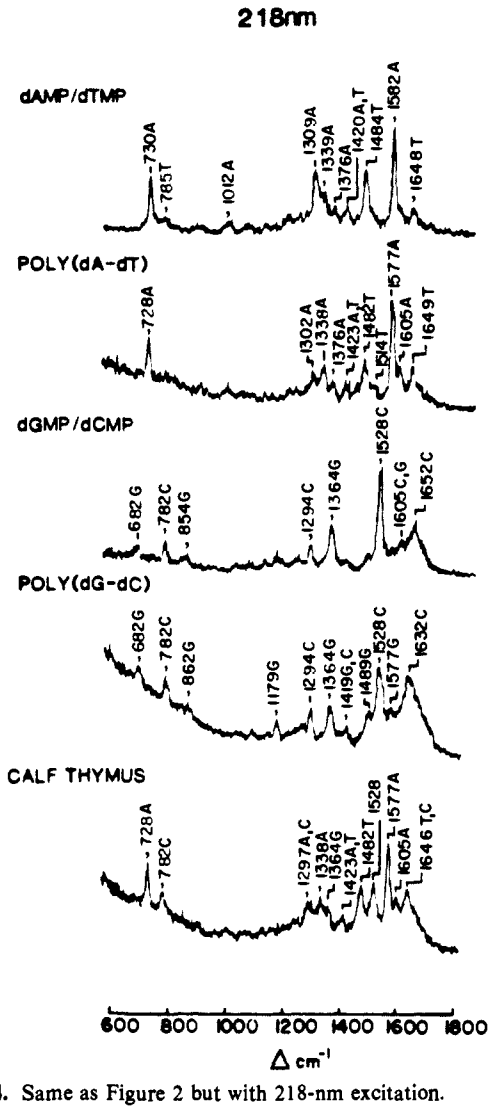

Figure 4. Same as Figure 2 but with 218-nm excitation.

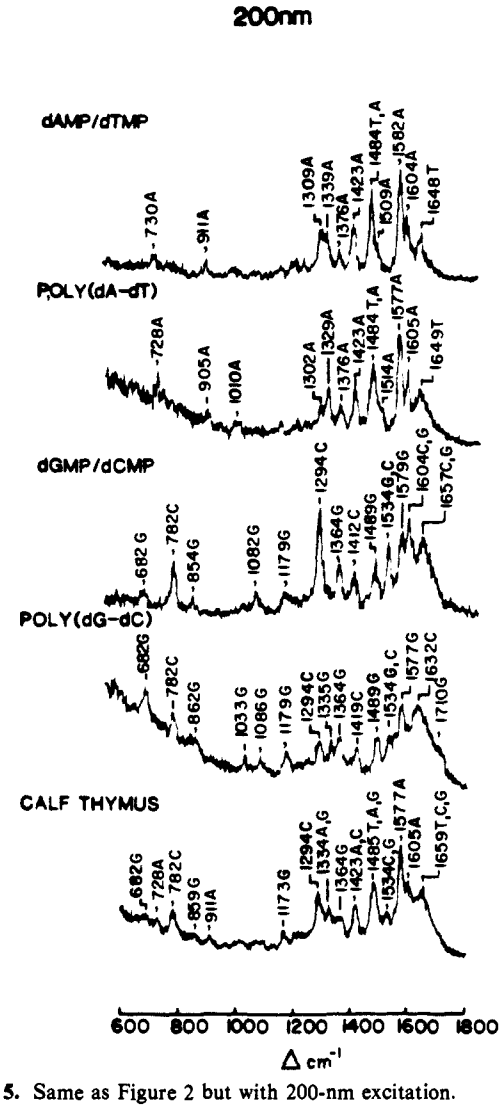

Figure 5. Same as Figure 2 but with 200-nm excitation.

while absorptivity is proportional to μ_e^2.

