A UV Resonance Raman Study of $d(A^+-G)_{10}$, a Single-Stranded Helix without Stacked or Paired Bases[†,‡]

Ishita Mukerji,[§] Mary Claire Shiber,[∥] Thomas G. Spiro,*[,§] and Jacques R. Fresco*[,∥]

Departments of Chemistry and Molecular Biology, Princeton University, Princeton, New Jersey 08544-1014

Received December 19, 1994; Revised Manuscript Received July 3, 1995[⊗]

ABSTRACT: UV resonance Raman spectroscopy has been utilized to directly observe structural features of the recently described nucleic acid single-stranded helix $d(A^+-G)_{10}$. An absence of base stacking is confirmed by invariant hypochromic ratios of dominant vibrational modes for the oligomer relative to its constituent monomers as the structure is thermally denatured. The N_1 of dA residues is protonated, as determined by similarity to the ring-stretching vibrations for protonated adenine and its derivatives. Selective resonance enhancement of Raman vibrational modes from dA and dG residues shows frequency shifts upon thermal denaturation that confirm the participation of the exocyclic amino of dA but not dG residues in H-bonding. Conformationally sensitive glycosyl bond modes suggest *anti* residue conformations.

An atypical nucleic acid secondary structure has recently been described for the oligonucleotide $d(A\text{-}G)_{10}$ in mildly acidic solution (Dolinnaya & Fresco, 1992; Dolinnaya et al., 1993; Shiber et al., 1995). A left-handed helical sense of twist encompasses a single strand with no stacked or paired bases. Instead, the helix is generated by ionic strength sensitive Coulombic interactions and H-bonds along its cylindrical surface involving nine of the ten alternating dA residues protonated on N_1 and their exocyclic amino hydrogens, respectively, with the P=O/P−O$^-$ centers of the backbone phosphates two residues upstream, while the intervening dG residues protrude into the solvent.

Here, we describe observations made on this helical structure by UV resonance Raman (UVRR) spectroscopy, a method which allows direct observation of some of the previously reported structural features that were deduced on the basis of indirect observations. Thus, while UV absorption spectroscopy indicated that the dA residues, and not the dG residues, are the sites of protonation (Dolinnaya & Fresco, 1992) and electrometric titration quantitated the number of such protonated residues (Dolinnaya et al., 1993), the assignment of N_1 as the site of protonation was made only by analogy with the protonation of adenine in the adenine hydrochloride crystal (Broomhead, 1948; Cochran, 1951) and in the poly(A^+•A^+) helix (Fresco, 1959; Rich et al., 1961). Moreover, UV perturbation spectroscopy showed the dG residues to be extrahelical (Shiber et al., 1995), and an energy-minimized molecular model pointed to the exocyclic amino hydrogens of the dA residues as the donors in the H-bonds to the phosphodiester backbone (Shiber et al., 1995); however, direct spectroscopic evidence for those weak but important interactions was lacking.

EXPERIMENTAL PROCEDURES

Nucleoside, Nucleotides, and d(A-G)$_{10}$. 2′-Deoxyadenosine 5′-monophosphate free acid and 2′-deoxyguanosine 5′-monophosphate (Peninsula Laboratories) and 8-bromoadenosine (Sigma) were used without further purification. The oligomer was prepared and purified as described (Shiber et al., 1995). After irradiation, oligomer integrity was checked by 5′-end-labeling and 16% denaturing PAGE; there was no evidence of degradation.

UVRR Spectroscopy. The spectrometer and light sources were as described (Rodgers et al., 1992). Samples in a stirred quartz cuvette were maintained at specified temperatures with an aluminum block cell holder and circulating bath, with sample temperature monitored by a copper−constantan thermocouple in the block. Spectra were calibrated against neat solutions of ethanol and *n*-pentane, for which the Raman band frequencies are well-known. The accuracy of the calibrated spectra is approximately ±1 cm^{-1}.

