Sugimoto, H., & Sawyer, D. T. (1985) *J. Am. Chem. Soc. 107*, 5712–5716.
Taylor, R. J., Jr., Stubbs, C. S., & Ellenbogen, L. (1969) *Biochem. Pharmacol. 18*, 587–594.
Waalks, T. P., & Udenfriend, S. (1957) *J. Lab. Clin. Med. 50*, 733–743.
Wallick, D. E., Bloom, L. M., Gaffney, B. J., & Benkovic, S. J. (1984) *Biochemistry 23*, 1295–1302.

Infrared and Raman Studies Show That Poly(dA)·Poly(dT) and d(AAAAATTTTT)$_2$ Exhibit a Heteronomous Conformation in Films at 75% Relative Humidity and a B-Type Conformation at High Humidities and in Solution[†]

E. Taillandier,[*,‡] J.-P. Ridoux,[‡] J. Liquier,[‡] W. Leupin,[§] W. A. Denny,[‖] Y. Wang,[⊥] G. A. Thomas,[⊥] and W. L. Peticolas[*,⊥]

Department of Chemistry and Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403, Laboratoire de Spectroscopie Biomoléculaire, UFR de Médecine, F-93012 Bobigny Cedex, France, Institut für Molekularbiologie and Biophysik, Eidgenossishe Technische Hochschule, CH-8093 Zurich, Switzerland, and Cancer Research Laboratory, University of Auckland School of Medicine, Auckland, New Zealand

Received November 3, 1986; Revised Manuscript Received January 29, 1987

ABSTRACT: The decadeoxynucleotide d(AAAAATTTTT)$_2$ in duplex form and the double-helical polynucleotide poly(dA)·poly(dT) have been studied by Raman and infrared (IR) spectroscopy under a variety of environmental conditions. The IR spectra have been taken of cast films and compared to the IR spectra of the alternating poly(dA-dT), which shows clear B-genus and A-genus vibrational spectra under conditions of high (>92%) and low (75%) relative humidity (RH). From the IR data, it is shown that d(AAAAATTTTT)$_2$ and poly(dA)·poly(dT) adopt a B-genus conformation in films with high water content. When the relative humidity of the film is decreased, the IR spectra reflect a gradual evolution of the geometry of both d(AAAAATTTTT)$_2$ and poly(dA)·poly(dT) into a form intermediate between the B genus and A genus, but the IR spectrum of a pure A genus has not been obtained. In these DNAs at 75% RH, the IR bands of adenosine have the same frequencies as those found in poly(dA-dT) at 75% RH where the local furanose conformation is C3′ endo/anti, but the thymidine frequencies do not resemble those of poly(dA-dT) at 75% RH but rather those of poly(dA-dT) at high humidities. It is concluded that both poly(dA)·poly(dT) and d(AAAAATTTTT)$_2$ adopt a fully heteronomous duplex geometry in cast films at low humidity. For studies in aqueous solution the Raman effect was employed. As a model for the heteronomous conformation in solution, the duplex poly(rA)·poly(dT) was used. Two methods were devised to estimate the amount of heteronomous conformation for d(AAAAATTTTT)$_2$ and poly(dA)·poly(dT) in solution from the intensities of a sugar–phosphate band and an adenine band obtained by a fit of the Raman spectrum to a sum of Lorentzians bands. These measurements indicate that about 30% of the adenine residues were attached to furanose rings with the C3′-endo/anti conformation on the time scale of the Raman effect. This would mean that no more than about 15% of the furanose rings in the duplex are in this conformation. If the conformation of poly(dA)·poly(dT) is fluctuating rapidly in solution, this small amount of C3′-endo ring pucker might be difficult to detect by other techniques such as NMR.