Table II gives molar scattering coefficients, j (relative to the sulfate ν_1 band), for the stronger Raman bands of poly(dG–dC) and the ratios, r_R, of their intensities to those of the corresponding bands of equimolar dGMP and dCMP. As expected, these ratios are all less than unity, but they range quite widely. In general the most intense bands have the lowest ratios. This tendency makes sense, since the strongest bands are expected to be those that gain the most intensity via A-term resonance with the local electronic transition. The weaker bands are likelier to gain an appreciable fraction of their intensity from higher lying electronic transitions either via the B term or simply via preresonance A-term enhancement. Provided these higher transitions experience less hypochromism than the local one, then r_R should be higher than it is for the more locally resonant modes. A good illustration of these effects is provided by the amide I and II modes in polypeptides.[19] The first amide π–π^* transition, at ~190 nm, shows hypochromism for α-helical polypeptides, and the RR intensity of the very strong amide II band, which involves stretching of the amide C–N bond, decreases with the square of the absorptivity. The weaker amide I (C=O stretching) intensity, however, is nearly independent of peptide conformation and is evidently gained mainly via higher electronic transitions.

Figure 11 compares absorption spectra for poly(dG–dC)·poly(dG–dC) and equimolar dGMP and dCMP. The hypochromism of the 260-nm band is clearly seen; at 240 nm the ratio of the absorptivities is 0.64. The square of this ratio, 0.41, is in good agreement with the lowest value of the Raman ratio, 0.36, recorded with 240-nm excitation. Thus the data are in accord with a simple model in which the Raman bands are wholly or partially resonant with the ~260 nm transitions and contain varying contributions from higher lying, less hypochromic transitions. (Indeed these states are expected to be hyperchromic.[6]) At 200 nm, the absorptivity ratio is 0.51. While most of the Raman ratios are at or above the square of this value, 0.26, two of them, 0.14 (782 cm^{-1}) and 0.08 (1294 cm^{-1}), are significantly lower. The most likely explanation for these deviations from the expected limiting value is that the locally resonant electronic transitions experience an energy shift, not just a lower intensity, upon G–C pairing and stacking, leading to a loss in resonance enhancement (via the $\Delta\nu_v + i\Gamma_v$ energy denominator.) Figure 11 shows that the absorption band at ~193 nm in the mononucleotide spectrum does indeed appear to shift significantly toward higher energy in the spectrum of the duplex. Since the Raman bands showing the very low intensity ratios are both cytosine modes, it may be that the main energy shift is associated with a cytosine electronic transition. Although the 260-nm absorption band maintains its peak wavelength, the structure that is seen in the mononucleotide spectra is lost in the duplex, suggesting some energy shifts of the component transitions. (There are at least one C and two G transitions in this band.[20]) Because of the

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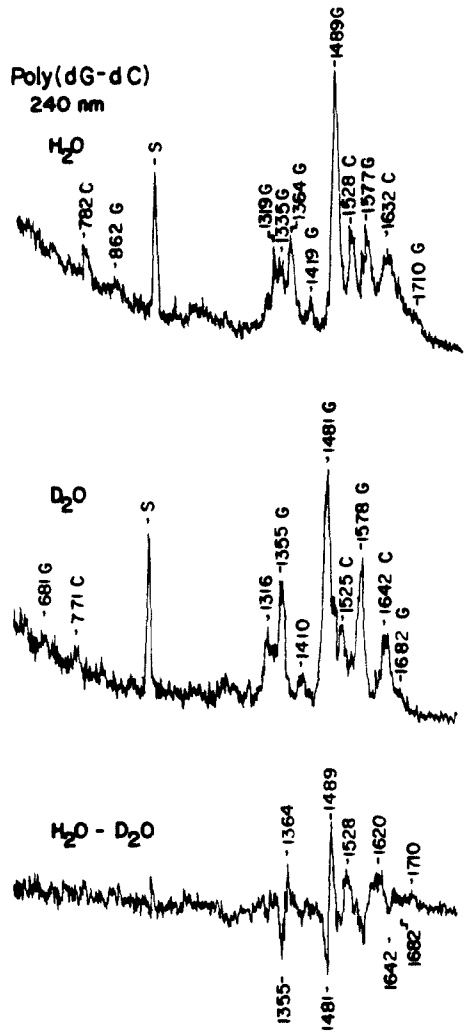