RESULTS AND DISCUSSION

Unstacked dA and dG Residues. Selective resonance enhancement of Raman vibrational modes arising from the dA and dG residues occurs predominantly at 250 and 240 nm, respectively, although both residues contribute to the Raman intensity at both wavelengths (Fodor et al., 1985; Perno et al., 1989)). Hence, by comparing spectra generated at different temperatures with 250 and 240 nm excitation, it is possible to assign thermally induced spectral shifts to changes in either dA or dG residues.

At pH 7.0 and high ionic strength the secondary structure of $d(A\text{-}G)_{10}$ involves helically stacked base tetrads (I. Mukerji, M. C. Shiber, J. R. Fresco, and T. G. Spiro, unpublished observations). Raman hypochromism, as measured by the intensity ratio of the oligomer relative to that for the constituent monomers, decreases some 30% for the helix at pH 7.0 when the temperature is raised from 4 to 40

[†] This work was supported by grants from the National Institutes of Health to T.G.S. (GM25158) and to J.R.F. (GM42936). I.M. was supported in part by a National Research Service Award (GM14324) from the National Institutes of Health.

[‡] This paper is no. 23 in the series entitled *Polynucleotides*, of which the last is Shiber et al. (1995).

* To whom correspondence should be addressed.

[§] Department of Chemistry.

[∥] Department of Molecular Biology.

[⊗] Abstract published in *Advance ACS Abstracts,* September 1, 1995.

0006-2960/95/0434-14300$09.00/0

Table 1: Hypochromic Ratios[a] Determined with 250 nm Excitation

	$d(A-G)_{10}$ at pH 7.0			$d(A^+-G)_{10}$ at pH 4.0		
temp (°C)	1337 cm^{-1}	1484 cm^{-1}	1576 cm^{-1}	1335 cm^{-1}	1485 cm^{-1}	1576 cm^{-1}
4	0.30	0.22	0.24	0.44	0.32	0.27
40	0.43	0.35	0.37	0.45	0.33	0.26

[a] Intensity of the indicated band relative to the corresponding band of the constituent monomers at the same degree of protonation.

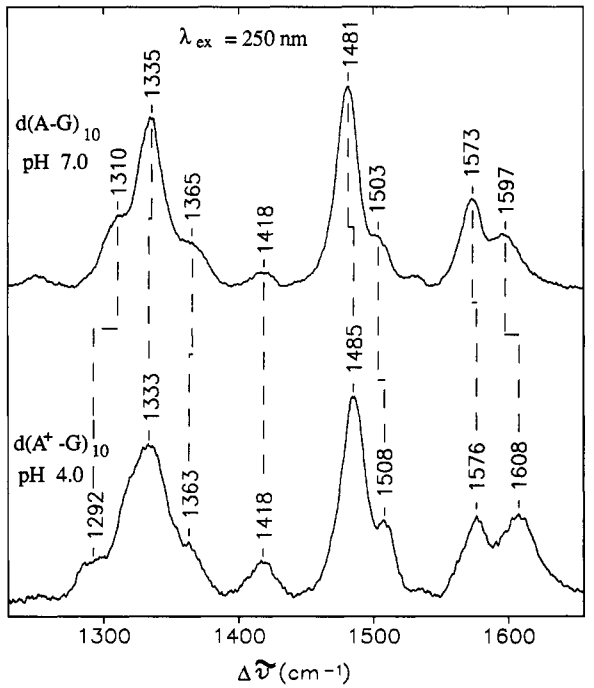

FIGURE 1: UV resonance Raman spectra obtained with 250 nm excitation of the multistranded structure of $d(A-G)_{10}$ in 0.3 M Na_2SO_4/0.01 M cacodylate, pH 7.0 (total $[Na^+] \cong 0.61$ M) and that of the single-stranded helix of $d(A^+-G)_{10}$ in 0.0019 M Na^+/0.013 M acetate/0.2 M cacodylate, pH 4.02 (total $[Na^+] = 0.0019$ M). Both samples were 2 mM in residues. Spectra are normalized to maximum peak height at 1481 and 1485 cm^{-1}.