Three main double-helical families are known for DNAs and synthetic oligo- or polynucleotides, the A, B, and Z families characterized by various physical techniques, X-ray single-crystal or fiber diffraction, NMR, vibrational spectroscopy (IR and Raman), and circular dichroism. Recently, many investigations have been undertaken to determine the detailed geometry of DNA of defined base sequence, because sequence-dependent structural variations could serve as recognition signals in the biological functions of the nucleic acids. Although both strands of the double helix in a given DNA conformation have identical geometries in either the A, the B, or the Z form, there is no absolute requirement that both polynucleotide chains in double-stranded DNA helixes should always be conformationally identical. Heteronomous duplex structures with different geometries for each strand have been suggested to exist in DNA–RNA complexes (Zimmerman et al., 1981; Arnott et al., 1986) and proposed in some cases of synthetic polynucleotides with homopurine and homopyrimidine strands (Thomas & Peticolas, 1983; Arnott et al., 1983; Nishimura et al., 1986). The poly(dA)·poly(dT) Raman spectrum recorded in aqueous solution at low temperature has a band that led Thomas and Peticolas to propose that poly(dA)·poly(dT) solutions contain at low temperature a measurable amount of C3′-endo/anti conformation in contrast to the uniform C2′-endo/anti conformation of the classical B double helix; at higher temperature this C3′-endo/anti Raman peak either disappears or becomes very broad (Thomas &

[†] This work was supported in part by grants from the National Institutes of Health (5 R01 GM 15547-18 and 5R01 GM33825-02) and the National Science Foundation (DMB-8417199), to W.L.P., and in part by grants from the Swiss Cancer League (to W.L.) and the Medical Research Council of New Zealand (to W.A.D.).

[‡] UFR de Médecine.

[§] Eidgenossishe Technische Hochschule.

[‖] University of Auckland School of Medicine.

[⊥] University of Oregon.

0006-2960/87/0426-3361$01.50/0

Peticolas, 1983). In the fibrous state, the X-ray fiber diffraction data of a polycrystalline low-humidity form of poly(dA)·poly(dT) are found to agree better with those of a calculated heteronomous structure with A-like conformation of the poly(dA) chain and B-like conformation of the poly(dT) chain, rather than with those of a more uniform structure with an identical B conformation on both chains (Arnott et al., 1983). Both 1D and 2D ^{1}H NMR spectroscopies at 500 MHz have also been used to examine the structure of poly(dA)·poly(dT) in solution (Sarma et al., 1985; Behling & Kearns, 1986). All NMR data for poly(dA)·poly(dT) are in disagreement with the heteronomous model of Arnott (Arnott et al., 1983) proposed for the fibrous state, but the same DNA molecule does not necessarily have the same structure in solution and in solid state at low humidity.

In view of this situation, the self-complementary decadeoxynucleotide d(AAAAATTTTT)$_2$ [will be called d(A_5T_5) from now on] has been synthesized and studied by 2D ^{1}H NMR, Raman, and IR spectroscopies. The NMR results will be published elsewhere (Leupin, 1987), and we report here IR and Raman data. IR and Raman spectroscopies have the advantage of being used for both long and short nucleic acid sequences; thus, vibrational spectra of d(A_5T_5) can easily be compared to poly(dA)·poly(dT) spectra recorded in the same conditions of hydration, counterions, and temperature. The IR and Raman spectra of poly(dA)·poly(dT) and poly(rA)·poly(dT) have enabled us to assign backbone sugar phosphodiester modes characteristic of a pure A-helix structure containing only A-T base pairs and vibrational modes characteristic of C3′-endo/anti conformations of dA and dT residues. The IR results indicate that in films different conformations are found depending on the degree of hydration. At high relative humidities d(A_5T_5) and poly(dA)·poly(dT) in films adopt a B-type conformation, in agreement with NMR results. On the other hand, the Raman results do indicate that in solution as high as 15% of the furanose rings of either d(A_5T_5) or poly(dA)·poly(dT) may be in the C3′-endo conformation. However, the low-humidity IR spectra of poly(dA)·poly(dT) are different from classical A-form IR spectra and may be interpreted in terms of two different geometries for the dA and dT strands, the dA residues being in C3′ endo/anti and the dT residues in C2′ endo/anti.

Correlation between IR and Raman spectra is discussed. It is known that poly(dA)·poly(dT) frequently occurs in terminal regions of genes (Schmidt, 1984). Thus, an unusual structure, such as an heteronomous duplex with one "A-like" strand, may play a role for initiation or termination of transcription.

MATERIALS AND METHODS

The self-complementary decadeoxynucleoside nonaphosphate d(AAAAATTTTT) was synthesized via a modified version of the phosphodiester method in liquid phase, starting from commercially available deoxynucleosides (Fluka AG) [see Denny, et al. (1982) and Leupin (1987) for a detailed description of the synthesis]. The decanucleotide was employed as its sodium salt.

