Ultraviolet Resonance Raman Spectra of Ribosyl C(1′)-Deuterated Purine Nucleosides: Evidence of Vibrational Coupling between Purine and Ribose Rings

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Abstract: Ultraviolet resonance Raman spectra of ribosyl C(1′)-deuterated guanosine and adenosine were measured. Most in-plane vibrations of the purine rings in the 1420–1100-cm^{-1} region showed frequency upshifts upon C(1′)-deuteration, while those in the 1100–700-cm^{-1} region showed downshifts. The purine ring vibrations above 1450 cm^{-1} were unaffected. The frequency shifts associated with the C(1′)-deuteration are explained by assuming couplings of the purine vibrations with a ribose C(1′)–H bending mode, which involves a hydrogen motion in the plane of N(9)–C(1′)–H. The purine vibrations that showed upshifts in the 1420–1100-cm^{-1} region are originally lowered in frequency by coupling with the ribosyl bending mode α(CH) around 1420 cm^{-1} and restore their intrinsic frequencies upon shifting of α(CH) to α(CD) around 1100 cm^{-1} in the C(1′)–D isotopomers. Some of the upshifted vibrations may be further pushed up by coupling with α(CD). On the other hand, the downshifted purine modes in the 1100–700-cm^{-1} region do not interact with α(CH) because of large frequency separations from α(CH) and retain their intrinsic frequencies in the C(1′)–H species. In the C(1′)–D species, however, α(CD) couples with these modes and pushes their frequencies downward. The observed C(1′)–D frequency shifts provide direct evidence of vibrational coupling between the base and ribose rings, suggesting that the purine base vibrations may be affected by the ribose ring puckering and glycosidic bond orientation. Actually, most of the purine vibrations that showed significant C(1′)–D shifts are known or proved to be conformational markers of purine nucleosides and nucleotides. The conformational sensitivity of purine vibrations really arises from vibrational coupling between the base and ribose rings.

Introduction

Vibrational spectroscopy is one of the powerful tools for investigating the structures of nucleic acids and nucleic acid–protein assemblies.[1-5] Hydrogen bonding,[6-8] base stacking,[8,9] and metal coordination[10-12] in nucleic acids can be revealed by studying the Raman and infrared spectra. Further, the frequencies of several Raman bands have been correlated with local conformations of nucleic acids, namely, the ribose ring puckering and glycosidic bond orientation. Such conformation marker bands were revealed by comparing the vibrational spectra of nucleoside and nucleotide crystals[13,14] or double-stranded polynucleotides[2,14-19] with known crystallographic structures. Interestingly, most of the conformation marker bands are in-plane vibrations of the base ring and their sensitivity to the ribose ring puckering and glycosidic bond orientation has been ascribed to vibrational coupling between base and ribose vibrations.[1-3] However, no direct experimental evidence has been reported for the base–ribose vibrational coupling. Since nucleic acid bases contain several proton-donor and -acceptor sites, base vibrational frequencies may also be affected by changes in hydrogen bonding associated with changes in conformation and intermolecular interaction.[7]

In order to confirm whether conformational sensitivity really arises from vibrational coupling between the base and ribose rings, we deuterated purine nucleosides at the ribosyl C(1′) position, which is directly connected to the base ring. If a significant vibrational coupling exists between the base and ribose rings, base vibrations would change in frequency upon C(1′)-deuteration as well as ribose vibrations. We employed ultraviolet resonance Raman (UVRR) spectroscopy to selectively enhance Raman scattering from the base in-plane vibrations[4,20-24] and to clearly distinguish them from unenhanced ribose vibrations. Many

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(1) Nishimura, Y.; Tsuboi, M. In *Spectroscopy of Biological Systems*; Clark, R. T., Hester, R. E., Eds.; John Wiley & Sons: New York, 1986; pp 177–232.

(2) Thomas, G. J., Jr. In *Spectroscopy of Biological Systems*; Clark, R. T., Hester, R. E., Eds.; John Wiley & Sons: New York, 1986; pp 233–309.

(3) Peticolas, W. L.; Kubasek, W. L.; Thomas, G. A.; Tsuboi, M. In *Biological Applications of Raman Spectroscopy*; Spiro, T. G., Ed.; John Wiley & Sons: New York, 1987; pp 81–133.

(4) Turpin, P.-Y.; Chinsky, L.; Laigle, A.; Jollès, B. *J. Mol. Struct.* **1989**, *214*, 43–70.

(5) Taillandier, E.; Liquier, J.; Ghomi, M. *J. Mol. Struct.* **1989**, *214*, 185–211.

(6) Kyogoku, Y.; Lord, R. C.; Rich, A. *J. Am. Chem. Soc.* **1967**, *89*, 496–504.

(7) Toyama, A.; Takeuchi, H.; Harada, I. *J. Mol. Struct.* **1991**, *242*, 87–98.

(8) Audet, P.; Simard, C.; Savoie, R. *Biopolymers* **1991**, *31*, 243–251.

(9) Small, E. W.; Peticolas, W. L. *Biopolymers* **1971**, *10*, 69–88.

(10) Lanir, A.; Yu, N.-T. *J. Biol. Chem.* **1979**, *254*, 5882–5887.

(11) Uchida, K.; Toyama, A.; Tamura, Y.; Sugimura, M.; Mitsumori, F.; Furukawa, Y.; Takeuchi, H.; Harada, I. *Inorg. Chem.* **1989**, *28*, 2067–2073.

(12) Perno, J. R.; Cwikel, D.; Spiro, T. G. *Inorg. Chem.* **1987**, *26*, 400–405.

(13) Nishimura, Y.; Tsuboi, M.; Sato, T.; Aoki, K. *J. Mol. Struct.* **1986**, *146*, 123–153.

(14) Katahira, M.; Nishimura, Y.; Tsuboi, M.; Sato, T.; Mitsui, Y.; Iitaka, Y. *Biochim. Biophys. Acta* **1986**, *867*, 256–267.

(15) Pohl, P. M.; Ranade, A.; Stockburger, M. *Biochim. Biophys. Acta* **1974**, *335*, 85–92.

(16) Thamann, T. J.; Lord, R. C.; Wang, A. H. J.; Rich, A. *Nucleic Acids Res.* **1981**, *9*, 5443–5457.

(17) Benevides, J. M.; Thomas, G. J., Jr. *Nucleic Acids Res.* **1983**, *11*, 5747–5761.

(18) Thomas, G. J., Jr.; Benevides, J. M. *Biopolymers* **1985**, *24*, 1101–1105.

(19) Nishimura, Y.; Torigoe, C.; Tsuboi, M. *Nucleic Acids Res.* **1986**, *14*, 2737–2748.

(20) Ziegler, L. D.; Hudson, B.; Strommen, D. P.; Peticolas, W. L. *Biopolymers* **1984**, *23*, 2067–2081.

(21) Kubasek, W. L.; Hudson, B.; Peticolas, W. L. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 2369–2373.

(22) Fodor, S. P. A.; Rava, R. P.; Hays, T. R.; Spiro, T. G. *J. Am. Chem. Soc.* **1985**, *107*, 1520–1529.

(23) Fodor, S. P. A.; Spiro, T. G. *J. Am. Chem. Soc.* **1986**, *108*, 3198–3205.