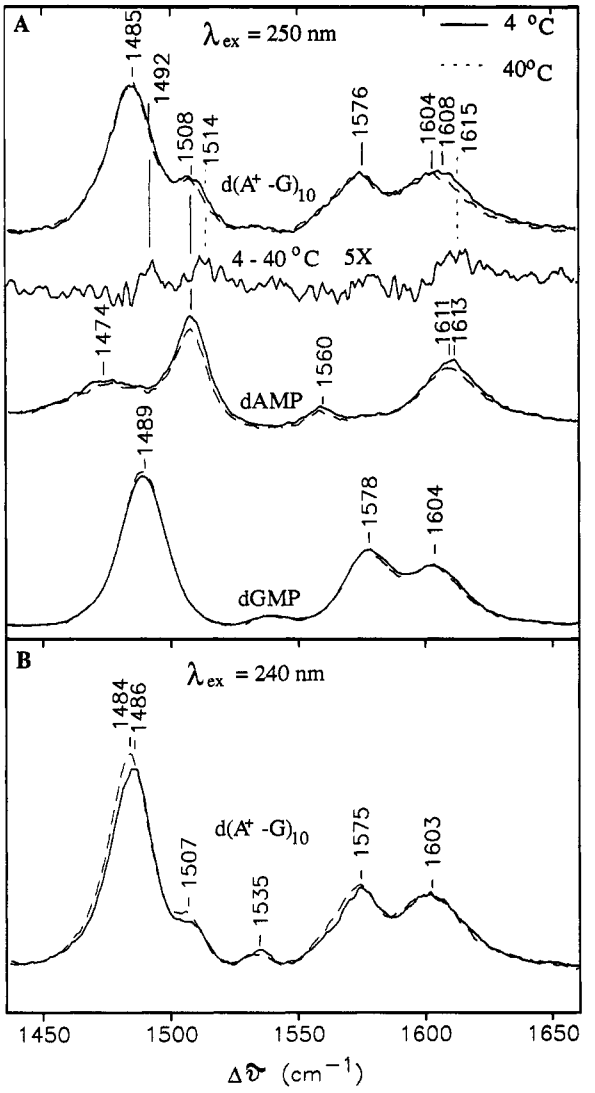

FIGURE 2: UV resonance Raman spectra of (A) $d(A^+-G)_{10}$, dAMP, and dGMP obtained with 250 nm excitation and (B) $d(A^+-G)_{10}$ obtained with 240 nm excitation. In panel A all spectra are plotted on the same scale. dGMP and $d(A^+-G)_{10}$ are multiplied by y-scaling factors of 0.5 and 1.5, respectively. $d(A^+-G)_{10}$ (2 mM residues) was in 0.0019 M Na^+/0.013 M acetate/0.2 M cacodylate, pH 4.02 (total $[Na^+] = 0.0019$ M). The 4−40 °C difference spectrum for $d(A^+-G)_{10}$ was generated using a subtraction factor of 1 and multiplied by a y-scaling factor of 5. dGMP (1 mM) was measured in the same buffer as the oligomer, while dAMP (1 mM) was in 0.001 M Na^+/0.013 M phosphate/0.2 M cacodylate, pH 3.02, to give the same degree of protonation as in the helix at pH 4.0. Spectra are shown for both 4 °C (—) and 40 °C (- - -).

°C, where the structure itself is 30% denatured (Table 1). This loss of hypochromicity can therefore be attributed to a thermally induced partial loss of base stacking. In contrast, for $d(A^+-G)_{10}$ at pH 4.0, the hypochromic ratio at 1485 cm^{-1} is 0.32 at 4 °C, where the helix is intact, and essentially the same at 40 °C, where it is almost completely denatured, as measured by a loss of CD intensity (Dolinnaya & Fresco, 1992; Dolinnaya et al., 1993) (Table 1). The invariance of the hypochromic ratios of dominant vibrational modes for this structure is in keeping with the absence of base stacking deduced earlier from UV absorbance observations (Dolinnaya & Fresco, 1992). In this connection, the low hypochromic ratios for both the pH 7.0 and 4.0 structures probably result at least in part from shifts in the UV absorption maxima of the helices relative to those of the monomers.