Poly(dA-dT) (lots 658/92 and 317810) and poly(dA)·poly(dT) (lot 517860) were purchased from P-L Biochemicals and used without further purification. The decanucleotide and the polynucleotides were dissolved in 500 mM NaCl (at a concentration of 10 OD/mL) and dialyzed overnight to a final Na^+ concentration of 1.5 Na^+ per nucleotide. The samples were then deposited on ZnSe windows and dried so as to obtain homogeneous films. The films were placed in sealed cells with controlled relative humidity. Selective C8-deuteriation of adenosine in poly(dA)·poly(dT) was obtained by incubation in 500 mM NaCl–D_2O for 24 h at 80 °C.

The Raman spectra of the solutions were taken with an argon laser on a SPEX 1301 monochromator as described previously (Thomas & Peticolas, 1983). The samples were placed in a capillary that was sealed and mounted in a copper block with the temperature set between 0 and 15 °C to prevent sample degradation in the laser beam. Repeated attempts to obtain the Raman spectrum of a film of fiber of poly(dA)·poly(dT) under conditions of low (75%) humidity failed due to decomposition of the polynucleotide in the laser beam.

The IR spectra were recorded with a Perkin-Elmer 180 spectrophotometer coupled with a Hewlett-Packard 9825 calculator. Data were treated as previously described (Taboury et al., 1985).

RESULTS AND INTERPRETATION

IR Data

Adenosine and Thymidine Local Conformation (*1550–1250-cm^{-1} Region*). The in-plane vibrations of the bases (single-bond stretching and bending motions), of the glycosidic bond, and of the sugar moiety can be observed by IR spectroscopy in the 1500–1250-cm^{-1} region. This spectral region is extremely sensitive to modifications in the nucleoside conformation and contains a set of IR marker bands corresponding to C2′-endo/anti and C3′-endo/anti geometries of adenosine and thymidine. This will be demonstrated from the IR spectra of poly (dA-dT) in well-known conformations and will be used to discuss new IR spectra recorded for d(A_5T_5) and poly(dA)·poly(dT) in low relative humidity conditions.

The IR spectrum of poly(dA-dT) recorded in high hydration conditions (RH 98%) in the presence of sodium counterions (one Na^+ per nucleotide) is presented (Figures 1I and 3, top I). These experimental conditions correspond to a classical B geometry of the polynucleotide, with C2′-endo/anti conformations for both dA and dT residues, and we observe on the IR spectrum four absorptions, which, as we shall discuss below, can be considered as marker bands for this C2′-endo/anti geometry of the nucleosides. These absorptions are located at 1281, 1295, 1327, and 1425 cm^{-1}.

The strong band observed at 1281 cm^{-1} in high-RH conditions can be assigned to C2′-endo/anti thymidine. This absorption is sensitive to deuterium exchange: exposure of the samples to D_2O shifts this band to 1303 cm^{-1} (spectra not shown), whereas selective adenine deuteriation does not displace it, as can be seen in Figure 3 (top I and top II). Moreover, in the IR spectrum of 2′dT one of the most intense absorptions is found at 1275 cm^{-1} and is also shifted to higher wavenumbers by deuterium exchange (=+25 cm^{-1}). By decreasing the relative humidity of poly(dA-dT) and poly(d[8-^{2}H]A-dT), the A-form IR spectrum is observed and the thymidine band is detected at 1273 cm^{-1}, corresponding to the C3′-endo/anti geometry (Figure 3, bottom I and bottom II).

The intense absorption observed in the poly(dA-dT) spectrum at 1425 cm^{-1} involves contributions from both adenine and thymine moieties. If we selectively deuteriate the C8 site of adenines, we observe a progressive shift of the adenine component from 1425 to 1410 cm^{-1} while the thymine component remains unaffected (Figure 2I,II). When the relative humidity is decreased, a new IR spectrum is obtained (Figure 1I) corresponding to a classical A form for poly(dA-dT) with C3′-endo/anti geometries of the nucleosides. We can observe on this IR spectrum that the 1425-cm^{-1} band is split into two components, the first one located at 1417 cm^{-1} and the second one at 1401 cm^{-1}. These two bands correspond respectively