Figure 6. Raman spectra, with 240-nm excitation, of poly(dG–dC)·poly(dG–dC) in H_2O and D_2O and the H_2O–D_2O difference spectrum. Conditions as in Figure 1.

complexity of the overlapping absorption bands, electronic energy changes associated with duplex formation have not been analyzed, as far as we are aware. Excitation profiles of the Raman bands[4] may be able to provide detailed information about these changes.

The Raman intensity gain associated with unstacking of the bases may be useful in monitoring single-stranded DNA. The effect is larger with UV than with visible excitation, and the high sensitivity associated with resonance enhancement will allow melting to be studied for dilute DNA samples. Since bands associated with individual bases can be isolated by adjusting the excitation wavelength, it should be possible to estimate both the extent and the composition of single-strand regions. The hypochromic ratios and molar scattering factors given in Table I for calf thymus DNA are expected to serve as reference data for such studies.

B. Exocyclic Frequencies. The vibrational frequencies associated with the carbonyl and amine groups on the purine and pyrimidine rings are expected to be perturbed upon duplex formation, since these groups are directly involved in H-bonding between the bases (Figure 1). Frequency shifts are expected because of electronic (bond strength) changes due to base pairing and stacking and also because of vibrational coupling across the H-bonds of the base pair. The latter is particularly important for G-C pairs, which have two, symmetrically disposed —C═O···H—NH— linkages (Figure 1). Coupling between the two C═O stretches was demonstrated by Miles and co-workers,[5] using GMP adducts of poly C. When ^{18}O was incorporated in the G

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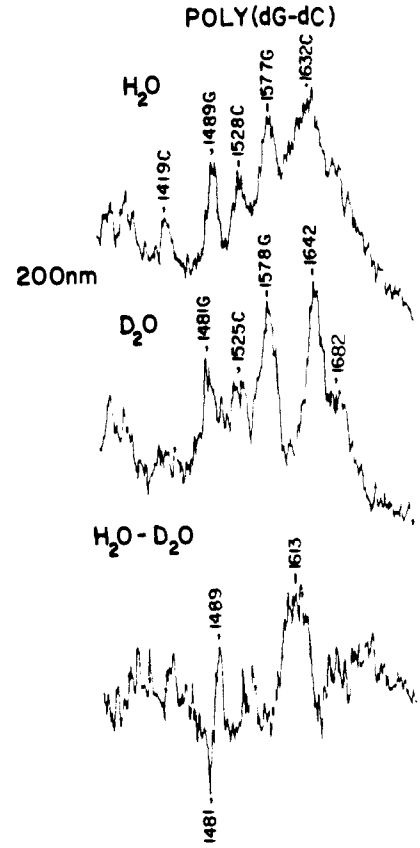

Figure 7. Same as Figure 7 but with 200-nm excitation.