Protonated and H-Bonded dA Residues. The frequencies of C=C and C=N ring stretching vibrations (above 1450 cm^{-1}) are 3−4 cm^{-1} higher for the single-stranded helix, $d(A^+-G)_{10}$, than for the multistranded helix, $d(A-G)_{10}$. Also, the UVRR intensities are higher for the 1508 and 1608 cm^{-1} bands relative to the 1485 cm^{-1} band at pH 4.0 than at pH 7.0 (Figure 1). IR and Raman studies of adenine and N9-substituted adenine derivatives have revealed similar frequency and intensity changes when the pH is lowered from neutrality to <4.0; these changes were attributed to protonation of adenine N_1 (Lord & Thomas, 1967; Toyama et al., 1991). Thus, the observed frequency differences and the altered intensity pattern indicate that the dA residues of the single-stranded helix are protonated at N_1.

The UVRR bands between 1400 and 1700 cm^{-1} were monitored with 250 and 240 nm excitation for changes due to a loss of H-bonding with increasing temperature. As shown in Figure 2A, the 1608 cm^{-1} band shifts to 1604 cm^{-1} between 4 and 40 °C. This band arises from the NH_2 scissors motion in dG and/or dA residues (Fodor et al., 1985). Normal mode analyses reveal the NH_2 scissors contribution to this vibrational mode to be at least 73%, with only minor contributions from other C=C and C—N stretching motions (Fodor et al., 1985). A similar shift is not observed in the

240 nm spectrum (Figure 2B), suggesting that, because of the selective enhancement with 250 nm excitation, the shift observed arises from the dA and not the dG residues. As a control for spectral changes due only to temperature effects, spectra were measured for dAMP at pH 3.0 (Figure 2A), where this monomer is 95% protonated, as are the dA residues in $d(A^+\text{-}G)_{10}$. The NH_2 scissors mode in dAMP shifts from 1613 cm^{-1} at 4 °C to 1611 cm^{-1} at 40 °C, only half the shift observed for the oligomer. The 4 cm^{-1} shift seen for the oligomer is an underestimate of the dA residue shift because the lower part of the composite band can be attributed to the dG residues, whose NH_2 scissors band is at 1604 cm^{-1} and is temperature-insensitive, as seen in the spectra of dGMP (Figure 2A). This is confirmed in the 4−40 °C difference spectrum of the oligomer, which exhibits a peak at 1615 cm^{-1} (Figure 2A). The frequency of the difference peak at 1615 cm^{-1} confirms the assignment to dA residues, since the monomer spectra show the amino mode of dAMP occurs at 1612 ± 1 cm^{-1}, while that of dGMP is observed at 1604 cm^{-1}. A similar difference peak is not observed with 240 nm excitation (data not shown), further suggesting that dA residues are primarily responsible for the frequency shift. The selective downshift in the NH_2 scissors frequency of the dA residues with increasing temperature is strong evidence for their diminished H-bonding.

We note that a difference of similar magnitude has been observed between poly(A·U) (1608 cm^{-1}) and AMP (1604 cm^{-1}), and for poly[d(A-U·U-A)], the NH_2 scissors mode occurs at 1606 cm^{-1} (Grygon & Spiro, 1990). In both those duplexes there are H-bonds between the exocyclic amino and carbonyl substituents of the A and U residues, respectively. Although the changes in the NH_2 scissors mode were not previously attributed to H-bond formation, the improved signal-to-noise ratio of the current observations permits a more reliable determination of frequency shifts of such small magnitude and, consequently, a more confident assignment. The observed frequency increase is consistent with stronger H-bonding as shown by previous UVRR studies of 9-ethyladenine in which participation in a donor H-bond shifted the NH_2 mode to higher frequency. Additionally, earlier IR and resonance Raman studies of crystalline adenine derivatives determined that the intrinsic NH_2 scissors frequency is increased by strong H-bonding (Toyama et al., 1991).