0002-7863/93/1515-11092$04.00/0

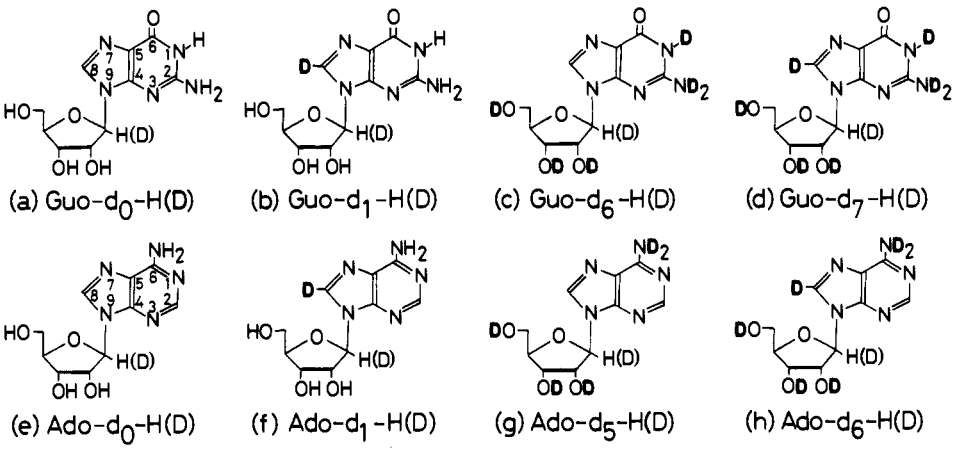

Figure 1. Schematic structures of ^{2}H-labeled nucleosides and their abbreviations: (a) guanosine (Guo-d_0), (b) (8-^{2}H)guanosine (Guo-d_1), (c) (1,N^2,N^2,$O^{2'}$,$O^{3'}$,$O^{5'}$-^{2}H$_6$)guanosine (Guo-d_6), (d) (1,N^2,N^2,8,$O^{2'}$,$O^{3'}$,$O^{5'}$-^{2}H$_7$)guanosine (Guo-d_7), (e) adenosine (Ado-d_0), (f) (8-^{2}H)adenosine (Ado-d_1), (g) (N^6,N^6,$O^{2'}$,$O^{3'}$,$O^{5'}$-^{2}H$_5$)adenosine (Ado-d_5), and (h) (N^6,N^6,8,$O^{2'}$,$O^{3'}$,$O^{5'}$-^{2}H$_6$)adenosine (Ado-d_6).

UVRR bands due to base in-plane modes in the 1420–700-cm^{-1} region have shown significant frequency shifts upon C(1')-deuteration, giving direct evidence of the base–ribose vibrational coupling. We will describe the mechanism of C(1')–D frequency shifts and the relation between the C(1')–D shift and conformational sensitivity.

Experimental Section

Materials. Adenosine (Ado) and guanosine (Guo) were purchased from Kojin Co. and were recrystallized from water. Poly(rA-rU) and poly(dA-dT) were purchased from Pharmacia and Sigma, respectively, and used without further purification. (1'-^{2}H)Adenosine (Ado-d_0-D) was synthesized from (1-^{2}H)ribose and adenine according to the literature.[25-27] (1-^{2}H)Ribose was prepared by reduction of ribonolactone (Sigma) with sodium amalgam in D_2O. The synthesis of (1'-^{2}H)guanosine (Guo-d_0-D) was performed enzymatically from (1'-^{2}H)2,6-diaminopurine-9-riboside,[28] which was prepared from (1-^{2}H)ribose and 2,6-diaminopurine as described.[25-27] NMR spectra of the (1'-^{2}H)nucleosides showed almost complete (>99%) deuteration at the C(1') position. Deuterations at the purine rings and ribose hydroxyl groups were carried out by use of hydrogen–deuterium exchange reactions.[29,30] The deuterated positions on the base and ribose rings for each isotopomer are shown in Figure 1. The nucleosides will be denoted by Ado-d_n-H(D) or Guo-d_n-H(D), where n stands for the total number of deuterium atoms on the base ring and in the ribose hydroxy groups, and H or D signifies the kind of atom, hydrogen or deuterium, attached to C(1').

UVRR Spectra. The fourth harmonic (266 nm) of a pulsed Nd:YAG laser (30 Hz, Quanta-Ray DCR-3G) was used to produce resonance Raman scattering from nucleosides and nucleotides. The fifth harmonic (213 nm) was also employed to excite the sample at a shorter wavelength. The laser power was 50 μJ/pulse at the sample position. UVRR spectra were recorded on a Jasco CT-80D double monochromator equipped with a Princeton Instruments D/SIDA-700 diode array detector (266-nm excitation) or on a fore-prism Raman spectrometer equipped with a Princeton Instruments LN/CCD-1152 charge-coupled device detector (213 nm).[31] The spectral slit width was 8 cm^{-1}.

Nucleosides were dissolved in H_2O or D_2O at a concentration of 1 mM, and the pH was adjusted to 6.2 with NaOH or HCl. Polynucleotides were dissolved in 10 mM Tris-HCl buffer (pH 7.6) containing 50 mM NaCl and 1 mM EDTA. The concentration was so adjusted as to be 2.0 in absorbance at 260 nm with a 1-mm light pass. H_2O solutions of nucleosides were circulated by use of a wire-guided flow apparatus. A spinning quartz cell was employed for D_2O solutions of nucleosides and H_2O solutions of polynucleotides. Scattering from the cell wall overlapped Raman bands in the 1100–1020-cm^{-1} region and below 950 cm^{-1} when the spinning cell was used.

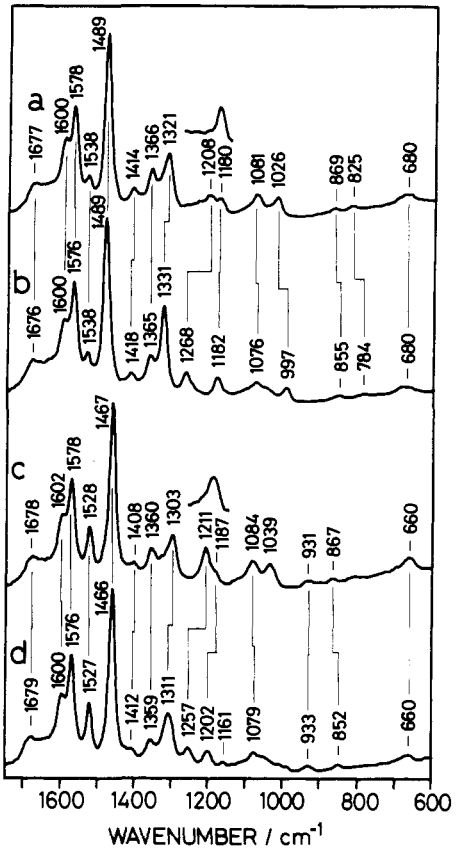

Figure 2. UVRR spectra of labeled guanosines in H_2O solution: (a) Guo-d_0-H, (b) Guo-d_0-D, (c) Guo-d_1-H, and (d) Guo-d_1-D. The excitation wavelength was 266 nm. The insets in (a) and (c) show the 213-nm-excited spectra in the 1280–1150-cm^{-1} region.