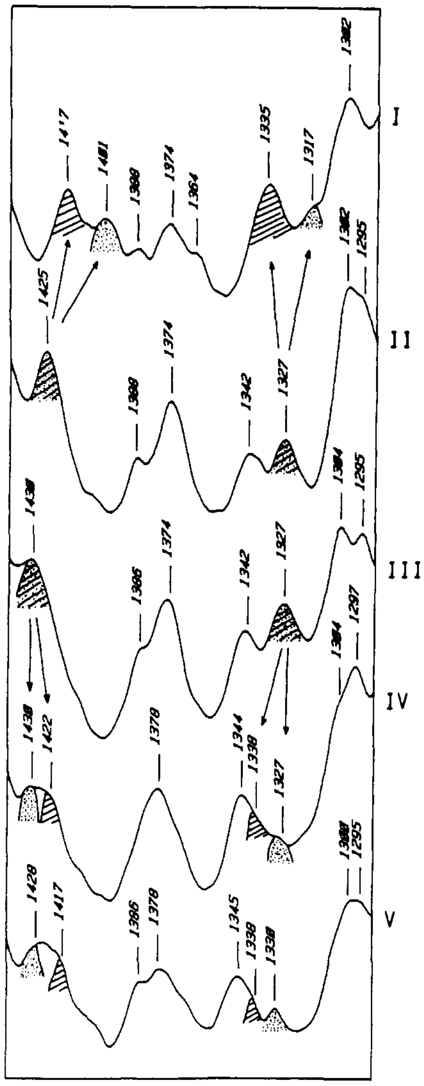

FIGURE 1: Infrared absorption spectra in the base deoxyribose vibrational region of (I) poly(dA-dT) A form at 75% RH, (II) poly(dA-dT) B form at 92% RH, (III) poly(dA)·poly(dT) B form at high RH, (IV) poly(dA)·poly(dT) at low RH, and (V) d(A_5T_5) at low RH. The hatched bands are assigned to adenosine while the stippled bands are due to thymidine.

to vibrational modes of C3′-endo/anti adenosine and C3′-endo/anti thymidine as is clearly shown by the same selective C8-deuteriation experiment (Figure 2III,IV); the 1417-cm^{-1} band is shifted further to 1408 cm^{-1} by C8-deuteriation, while the 1401-cm^{-1} thymidine contribution is now mostly overlapped by the [8-^{2}H]A band.

The IR band found at 1327 cm^{-1} in B-form poly(dA-dT) also involves contributions of C2′-endo/anti adenosine and C2′-endo/anti thymidine (Figure 1II). In the A-form spectrum (Figure 1I), contributions of both nucleosides are now again separated: the C3′-endo/anti thymidine absorption is observed at 1317 cm^{-1} while the C3′-endo/anti adenosine band is found at 1335 cm^{-1}, superimposed on another adenosine absorption that was located at 1342 cm^{-1} in the B-form spectrum. (This is why the relative intensity of this band seems to increase so much under the B → A transition.) Finally a thymidine absorption is detected at 1295 cm^{-1} in the B-form spectrum of poly(dA-dT) (Figure 1II) and poly(d[8-^{2}H]A-dT) (overlapped by the adenosine band shifted by selective deuteriation from 1303 to 1299 cm^{-1}, Figure 3, top I and top II). It is clearly absent from the A-form spectra (Figures 1I and 3, bottom I and bottom II), thus assignable to the C2′-endo/anti geometry of the thymidine.

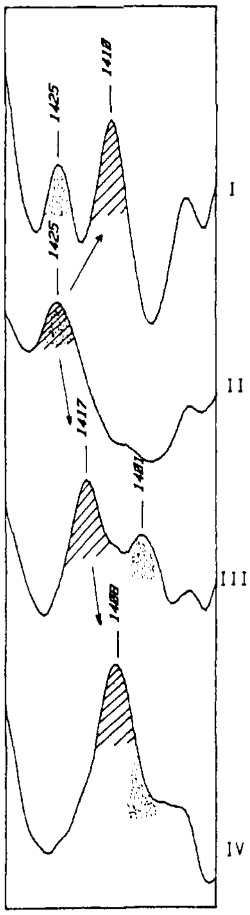

FIGURE 2: Effect of selective C8-deuteriation of adenosine in poly(dA-dT). The C8-deuteriation changes the frequencies of the adenosine slightly, allowing the assignment of the adenine and thymine contributions to a vibration mode (see text). (I) Poly(dD8A-dT), B form; (II) poly(dA-dT), B form; (III) poly(dA-dT), A form; (IV) poly(d[8-^{2}H]A-T), A form.