Augmented H-bonding by the exocyclic NH_2 of dA residues can also account for the downshift in the 1310 cm^{-1} band at pH 7.0 to 1292 cm^{-1} at pH 4.0 (Figure 1). This band has been shown to be sensitive to solvent H-bonding in UVRR spectra of 9-ethyladenine (Toyama et al., 1991).

anti dA and dG Residues. Purine base vibrations are sensitive to glycosyl bond orientation and sugar pucker as a consequence of vibrational coupling between the base and ribose rings (Toyama et al., 1993). In Z-DNA the sugar of dG residues occurs in the $C_{3'}$-*endo* conformation and the base is oriented *syn*, while in B-DNA the sugar pucker is $C_{2'}$-*endo* and the base is oriented *anti*. UVRR studies of poly[d(G-C)] have revealed two conformationally sensitive Raman dG bands with frequencies at 682 and 862 cm^{-1} for the B form or at 625 and 842 cm^{-1} for the Z form (Fodor & Spiro, 1986). At 4 °C, these Raman bands for the dG residues of $d(A^+\text{-}G)_{10}$ are observed at 681 and 864 cm^{-1} (Figure 3), indicating an *anti* base orientation and a $C_{2'}$-*endo* sugar conformation. The absence of a Raman band at 625 cm^{-1} also argues against the *syn* orientation for the dG residues in the single-stranded helix. The mode at 681 cm^{-1} broadens considerably with increasing temperature (data not shown), consistent with a mixture of conformations upon thermal denaturation.

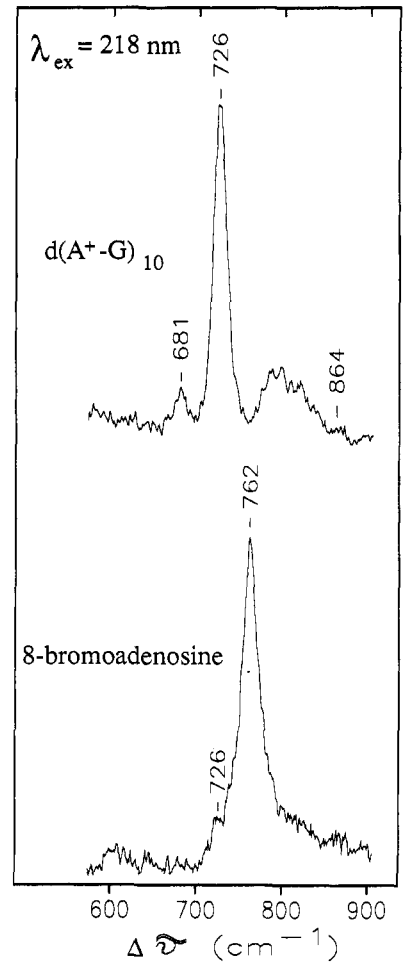

FIGURE 3: UV resonance Raman spectra of $d(A^+\text{-}G)_{10}$ and 8-bromoadenosine obtained with 218 nm excitation. Spectra are normalized to maximum peak height at 726 and 762 cm^{-1}. $d(A^+\text{-}G)_{10}$ (2 mM residues) was in 0.0019 M Na^+/0.004 M acetate, pH 4.00 (total $[Na^+]$ = 0.0019 M). 8-Bromoadenosine (1 mM) was in 0.001 M Na^+/0.038 M phosphate, pH 1.92, to give the same degree of protonation as in the helix at pH 4.0. Spectra shown are at 4 °C.

In Raman studies of oligomeric DNA crystals and fibers, the ring-breathing mode of dA residues in the $C_{2'}$-*endo/anti* configuration was detected at 728 cm^{-1}. For dA residues with the base in the *syn* orientation about a $C_{3'}$-*endo* sugar, this band was observed at 730 cm^{-1}, and an additional band was detected at 622 cm^{-1} (Benevides et al., 1984; Ridoux et al., 1988). In $d(A^+\text{-}G)_{10}$, the dA ring-breathing mode occurs at 726 cm^{-1} (Figure 3), and no bands are detected at 622 cm^{-1}, suggesting the dA residues are *anti*.