Results

Guanosine. Figure 2 shows 266-nm-excited Raman spectra of Guo-d_0-H (part a) and Guo-d_1-H (c) and their C(1')–D isotopomers (b and d) in H_2O solution. Figure 3 shows the spectra of these compounds in D_2O solution, where all the labile hydrogen atoms in the NH, NH_2, and OH groups are exchanged with deuterium atoms. The excitation wavelength is in resonance with the guanine ring in-plane absorption around 253 nm, and all the UVRR bands in the figures arise from in-plane vibrations of the

(24) Nishimura, Y.; Tsuboi, M.; Kubasek, W. L.; Bajdor, K.; Peticolas, W. L. *J. Raman Spectrosc.* **1987**, *18*, 221–227.
(25) Itoh, T.; Mizuno, Y. *Heterocycles* **1976**, *5*, 285–292.
(26) Saneyoshi, M.; Satoh, E. *Chem. Pharm. Bull.* **1979**, *27*, 2518–2521.
(27) Kissman, H. M.; Pidacks, C.; Baker, B. R. *J. Am. Chem. Soc.* **1955**, *77*, 18–24.
(28) Howard, F. B.; Miles, H. T. *J. Biol. Chem.* **1965**, *240*, 801–805.
(29) (a) Livramento, J.; Thomas, G. J., Jr. *J. Am. Chem. Soc.* **1974**, *96*, 6529–6531. (b) Thomas, G. J., Jr.; Livramento, J. *Biochemistry* **1975**, *14*, 5210–5218.
(30) Lane, M. J.; Thomas, G. J., Jr. *Biochemistry* **1979**, *18*, 3839–3846.
(31) (a) Takeuchi, H.; Harada, I. *J. Raman Spectrosc.* **1990**, *21*, 509–515. (b) Hashimoto, S.; Ikeda, T.; Takeuchi, H.; Harada, I. *Appl. Spectrosc.* **1993**, *47*, 1283–1285.

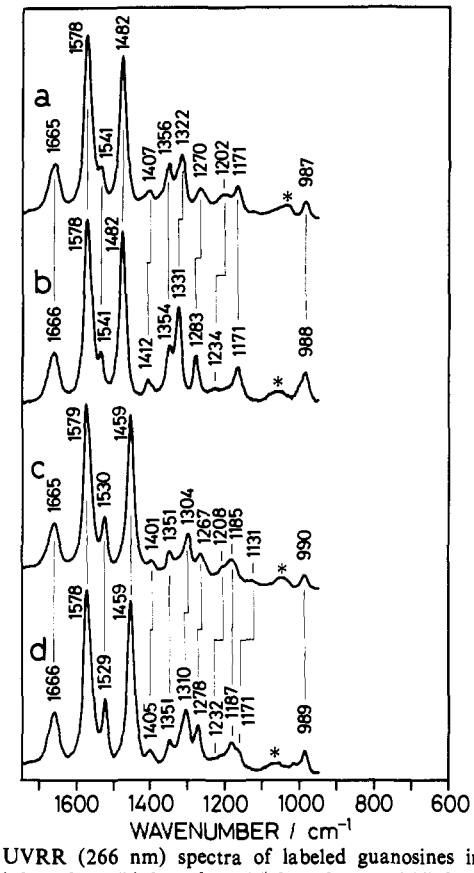

Figure 3. UVRR (266 nm) spectra of labeled guanosines in D_2O solution: (a) Guo-d_6-H, (b) Guo-d_6-D, (c) Guo-d_7-H, and (d) Guo-d_7-D. Broad bands marked with asterisks are due to scattering from the quartz cell wall.

Table I. Frequencies (cm^{-1}) of Guanosine In-Plane Vibrations and Their Isotopic Shifts

frequency			isotope shift				
Guo-d_0	B-DNA[a]	Z-DNA[a]	C(1′)–D	C(8)–D	7,9-^{15}N[b]	*N*,*O*-D[c]	1,2,3-^{15}N[b]
1677			−1	+1		−12	
1600	1620	1620	0	+2		−330/−429[d]	
1578	1577	1579	−2	0	0	0	−7
1538			0	−10	−7	+3	
1489	1489	1485	0	−22	−8	−7	−2
1414	1419	1406	+4	−6	−7	−7	−2
1366	1364	1355	−1	−6	−11	−10	−3
1321	1335/1319	1318	+10	−18	−15	+1	−3
1208			+60	+3		−6	
1180	1179	1179	+2	+7	−4	−9	−13
1081	1086	1074	−5	+3			
1026	1033		−29	+13			
869	862	842	−14	−2	0		0
825			−41	−2[e]			
680	682	624	0	−20	0		0

[a] Poly(dG-dC) duplexes (ref 23). [b] Reference 32. [c] (1,N^2,N^2,O^2',O^3',O^5')-deuteration. [d] Two possible assignments for the ND_2 scissors. [e] Observed with 213-nm excitation.

9-substituted guanine ring. Most bands in the UVRR spectra of C(1′)–H species have already been observed with visible excitation, though some ribose vibrational bands overlap the 1100–800-cm^{-1} region in the visible Raman spectra. By comparing the spectra of Guo-d_0-H and Guo-d_0-D, we can see the effects of C(1′)-deuteration. Significant frequency change occurs for several bands in the 1420–700-cm^{-1} region, whereas the bands outside that region remain practically unchanged. Table I summarizes the C(1′)–D frequency shifts together with other isotopic shifts including ^{15}N-substitution effects[32] observed in visible Raman spectra.

There are five bands above 1420 cm^{-1} in each spectrum of Figure 2, and their vibrational origins are rather clear. The 1677-cm^{-1} band of Guo-d_0-H has been assigned to the C(6)=O stretch because the corresponding infrared band in D_2O solution showed a large C(6)=^{18}O shift.[28] The 1600-cm^{-1} band disappears upon dissolution in D_2O (see Figure 3) and has been assigned to the NH_2 scissors.[22] The 1578-cm^{-1} band is ascribed to a pyrimidine ring vibration on the basis of a large 1,2,3-^{15}N shift but no C(8)–D and 7,9-^{15}N shifts (Table I). The effects of C(8)–D and 7,9-^{15}N substitutions are large for both of the remaining bands at 1538 and 1489 cm^{-1}, suggesting significant contributions from imidazole modes. The 1538-cm^{-1} mode seems to involve only a part of the imidazole ring adjacent to C(6) because its counterpart in Guo-d_7-H showed a large C=^{18}O shift.[11] The 1489-cm^{-1} band is sensitive to protonation and metal coordination at N(7)[11,12] and is ascribed to a vibration of the N(7) moiety. As described above, the five vibrational modes above 1420 cm^{-1} do not involve large atomic displacements around N(9), to which the C(1′) atom is attached, and are insensitive to C(1′)-deuteration.