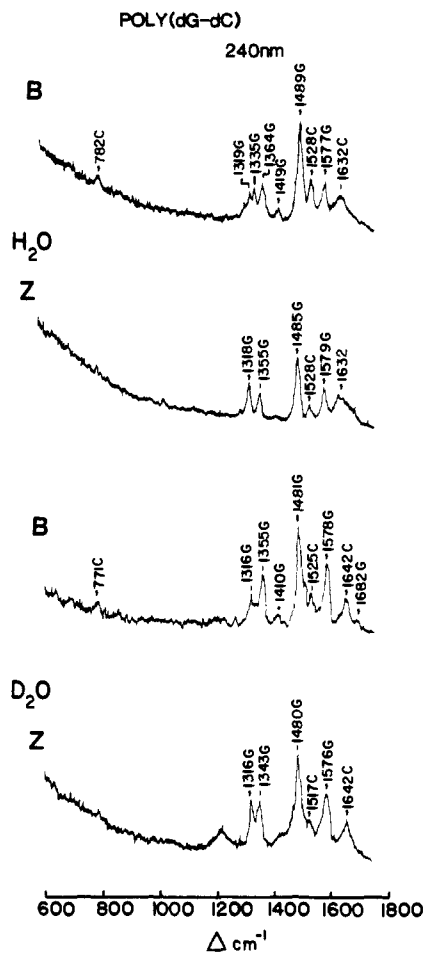

Figure 8. Raman spectra, 1.4 mM with 240-nm^{-1} excitation, for poly(dG–dC)·poly(dG–dC) (1.4 mm in base pairs) in low salt (B) and in 4 M NaCl (Z) and in H_2O and D_2O. Conditions as in Figures 3 and 4.

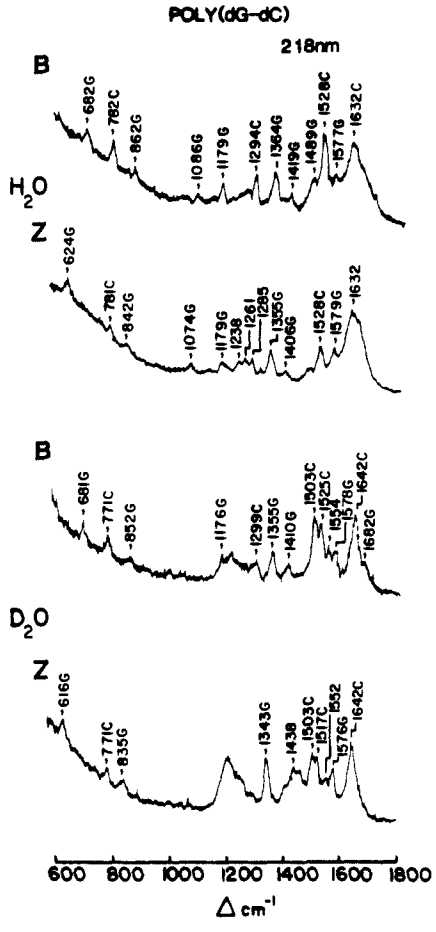

Figure 9. Same as Figure 8 but with 218-nm excitation.

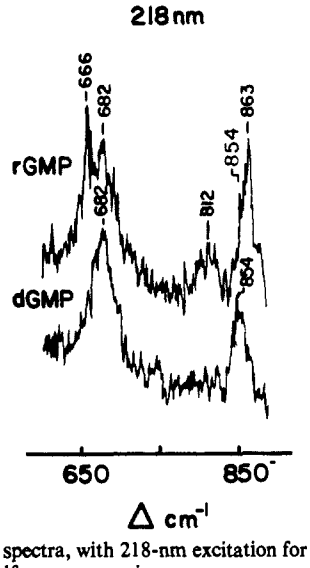

Figure 10. Raman spectra, with 218-nm excitation for dGMP and rGMP (5 mM) in the midfrequency region.

carbonyl, infrared frequency shifts were seen for the C=O stretching bands of both the G *and* the C residues. Using the isotope data, they were able to sort out the frequency shifts on duplex formation associated with electronic and vibrational coupling effects. The latter are dominant and force the C=O frequencies apart, the C frequency shifting down from 1656 to 1646 cm^{-1} and the G frequency shifting up from 1664 to 1682 cm^{-1} between poly C and GMP on the one hand and GMP·poly C on the other.