As an additional standard, we examined 8-bromoadenosine, which has been shown by X-ray crystallography to be in the $C_{2'}$-*endo/syn* conformation (Tavale & Sobell, 1970). Its ring-breathing mode is observed at 762 cm^{-1}, ca. 40 cm^{-1} upshifted from its position in the oligomer (Figure 3). By analogy with Raman studies of Z-RNA, in which the ring-breathing mode of the rG residues in Z form is relatively unchanged upon bromination at C8 (Hardin et al., 1987; Trulson et al., 1987), this large upshift is attributed to the

$C_{2'}$-*endo/syn* conformation and not to bromine substitution. The large difference in position of the dA ring-breathing mode in $d(A^+-G)_{10}$ (726 cm^{-1}) relative to 8-bromoadenosine (762 cm^{-1}) further suggests that dA residues are in the *anti* orientation.

In interpreting these assignments of orientation about the glycosyl bonds, it is important to realize that the oligomeric standards against which the UVRR bands have been evaluated are all in well-defined *syn* or *anti* conformations. It is not necessarily the case, however, that intermediate orientations about the glycosyl bonds will manifest spectroscopic properties that identify orientations consistent with the *syn*–*anti* nomenclature assigned by crystallographers.

In sum, UVRR observations on $d(A^+-G)_{10}$ confirm the absence of significant base stacking, involvement of the exocyclic amino hydrogens of dA but not of dG residues in H-bonds, and protonation at N_1 of the dA residues. In addition, this study provides information on the orientation of the base residues and their sugar conformations. These direct spectroscopic observations add confidence to the secondary structure delineated earlier based upon other physical–chemical solution properties.

REFERENCES

Benevides, J. M., Wang, A. H.-J., van der Marel, G. A., van Boom, J. H., Rich, A., & Thomas, G. J., Jr. (1984) *Nucleic Acids Res. 12*, 5913–5925.

Broomhead, J. M. (1948) *Acta Crystallogr. 1*, 324–329.

Cochran, W. (1951) *Acta Crystallogr. 4*, 81–92.

Dolinnaya, N. G., & Fresco, J. R. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 9242–9246.

Dolinnaya, N. G., Braswell, E. H., Fossella, J. A., Klump, H., & Fresco, J. R. (1993) *Biochemistry 32*, 10263–10270.

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

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

Fresco, J. R. (1959) *J. Mol. Biol. 1*, 106–110.

Grygon, C. A., & Spiro, T. G. (1990) *Biopolymers 29*, 707–715.

Hardin, C. C., Zarling, D. A., Puglisi, J. D., Trulson, M. O., Davis, P. W., & Tinoco, I., Jr. (1987) *Biochemistry 26*, 5191–5199.

Lord, R. C., & Thomas, G. J., Jr. (1967) *Spectrochim. Acta 23A*, 2551–2591.

Perno, J. R., Grygon, C. A., & Spiro, T. G. (1989) *J. Phys. Chem. 93*, 5672–5678.

Rich, A., Davies, D. R., Crick, F. H. C., & Watson, J. D. (1961) *J. Mol. Biol. 3*, 71–86.

Ridoux, J. P., Liquier, J., & Taillandier, E. (1988) *Biochemistry 27*, 3874–3878.

Rodgers, K. R., Su, C., Subramaniam, S., & Spiro, T. G. (1992) *J. Am. Chem. Soc. 114*, 3697–3709.

Shiber, M. C., Lavelle, L., Fossella, J. A., & Fresco, J. R. (1995) *Biochemistry 34*, 14293–14299.

Tavale, S. S., & Sobell, H. M. (1970) *J. Mol. Biol. 48*, 109–123.

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

Toyama, A., Takino, Y., Takeuchi, H., & Harada, I. (1993) *J. Am. Chem. Soc. 115*, 11092–11098.

Trulson, M. O., Cruz, P., Puglisi, J. D., Tinoco, I., Jr., & Mathies, R. A. (1987) *Biochemistry 26*, 8624–8630.

BI9429039