Many peaks in the 1420–700-cm^{-1} region change in frequency upon C(1′)-deuteration, and most of the peak shifts are easily traced as shown with vertical lines in Figures 2 and 3. Among the three bands in the 1420–1300-cm^{-1} region, the lowest-frequency (1330–1300 cm^{-1}) band, at 1321 cm^{-1} for Guo-d_0-H, shows marked C(1′)–D upshift (6–10 cm^{-1}). This band shows large C(8)–D and 7,9-^{15}N shifts as well (Table I). Further, the frequency of the band is sensitive to substitution at N(9) (1266 cm^{-1} for guanine[33] and 1306 cm^{-1} for 9-ethylguanine[24]). Thus, the 1321-cm^{-1} band is assigned to an imidazole ring mode with a significant contribution from the C(8)–N(9) stretch. The C(8)–N(9) stretch in guanosine may be coupled with a C(1′)–H motion through the N(9)–C(1′) linkage leading to a large C(1′)–D shift. The 1414-cm^{-1} band of Guo-d_0-H shows C(1′)–D, C(8)–D, and ^{15}N shifts similar to those of the 1321-cm^{-1} band, though the shifts are smaller for the 1414-cm^{-1} band. This band is also assigned to an imidazole ring mode coupled with a C(1′)-H motion. On the other hand, the 1366-cm^{-1} band in Guo-d_0-H is insensitive to C(1′)-deuteration. This band was once assigned to an imidazole ring mode on the basis of a large (11 cm^{-1}) shift upon 7,9-^{15}N substitution.[32] Unlike the 1321-cm^{-1} band, however, its frequency is not much affected by substitution at N(9) (1361 cm^{-1} for guanine[33] and 1369 cm^{-1} for 9-ethylguanine[24]), suggesting a small N(9) motion. The large 7,9-^{15}N shift of the 1366-cm^{-1} band is attributable mainly to an N(7) motion. Fodor et al. have assigned this band to a stretching mode of the C(2)=N(3)—C(4)=C(5)—N(7)=C(8) triene linkage on the basis of the excitation profile of UVRR intensity.[22] The spectral features in the 1420–1300-cm^{-1} region in D_2O solution (Figure 3) are very similar to those in H_2O solution, and the C(1′)–D frequency shifts are also analogous in both solutions.

The effect of C(1′)-deuteration is most significant in the 1300–1100-cm^{-1} region. As Figure 2 shows, there are two weak and closely lying bands at 1208 and 1180 cm^{-1} for Guo-d_0-H, two separate bands at 1268 and 1182 cm^{-1} for Guo-d_0-D, one band at 1211 cm^{-1} with a shoulder at 1187 cm^{-1} for Guo-d_1-H, and two bands at 1257 and 1202 cm^{-1} for Guo-d_1-D. Of the paired bands of Guo-d_0-H, the low-frequency (1180 cm^{-1}) one becomes much stronger than the other with 213-nm excitation (see the inset in Figure 2). Likewise, only the 1187-cm^{-1} band appears in the 213-nm spectrum of Guo-d_1-H. The same intensity behavior with 213-nm excitation indicates that the low-frequency components arise from nearly the same vibrational mode. The high-frequency component at 1208 cm^{-1} in Guo-d_0-H (Figure 2a) shifts to 1268 cm^{-1} upon C(1′)-deuteration (Figure 2b). This large (60 cm^{-1}) upshift indicates a significant contribution from a C(1′)–H motion. Most probably, the 1208-cm^{-1} band arises from the N(9)–C(1′) stretch coupled with a C(1′)–H motion. The corresponding band in the C(8)–D species (Guo-d_1-H) appears at 1211 cm^{-1}

(32) Delabar, J.-M.; Guschlbauer, W. *Biopolymers* **1979**, *18*, 2073–2089.

(33) Delabar, J.-M.; Majoube, M. *Spectrochim. Acta, Part A* **1978**, *34A*, 129–140.

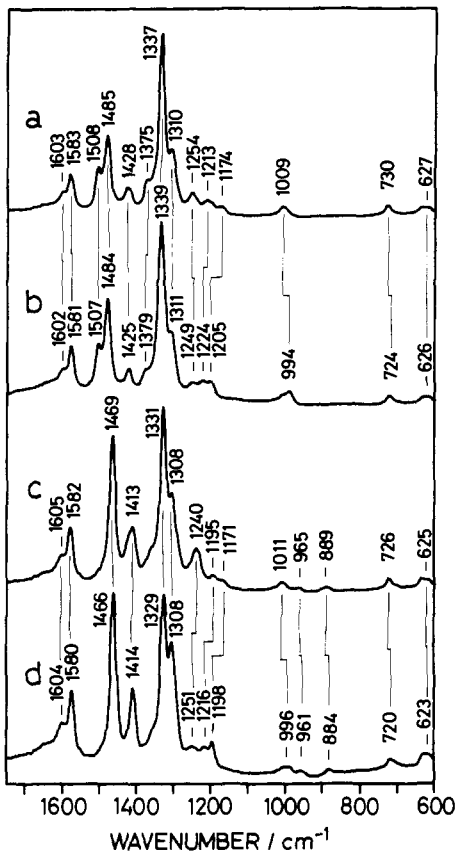

Figure 4. UVRR (266 nm) spectra of labeled adenosines in H_2O solution: (a) Ado-d_0-H, (b) Ado-d_0-D, (c) Ado-d_1-H, and (d) Ado-d_1-D.

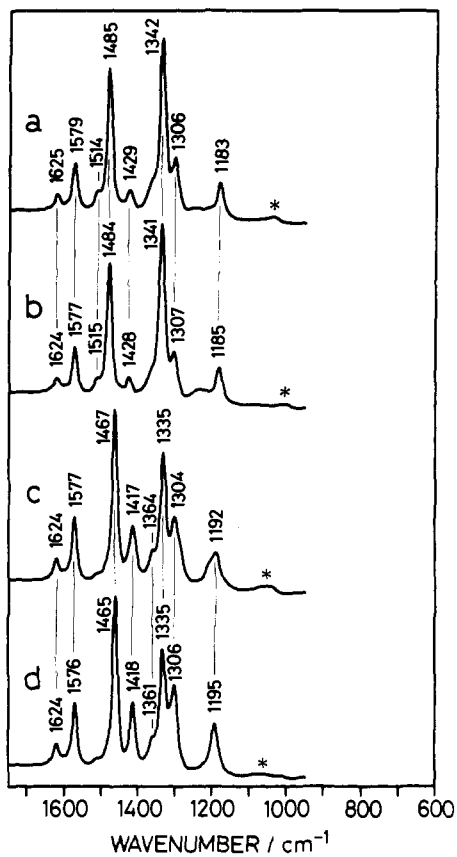

Figure 5. UVRR (266 nm) spectra of labeled adenosines in D_2O solution: (a) Ado-d_5-H, (b) Ado-d_5-D, (c) Ado-d_6-H, and (d) Ado-d_6-D. Broad bands marked with asterisks are due to scattering from the quartz cell wall.

(partly overlapping the 1187-cm^{-1} band in Figure 2c) and shows a large upshift to 1257 cm^{-1} on C(1')-deuteration (Figure 2d).