The C=O frequencies have been identified in the UVRR spectra[3] of dCMP and dGMP at 1652 and 1679 cm^{-1} in H_2O and 1649 and 1661 cm^{-1} in D_2O, the relatively large D_2O shift for

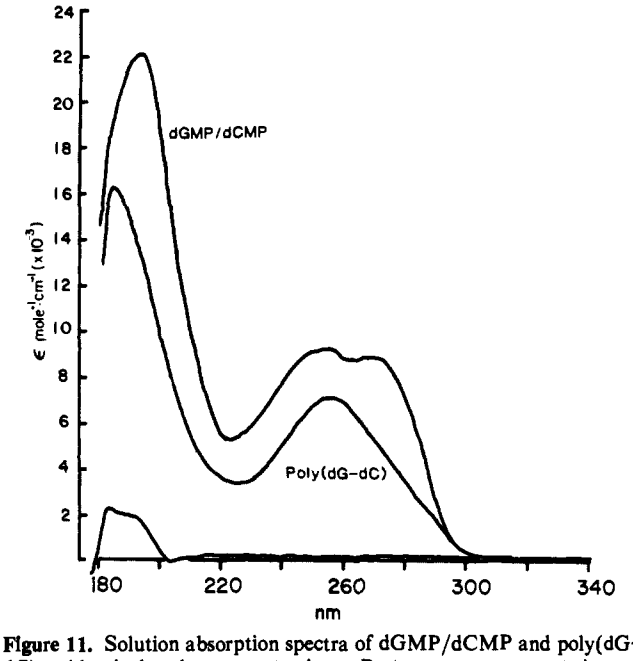

Figure 11. Solution absorption spectra of dGMP/dCMP and poly(dG-dC) at identical molar concentrations. Bottom trace represents instrument response function.

G being attributable to coupling with the NH bend of the adjacent imine group. The 240-nm excited RR spectrum of poly(dG-dC) in D_2O shown in Figure 6 has bands at 1645 and 1682 cm^{-1}, assignable to the C and G C=O stretches, based on the IR results for GMP·polyC and polyG·polyC. (All of the IR frequencies seem to be ~ 5 cm^{-1} higher than the Raman frequencies, as might be expected from the different selection rules.) We note that Benevides and Thomas[10] suggest assignments of these modes to bands in the visible excitation Raman spectrum of poly(dG-dC) in D_2O at 1635 and 1688 cm^{-1}, in fair agreement with the UVRR and IR values. The fact that the frequencies are quite similar for poly(dG-dC) and for polyG·polyC is consistent with vibrational coupling being more important than electronic effects in determining the duplex frequencies, since the stacking interactions are quite different in the two duplexes. The H_2O spectrum is less well resolved, but there are discernable peaks at 1632 and 1710 cm^{-1}, which we assign to C and G, respectively. These assignments, summarized in Table IV, suggest that the vibrational coupling is larger in H_2O than in D_2O, since the frequency shifts between the mononucleotides and the duplex are larger. (IR data are unavailable in H_2O because of interfering H_2O absorption.)

The larger H_2O effect may be due to the involvement of the δ_{NH_2} scissors modes in the vibrational coupling. Evidence for this involvement can be seen in the H_2O–D_2O difference UVRR spectra for poly(dG-dC), seen in Figures 6 and 7. In the mononucleotide spectra this mode is seen at 1603–1605 cm^{-1} for both G and C, identifiable by its disappearance in D_2O.[3] The band is strongest at 200 nm for C but at 240 and 266 nm for G. The 240-nm poly(dG-dC) spectrum (Figure 6) shows no band at 1605 cm^{-1}, but the 1632-cm^{-1} band, assigned to C=O stretching for C, is broad, whereas the corresponding 1642-cm^{-1} band in the D_2O spectrum is sharp. Subtraction of the spectra reveals a 1620-cm^{-1} band in H_2O, which we assign to the δ_{NH_2} mode of G. The 200-nm duplex spectrum (Figure 7) likewise has no 1605-cm^{-1} band, but now the 1632-cm^{-1} band is very broad. Subtraction of the D_2O spectrum reveals a band centered at 1613 cm^{-1}, which extends from 1620 to 1595 cm^{-1}. We attribute this broad feature to overlapping δ_{NH_2} bands of G, at 1620 cm^{-1}, and C, at ~1595 cm^{-1}. Thus there is evidence of the same up- and downshifting, relative to the mononucleotides, for δ_{NH_2} as for $\nu_{C=O}$: 1605 → 1620 cm^{-1} for G, and 1603 → ~1595 cm^{-1} for C. Since the C=O and NH_2 groups in question are connected by H-bonds, it is not surprising that their frequency shifts are concerted.