In D_2O solution (Figure 3), the 1300–1100-cm^{-1} region becomes complicated due to possible overlap of the ND_2 scissors band. Actually, there are three distinct bands in Figure 3a (in contrast to two peaks in Figure 2a). Unlike the observation in H_2O solution, all the three bands are equally enhanced with 218-nm excitation,[22] indicating that the modes of vibration in D_2O solution are different from those in H_2O solution. Upon C(1')-deuteration, the 1202-cm^{-1} band of Guo-d_6-H (Figure 3a) shifts to 1234 cm^{-1} (Figure 3b). Although this band was considered to arise from the ND_2 scissors,[22] the large C(1')–D shift suggests another vibrational mode, possibly a mode involving the N(9)–C(1') stretch and C(1')–H motion as proposed for the 1208-cm^{-1} band of Guo-d_0-H. The corresponding band in Guo-d_7-H is located at 1208 cm^{-1} (Figure 3c), but the location of the C(1')–D shifted band is not clear in the spectrum of Guo-d_7-D (Figure 3d). The 1171-cm^{-1} shoulder peak of Guo-d_7-D may result from an upshift of a weak band of Guo-d_7-H at 1131 cm^{-1}.

Four bands appear in the 1100–700-cm^{-1} region of Guo-d_0-H (Figure 2a). A relatively strong band at 1026 cm^{-1} shows a large C(1')–D shift to 997 cm^{-1} in Guo-d_0-D and is likely to arise from an imidazole ring deformation coupled with a ribose mode. Similar assignment also applies to the 825-cm^{-1} band, which shifts down to 784 cm^{-1} in Guo-d_0-D. The 931-cm^{-1} band in the spectrum of Guo-d_1-H (Figure 2c) is assigned to the C(8)–D in-plane deformation and is not affected by C(1')-deuteration (Figure 2d). A broad band at 680 cm^{-1} (660 cm^{-1} for C(8)–D isotopomers) is ascribed to the guanine ring-breathing vibration,[34,35] and its frequency does not change upon C(1')–deuteration. In the 1100–950-cm^{-1} region of the D_2O solution spectra (Figure 3), only one band is seen around 990 cm^{-1}, which is insensitive to C(1')-deuteration.

Adenosine. Figure 4 shows the 266-nm Raman spectra of Ado-d_0-H (part a) and Ado-d_1-H (c) and their C(1')–D isotopomers, Ado-d_0-D (b) and Ado-d_1-D (d), in H_2O solution. The Raman spectra of their D_2O solutions (Ado-d_5-H, -d_5-D, -d_6-H, and -d_6-D) are shown in Figure 5. Figure 6 shows the 213-nm Raman spectra of these isotopomers in the 1400–1100-cm^{-1} region. The deuteration shifts of the Ado-d_0-H vibrations are summarized in Table II together with the 8-^{13}C and 1,3-^{15}N shifts for adenine.[36] As was the case for guanosine, significant (>3 cm^{-1}) C(1')–D shifts are not observed above 1420 cm^{-1}, probably due to the same reason that the vibrations in this frequency region do not involve atomic motions around the N(9)–C(1') linkage.

In the 1420–1100-cm^{-1} region, C(1')–D shifts are significant and most of them are upshifts. The 1375-cm^{-1} shoulder of Ado-d_0-H shows a 4-cm^{-1} upshift to 1379 cm^{-1} in Ado-d_0-D. In the 1350–1280-cm^{-1} region, the effect of C(1')-deuteration on the 266-nm Raman bands is very small (Figures 4 and 5). This is consistent with the observation that two bands of adenine corresponding to the 1337- and 1310-cm^{-1} bands of adenosine (Figure 4a) show very large 1,3-^{15}N shifts (Table II)[36] suggestive of dominant contributions of pyrimidine modes. With 213-nm excitation, however, the spectral pattern in the 1350–1280-cm^{-1} region changes drastically upon C(1')-deuteration (Figure 6). The 1309-cm^{-1} band of Ado-d_0-H (Figure 6a) upshifts to 1326 cm^{-1} in Ado-d_0-D (Figure 6b). The upshifted band is not the counterpart of the unshifted 1310-cm^{-1} band in the 266-nm spectrum, indicating the existence of a new vibration that is unseen with 266-nm excitation. Similarly, a new band is found at 1292 cm^{-1} in the 213-nm spectrum of Ado-d_1-H (Figure 6c), which upshifts to 1316 cm^{-1} in Ado-d_1-D. The 1307-cm^{-1} band of Ado-d_5-H and the 1291-cm^{-1} band of Ado-d_6-H are also new bands, which upshift to 1338 and 1310 cm^{-1}, respectively, upon C(1')-

(34) Nishimura, Y.; Tsuboi, M.; Sato, T. *Nucleic Acids Res.* **1984**, *17*, 6901–6908.

(35) Ghomi, M.; Letellier, R.; Taillandier, E. *Biochimie* **1988**, *70*, 841–846.

(36) Hirakawa, A. Y.; Okada, H.; Sasagawa, S.; Tsuboi, M. *Spectrochim. Acta, Part A* **1985**, *41A*, 209–216.

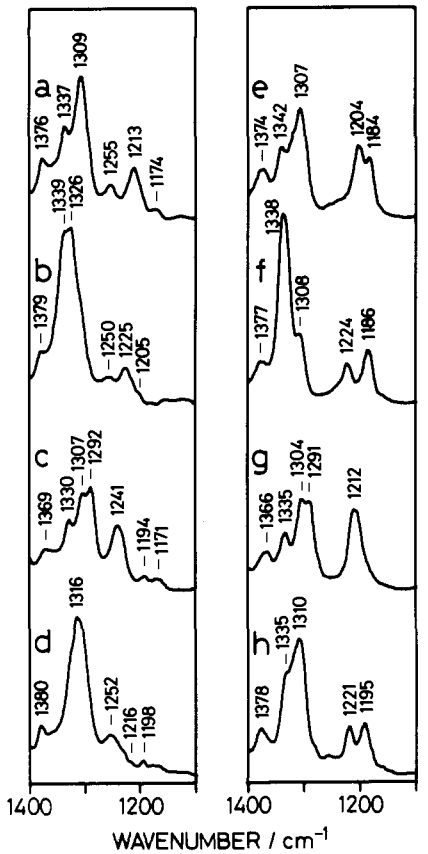

Figure 6. UVRR (213 nm) spectra of labeled adenosines in the 1400–1100-cm^{-1} region: (a) Ado-d_0-H, (b) Ado-d_0-D, (c) Ado-d_1-H, (d) Ado-d_1-D, (e) Ado-d_5-H, (f) Ado-d_5-D, (g) Ado-d_6-H, and (h) Ado-d_6-D.

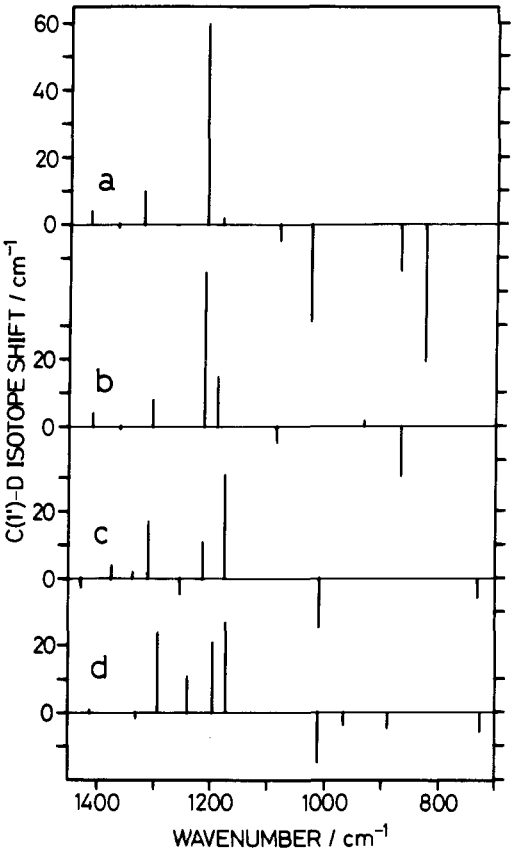

Figure 7. Schematic drawing of the C(1′)–D frequency shifts observed for UVRR bands of guanosine and adenosine and their C(8)–D isotopomers: (a) Guo-d_0-H, (b) Guo-d_1-H, (c) Ado-d_0-H, and (d) Ado-d_1-H. Bars extending upward and downward signify upshifts and downshifts, respectively.

Table II. Frequencies (cm^{-1}) of Adenosine In-Plane Vibrations and Their Isotopic Shifts

frequency			isotope shift				
Ado-d_0	A-RNA[a]	B-DNA[b]	C(1′)–D	C(8)–D	8-^{13}C[c]	N,O-D[d]	1,3-^{15}N[c]
1603	1603	1605	−1	+2		−318	
1583	1579	1579	−2	−1	−2,−3.6[e]	−4	−7.7,−6.0[e]
1508	1503	1504	−1	−13[f]	−3.2	+6	−5.4
1485	1481	1481	−1	−16	−7.8	0	−6.7
1428	1422	1422	−3	−15	−9.6	+1	−1.6
1375	1376[f]	1376[f]	+4	−7[f]	−1.5	−2[f]	−3.6
1337	1336	1337	+2	−6	−3.3	+5	−16.6
1310	1308	1307	+1	−2	−2.4	−4	−11.3
1309[f]	1308[f]	1342[f]	+17[f]	−17[f]		−2[f]	
1254	1257[f]	1254[f]	−5	−14	−4.5		−5.7
1213	1219[f]	1226[f]	+11	−18			
1174	1176[f]	1169[f]	+31	−3	−15.0		−2.6
1009	1006[f]	1013[f]	−15	+2			
730	726[f]	728[f]	−6	−4	−3.2		−7.4
627			−1	−2	−0.9		−7.8

[a] A-form poly(rA-rU)·poly(rA-rU). [b] B-form poly(dA-dT)·poly(dA-dT). [c] Observed for crystalline adenine. From ref 36. [d] (N^6,N^6,O^2',O^3',O^5')-deuteration. [e] Crystal field splitting. [f] Observed with 213-nm excitation.

deuteration. These new bands are particularly enhanced with 213-nm excitation but are buried under stronger bands in the spectra excited at 266 nm.

The 266-nm Raman spectrum of Ado-d_0-H shows three bands in the 1280–1100-cm^{-1} region, all of which change in frequency upon C(1′)-deuteration. With 213-nm excitation (Figure 6), only one of the three bands is selectively enhanced. The 1213-cm^{-1} band of Ado-d_0-H shifts to 1225 cm^{-1}, and the 1241-cm^{-1} band of Ado-d_1-H shifts to 1252 cm^{-1}. For the remaining two bands, it may be reasonable to assume a correspondence in the order of frequency. Thus, the 1255- and 1174-cm^{-1} bands of Ado-d_0-H shift to 1250 and 1205 cm^{-1}, respectively, upon C(1′)-deuteration (Figure 6a,b). Similarly, the 1194- and 1171-cm^{-1} bands shift to 1216 and 1196 cm^{-1} (Figure 6c,d). Among the three bands in each spectrum of Ado-d_0-H and Ado-d_1-H, the one with the largest C(1′)–D shift (at 1174 and 1171 cm^{-1}, respectively) is assigned to a coupled mode of the N(9)–C(1′) stretch and a C(1′)–H motion by analogy with the 1208-cm^{-1} band of guanosine. In D_2O solutions, 1204-cm^{-1} band of Ado-d_5-H and the 1212-cm^{-1} band of Ado-d_6-H show large C(1′)–D upshifts (Figure 6e–h). These bands may correspond to the 1174- and 1171-cm^{-1} bands in H_2O solution. A band around the 1185-cm^{-1} region has been assigned to the ND_2 scissors,[22] and it does not show a significant C(1′)–D shift.

Below 1100 cm^{-1} (Figure 4), an Ado-d_0-H band at 1009 cm^{-1} (1011 cm^{-1} for Ado-d_1-H) shows a large downshift (−15 cm^{-1}) upon C(1′)-deuteration. The 730-cm^{-1} band of Ado-d_0-H, which is one of the strongest bands in the visible Raman spectra and has been assigned to the ring-breathing mode,[35] shows a 6-cm^{-1} downshift, in contrast to no C(1′)–D shift of the guanine ring-breathing mode at 680 cm^{-1}.

Discussion

Mechanism of C(1′)–D Shifts of Purine Vibrations. The effects of C(1′)-deuteration on the guanosine and adenosine vibrational frequencies are depicted schematically in Figure 7. Most bands in the 1420–1100-cm^{-1} region shift upward and those in the 1100–700-cm^{-1} region shift downward for both nucleosides, though the shifts are smaller for adenosine. If the isotopic substitution simply increases the effective mass of vibration, the vibrational frequency would decrease. The observation of frequency upshifts in the 1420–1100-cm^{-1} region cannot, therefore, be explained by the simple mass effect and requires more complex mechanisms of vibrational coupling. Significant coupling of two vibrations occurs if the directions of vibrational displacement and the locations of vibrating atomic groups as well as the frequencies of vibrations are close to each other. The frequencies of the two vibrations are pushed apart by the coupling, the magnitude of the frequency

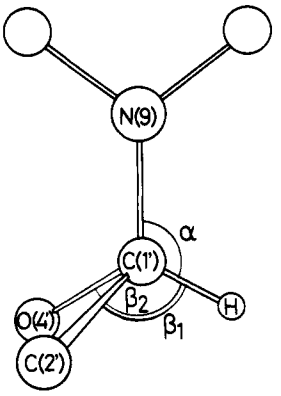

Figure 8. Definitions of two C(1′)–H bending modes: $\alpha(CH) = a\Delta\alpha - b(\Delta\beta_1 + \Delta\beta_2)$ and $\beta(CH) = (\Delta\beta_1 - \Delta\beta_2)/2^{1/2}$. For the tetrahedral geometry, the coefficients, a and b, in the definition of $\alpha(CH)$ are $2/5^{1/2}$ and $1/5^{1/2}$, respectively.

change being dependent on both the strength of interaction and the separation of their intrinsic frequencies. In any case, the low-frequency component becomes lower and the high-frequency one becomes higher.