The situation is quite different for poly(dA-dT)·poly(dA-dT), whose base pairs have only one C=O···H_2N interaction (Figure

Table V. Association of Conformationally Sensitive G Frequencies (cm^{-1}) with Sugar Pucker and Guanine Orientation

C2′-endo, anti		
rGMP	682	854
dGMP	682	854
B poly(dG–dC)·poly(dG–dC)	682	860
C3′-endo, anti		
rGMP	666	863
C3′-endo, syn		
Z poly(dG–dC)·poly(dG–dC)	625	842

1). All of the duplex spectra show a clear 1605-cm^{-1} band, due to the δ_{NH_2} mode of A, unshifted from that of dAMP. On the other hand, two higher frequency bands are seen at 1649 and 1669 cm^{-1}, with 266- and 240-nm excitation (Figures 2 and 3), while the mononucleotides show only one, at 1655 cm^{-1}. The latter is known, from D_2O studies for dTMP,[3] to contain $\nu_{C=O}$ (1655 cm^{-1}) and $\nu_{C=C}$ (1648 cm^{-1}) contributions, and it therefore appears that duplex formation shifts the T $\nu_{C=O}$ frequency up by 14 cm^{-1}. The extent and direction of this shift are similar to that calculated by Howard et al.[5] for the association shift +9.4 cm^{-1} (i.e., electronic effect), as distinct from the coupling shift, of the G $\nu_{C=O}$ mode in GMP-polyC (although the association shift for the C $\nu_{C=O}$ mode was only −1.2 cm^{-1}). The effect can also be interpreted as a transition dipole coupling between C_4=O groups of T residues on the opposite strands, as discussed by Tsuboi and Nishimura.[24] In any event, much smaller effects of duplex formation on the exocyclic modes are seen for A·T than for G·C, again emphasizing the importance of vibrational coupling in the latter.

C. Z DNA: Conformationally Sensitive Bands. When poly(dG–dC) is exposed to high concentrations of salts, it converts from the normal B DNA confomation to the left-handed Z form.[9] The orientation of the guanine bases changes from anti to syn, and the conformation of the sugar attached to G changes from C2′-endo to C3′-endo. The stacking arrangement of the bases is also quite different in the two forms. It is well-known that a guanine Raman band at 682 cm^{-1} shifts down to 625 cm^{-1} when poly(dG–dC) is converted from the B to the Z form, presumably because this mode is coupled to vibrations of the deoxyribose ring and the coupling is sensitive to the gaunine–sugar conformation. Other Raman spectral differences between the B and Z forms have been noted by Benevides and Thomas,[10] using visible laser excitation.

Figures 8 and 9 compare RR spectra with 240- and 218-nm excitation of poly(dG–dC) in low salt (B form) and in 4 M NaCl, well above the critical concentration (2.4 M) for the B → Z transition.[8] The high concentration of chloride enhances the broad H_2O bending mode at ~1650 cm^{-1},[21] tending to obscure the nucleotide bands (exocyclic modes) in this region. The chloride effect appears subject to resonance enhancement (Cl^- has a strong absorption band at ~180 nm, due to charge transfer to solvent[22]), and spectra with 200-nm excitation (not shown) were badly obscured. In D_2O, the bending mode is shifted[3] to ~1210 cm^{-1}, and a broad band at this frequency can be seen in the D_2O spectra. Several nucleotide frequencies are shifted in D_2O, due to the exchange of exocyclic NH_2 (G and C) or ring NH (G) protons, and there are also some large intensity changes. Correlations with the H_2O spectra follow the analysis given for the mononucleotides in ref 3. The frequencies are listed in Table III.

There is a large downshift in the G C=O stretch in the Z form. This can be seen most clearly in the 218-nm D_2O spectra (Figure 9). The 1682-cm^{-1} band assigned to this mode disappears under the stronger 1642-cm^{-1} C=O stretch of C, which does not shift. The shifted band is seen at 1662 cm^{-1} in the visible excitation spectrum of Benevides and Thomas,[10] in which the neighboring C band is much weaker. The situation in H_2O is unclear, this region being obscured by the ~1650 cm^{-1} H_2O band both in the visible and UV (at high salt) excitation spectra. Since Watson–Crick base-pairing is present in the Z as well as the B form, the interbase vibrational coupling discussed in the previous section is expected to be unaltered, and it seems unlikely that the sugar–guanine conformation could significantly affect the exocyclic C=O stretch. Consequently, the downshift of this frequency probably reflects an electronic influence of the altered stacking arrangement of the bases. It is curious that no shift is seen for the C=O stretch of the cytosine residues. Conceivably this asymmetry is related to the fact that in the Z structure C is stacked with residues on both sides, but G is stacked (on C) only in the GpC direction; for CpG, the interaction of G is with the sugar of the adjacent C residue. We were unable to monitor the exocyclic NH_2 scissors modes because of the water band interference at high salt.

Most of the base ring modes do not show appreciable B–Z frequency differences. The 218-nm D_2O spectra do, however, reveal an 8-cm^{-1} downshift of the 1525-cm^{-1} band, assigned to a ring mode of C[3]. This is the first report of a cytidine band shift associated with Z DNA. Since the conformation of cytidine, in contrast to guanidine, does not change significantly between the B and Z forms, this shift may also reflect the altered stacking arrangements in Z. The 1526-1517-cm^{-1} band is prominent with 218-nm excitation and might prove useful as a B–Z marker for natural DNA, the nearby invariant 1503-cm^{-1} band serving as a reference. A–T pairs do not contribute significantly in this region.

A substantial shift is seen for the 1364 → 1355-cm^{-1} (1355 → 1343 cm^{-1} in D_2O) band of G, as has been reported by Benevides and Thomas. Another downshift is seen for the weak 1419 → 1410-cm^{-1} G band. We note that this is not the same as the 1418-cm^{-1} band seen with visible excitations,[8,10] which shifts *up* to 1428 cm^{-1} in Z and is assigned to a deoxyribose mode.

The well-known Z-sensitive 683 → 624-cm^{-1} band of G shows up distinctly in the 218-nm spectra. In D_2O the Z, but not the B, frequency is sensitive (624 → 616-cm^{-1}) to H/D exchange, as reported by Benevides and Thomas,[10] reflecting an alteration in the normal mode composition due to the change in guanidine conformation. The 218-nm spectra also reveal, for the first time, an additional conformationally sensitive band at 862 → 842 cm^{-1} (852 → 835 cm^{-1} D_2O). This band is associated with the 854-cm^{-1} (843 cm^{-1} in D_2O) band of dGMP.[3] Like the 682-cm^{-1} mode it is probably a ring mode coupled to the sugar conformation. The relationship between these modes is emphasized by the 218-nm spectrum of rGMP, which is compared with that of dGMP in Figure 10. It is known[14] that rGMP in solution contains an equilibrium mixture of C2′- and C3′-*endo* sugar conformers, in contrast to dGMP, which is more rigid and has predominantly the C2′-endo conformer. Consistent with this difference rGMP shows a doubling of both conformationally sensitive G bands: 682/666 cm^{-1} and 854/863 cm^{-1}. The origin of the 812-cm^{-1} rGMP band is uncertain. The association of these frequencies with sugar conformation and guanine orientation is given in Table V. All of the C2′-endo, anti conformations give similar frequencies although the 860-cm^{-1} band is slightly bigger in poly(dG–dC) than in dGMP.

Acknowledgment. This work was supported by NIH Grant GM25158.

Registry No. rGMP, 85-32-5; dAMP, 653-63-4; dTMP, 365-07-1; dGMP, 902-04-5; dCMP, 1032-65-1; poly(dA-dT), 26966-61-0; poly(dG-dC), 36786-90-0.

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