As described above, several purine vibrations show large frequency upshifts upon C(1′)-deuteration, indicating a strong vibrational coupling between the purine base and ribose. Any purine vibration that showed a significant C(1′)–D frequency shift must be close to a ribose vibration in direction, location, and frequency. Further, such a ribose vibration must involve an appreciable C(1′)–H motion. If these conditions are fulfilled, the C(1′)-deuteration decreases the frequency of the ribose vibration and concomitantly the purine vibrational frequency will upshift or downshift as a result of changes in vibrational coupling. The largest C(1′)–D shift of purine vibrations is 60 cm^{-1}, implying that the ribose vibration that couples with purine vibrations should undergo a much larger C(1′)–D shift. Most likely candidates for such a ribose vibration are the two C(1′)–H bending vibrations, α(CH) and β(CH), defined in Figure 8.

In α(CH), the hydrogen atom moves in the plane of N(9)–C(1′)–H and the atomic displacements of α(CH) and the N(9)–C(1′) stretch are in the same plane, while the hydrogen motion in β(CH) is perpendicular to the plane and to the atomic displacements of the N(9)–C(1′) stretch. Thus the N(9)–C(1′) stretch can couple with α(CH) but not with β(CH). The strength of vibrational coupling between the C(1′)–H bends and purine ring modes depends on the glycosidic bond orientation defined by the C(4)–N(9)–C(1′)–O(4′) torsional angle (χ). Usually the χ angle is around 55° (syn) or −125° (anti).[37] In either orientation, the C(1′)–H hydrogen atom lies close to the purine ring plane and α(CH) can mix with purine ring in-plane vibrations. In order for β(CH) to mix with purine in-plane vibrations, the N(9)–C(1′)–H plane should be nearly perpendicular to the purine ring plane with a χ angle around −35° or 145°. This perpendicular orientation is, however, rarely found for nucleosides and their derivatives.[37] Hence, only α(CH) is geometrically able to couple with purine ring in-plane vibrations as well as with the N(9)–C(1′) stretch.

The α(CH) mode is a bending vibration of an isolated C–H bond, and its frequency is expected to be around 1400 cm^{-1}. For instance, the corresponding C–H bending mode in cyclopentanol, a five-membered-ring compound like ribose, has a frequency of 1382 cm^{-1} and downshifts to 1027 cm^{-1} upon deuteration of the C–H group [vibrations corresponding to β(CH) and β(CD) were found at 1074 and 812 cm^{-1}, respectively].[38] We have attempted to find the α(CH) and α(CD) bands of adenosine and guanosine in the UVRR, visible Raman, and infrared spectra. However, these bending bands were too weak to be identified with confidence. Although the exact frequencies of α(CH) and α(CD) are unknown, they may not be much different from those in cyclopentanol.

It is interesting to note that the highest frequency of the upshifted bands is close to the expected frequency of α(CH) and the boundary of upshift and downshift regions is close to the frequency expected for α(CD). This coincidence may not be accidental but suggests that the observed C(1′)–D shifts are caused by vibrational coupling with α(CH) and α(CD), which are located around 1420 and 1100 cm^{-1}, respectively. By assuming such vibrational coupling, the observed C(1′)–D shifts can be explained as follows. The N(9)–C(1′) stretching mode of Guo-d_0-H showed the largest (+60 cm^{-1}) C(1′)–D shift from 1208 to 1268 cm^{-1}. Geometrically, the N(9)–C(1′) stretch can couple with α(CH) as described above. The only remaining factor that will affect the coupling scheme is their intrinsic frequencies. Since the N(9)–C(1′) stretch is lower in frequency than α(CH), the coupling between them pushes the N(9)–C(1′) stretching frequency downward. On the other hand, coupling with α(CD) in the C(1′)–D species pushes the N(9)–C(1′) frequency upward because the order of frequency is reversed in the C(1′)–D species. The frequency lowering in the C(1′)–H species and the raising in the C(1′)–D species result in an upshift of 60 cm^{-1} upon C(1′)-deuteration. In this case, both the couplings with α(CH) and α(CD) may contribute comparably to the C(1′)–D shift. On the other hand, the contribution from the coupling with α(CH) may be dominant for higher-frequency vibrations such as the 1414- and 1321-cm^{-1} modes of Guo-d_0-H because they are close to α(CH) in frequency but far away from α(CD). These vibrations are pushed down by coupling with α(CH) in the C(1′)–H species but restore their intrinsic frequencies in the C(1′)–D species. Conversely, coupling with α(CH) is negligible for the modes below 1100 cm^{-1} and their C(1′)–D downshifts are ascribed solely to the frequency lowering by coupling with α(CD). The modes above 1420 cm^{-1} lack such coupling because they are vibrations far away from the N(9)–C(1′) moiety as described in the Results. Similar explanations apply to the C(1′)–D shifts in the other isotopomers of guanosine as well as in adenosine.

Relation between C(1′)–D Shift and Conformational Sensitivity. The purine vibrations that shift upon C(1′)-deuteration are coupled with α(CH) and/or α(CD) as described above. It is probable that such purine vibrations are also coupled with other ribose vibrations. Changes in ribose ring puckering and glycosidic bond orientation will affect the modes and frequencies of ribose vibrations including α(CH), resulting in changes in vibrational coupling with the purine vibrations. Thus, the purine vibrations sensitive to C(1′)-deuteration are expected to serve as conformation markers of purine nucleosides and nucleotides.

1. Guanosine. Seven vibrations of guanosine showed C(1′)–D shifts larger than 3 cm^{-1} (Table I). Among them, three bands at 1414, 1081, and 869 cm^{-1} are known to be conformation-sensitive. The guanine–ribose conformation in double-stranded poly(dG-dC) changes from C2′-endo/anti to C3′-endo/syn on going from the right-handed B form to the left-handed Z form. Concomitantly, UVRR bands of the guanine residue at 1419, 1086, and 862 cm^{-1} shift to 1406, 1074, and 842 cm^{-1}, respectively[23] (see Table I). In addition, a single band at 1321 cm^{-1} of guanosine, which showed a +10-cm^{-1} C(1′)–D shift, becomes a doublet at 1335 and 1319 cm^{-1} in B-form poly(dG–dC) and reverts to a singlet at 1318 cm^{-1} in the Z form. Therefore, this vibration is also sensitive to the conformation. The 1208-cm^{-1} band showed the largest (+60 cm^{-1}) C(1′)–D shift. The frequency of this band varies with conformation in crystalline guanine nucleosides and nucleotides.[13] Guanosine-2′-monophosphate bound to ribonuclease T_1[39] or F_1[40] takes the C3′-endo/syn conformation and gives a characteristic band at 1218 cm^{-1}. These

(37) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1983.
(38) Durig, J. R.; Karriker, J. M.; Harris, W. C. *Spectrochim. Acta, Part A* **1971**, *27A*, 1955–1971.
(39) Takeuchi, H.; Satoh, Y.; Harada, I. *J. Mol. Struct.* **1991**, *242*, 49–59.
(40) Takeuchi, H.; Harada, I.; Yoshida, H. *Biochim. Biophys. Acta* **1991**, *1078*, 307–312.

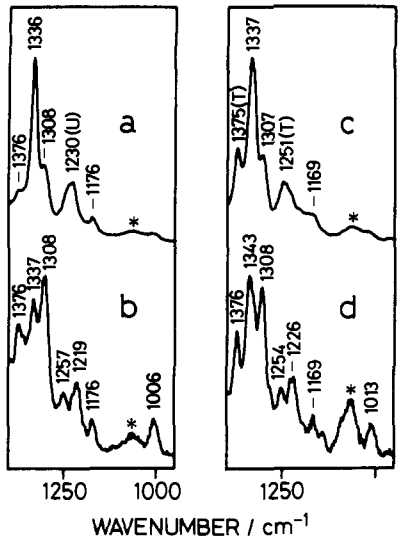

Figure 9. UVRR spectra of double-stranded polynucleotides: (a) and (b) poly(rA-rU)·poly(rA-rU), (c) and (d) poly(dA-dT)·poly(dA-dT). The excitation wavelength was 266 nm (a and c) or 213 nm (b and d).

observations indicate that the 1208-cm^{-1} band also bears conformational sensitivity. In double-stranded poly- and oligonucleotides, however, stronger Raman bands due to the cytosine residue overlap the guanosine 1208-cm^{-1} band and the guanosine conformation cannot be deduced from this vibration. The remaining two bands at 1026 and 825 cm^{-1} are weak and are sometimes overlapped by other bands in nucleotides. Although the conformational sensitivities of these bands are not known, they may potentially be conformation markers as are the other guanosine vibrations discussed above.

The guanine ring-breathing mode at 680 cm^{-1} is well characterized as a marker of the ribose ring puckering and the glycosidic bond orientation.[1-3,34,35] The frequency change associated with the conformational change is very large as shown in Table I (682 cm^{-1} for C2′-endo/anti and 624 cm^{-1} for C3′-endo/syn). However, this vibration did not show any C(1′)–D shift. The large frequency separation from α(CH) and α(CD) may be responsible for the lack of C(1′)–D shift. It is highly probable that the ring-breathing vibration is strongly coupled with a ring deformation vibration of ribose around 650 cm^{-1}, which makes the breathing mode conformation-sensitive.[41] The 1361-cm^{-1} band of guanosine is also regarded as a conformation marker, though its frequency is insensitive to C(1′)-deuteration. This mode does not involve a significant N(9) motion, as described in the Results, and its direct coupling with ribose vibrations is unlikely to happen. Possibly, this mode couples with the 1321-cm^{-1} one, which is a conformation marker involving a large N(9) motion, and gains conformational sensitivity through coupling with the 1321-cm^{-1} mode.

2. Adenosine. As revealed by the 213-nm spectra, adenosine has three vibrations in the 1340–1300-cm^{-1} region. Among the three, only the 1309-cm^{-1} mode showed a large C(1′)–D shift (+17 cm^{-1}) and the remaining two modes at 1337 and 1310 cm^{-1} were unaffected. This observation suggests that the 1309-cm^{-1} mode is conformation-sensitive while the others are not. Figure 9 compares UVRR spectra of A-form poly(rA-rU)·poly(rA-rU)[42] and B-form poly(dA-dT)·poly(dA-dT).[14] The adenine residue takes the C3′-endo/anti conformation in the A form and C2′-endo/anti in theB form.[1-3,37] With 266-nm excitation (Figure 9a,c), the A-RNA gives two Raman bands at 1336 and 1308 cm^{-1}, which are practically identical to those of the B-DNA. This is because the expected conformation-sensitive mode at 1309 cm^{-1} is not resonance enhanced by 266-nm excitation and only the two modes insensitive to the conformation appear in the spectra. On the other hand, the 213-nm spectra of A-RNA and B-DNA largely differ from each other (Figure 9b,d). A band at 1308 cm^{-1}, which corresponds to the 1309-cm^{-1} band of adenosine, is dominant in the 213-nm spectrum of A-RNA. The intensity maximum shifts to 1343 cm^{-1} for the B-DNA, where no peak was observed with 266-nm excitation. This large spectral change is accounted for by a shift of the conformation-sensitive vibration from 1308 (C3′-endo/anti) to 1343 cm^{-1} (C2′-endo/anti). In visible Raman spectra, a band around 1335–1340 cm^{-1} has been proposed to be a conformation marker of adenine residues in polynucleotides.[1,2,14] The band shifted from ~1335 (C3′-endo/anti) to ~1340 cm^{-1} (C2′-endo/anti). This observation can be explained as follows. The actual conformation marker band at 1308 cm^{-1} in the C3′-endo/anti conformation is weak with visible excitation and is overlapped by a conformation-insensitive band at 1310 cm^{-1}. Thus, a stronger band around 1335 cm^{-1}, which is also conformation-insensitive, was regarded as a marker of that conformation, In the C2′-endo/anti conformation, on the other hand, the conformation-sensitive band at 1343 cm^{-1} becomes stronger and overlaps the insensitive band around 1335 cm^{-1}, Accordingly, the 1340-cm^{-1} band looked as if it shifted from ~1335 cm^{-1} in the visible Raman spectrum.

Three bands of adenosine at 1209, 1177, and 1009 cm^{-1} showed large C(1′)–D shifts, suggesting a possibility of their being conformation-sensitive. Actually, these bands are observed at 1219, 1176, and 1006 cm^{-1} in A-form poly(rA-rU)·poly(rA-rU) (Figure 9b) and shift to 1226, 1169, and 1013 cm^{-1} in B-form poly(dA-dT)·poly(dA-dT) (Figure 9d). The adenine ring-breathing mode at 730 cm^{-1} also showed a C(1′)–D shift of –6 cm^{-1}. This shift is attributable to a frequency lowering by coupling with α(CD) and not to coupling with α(CH). If the ring-breathing mode does not couple with any other C(1′)-H ribose vibrations either, it will not be sensitive to the conformation. Actually, the ring-breathing mode of adenosine is not regarded as a conformation marker, indicating a lack of such coupling.

Conclusions

Deuteration at the ribose C(1′) position has provided experimental evidence of vibrational coupling between the purine and ribose rings. Generally, purine in-plane vibrations in the 1420–1100-cm^{-1} region couple with a C(1′)–H bend, α(CH), around 1420 cm^{-1}, leading to frequency upshifts upon C(1′)-deuteration. On the other hand, α(CD) in the C(1′)–D species pushes purine vibrational frequencies downward in the 1100–700-cm^{-1} region and, possibly, upward in the 1300–1200-cm^{-1} region through vibrational coupling. The observed C(1′)–D shifts are well explained by the coupling scheme described above. Such vibrational coupling is absent for the purine vibrations above 1420 cm^{-1} for the geometrical reason that their vibrational modes do not involve atomic motions in the N(9)–C(1′) moiety. Most of the purine UVRR bands that showed significant C(1′)–D shifts are conformation markers that have been established already or proved to be so in this work. It is concluded that the conformational sensitivity of purine in-plane vibrations really arises from vibrational coupling with ribose.

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(41) Benevides, J. M.; Lemeur, D.; Thomas, G. J., Jr. *Biopolymers* **1984**, *23*, 1011–1024.
(42) Morikawa, K.; Tsuboi, M.; Takahashi, S.; Kyogoku, Y.; Mitsui, Y.; Iitaka, Y.; Thomas, G. J., Jr. *Biopolymers* **1973**, *23*, 1011–1024.