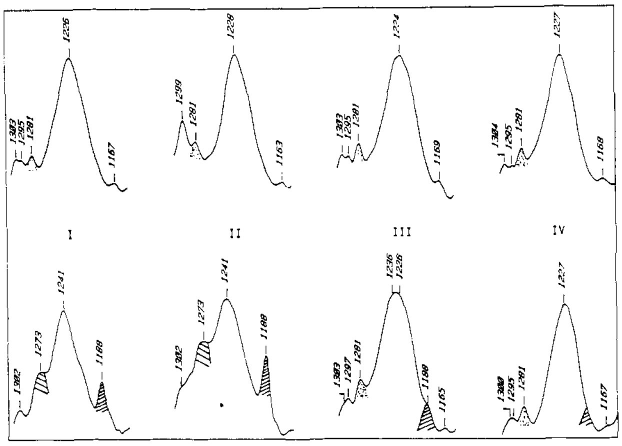

FIGURE 3: Deoxyribose and antisymmetric phosphate vibrations of (I) poly(dA-dT), (II) poly(d[8-^{2}H]A-dT), (III) poly(dA)·poly(dT), and (IV) d(A_5T_5). (Upper) High-RH spectra (all B form); (lower) low-RH spectra. The stippled vibration is the thymidine C2′-endo/anti marker band while the thymidine C3′-endo/anti marker band is hatched up to the left and the A-form deoxyribose marker band is hatched up to the right.

A recent calculation (unpublished results) using a normal coordination analysis of the adenosine and thymidine residues involved in oligonucleotides and polynucleotides shows that the conformation-sensitive mode at 1281 cm^{-1} (B form) and 1273 cm^{-1} (A form) involved mainly the N1–C6–H and C5–C6–H deformations and that those at 1425 cm^{-1} (B form) and 1401 cm^{-1} (A form) the N1–C6 and C1–N1 stretching vi-

Table I: IR Marker Band Frequencies of the Adenosine and Thymidine Residues (in cm^{-1}) for the C3′-Endo/Anti and C2′-Endo/Anti Furanose Ring Geometries

	deoxyadenosine		thymidine		
	high RH	low RH	high RH	low RH	
DNA	C2′ endo	C3′ endo	C2′ endo	C3′ endo	C2′ endo
poly(dA-dT)	1425	1417	1425	1401	
poly(d[8-^{2}H]A-dT)	1425	1417	1425	1401	
poly(dA)·poly(dT)	1430	1422	1430		1430
d(A_5T_5)	1428	1417	1428		1428
poly(dA-dT)	1327	1335	1327	1317	
poly(d[8-^{2}H]A-dT)	1327	1335	1327	1317	
poly(dA)·poly(dT)	1327	1338	1327		1327
d(A_5T_5)	1328	1338	1328		1330
poly(dA-dT)			1281	1273	
poly(d[8-^{2}H]A-dT)			1281	1273	
poly(dA)·poly(dT)			1281		1281
d(A_5T_5)			1281		1281

brations of the thymidine. Moreover, the 1327-cm^{-1} (B form) and 1317-cm^{-1} (A form) bands are due mainly to the C4′–C5′ and C4′–O1′ deoxyribose vibrations of dT, and the 1295-cm^{-1} IR band in the B geometry, not observed in the case of the A-form spectrum, is correlated to the sugar CH_2 scissoring vibration mode frequency shift upon the C2′-endo → C3′-endo conformational transition of the dT residue. The observed and calculated band shifts agree well with the C2′-endo/anti–C3′-endo/anti structural modification of dT residues upon B → A transition.

The IR spectrum of poly(dA)·poly(dT) recorded at high RH in the presence of sodium is presented in Figure 1III and Figure 3, top III. As can be easily observed, it is quite comparable to that of poly(dA-dT) recorded in the same experimental conditions (Figure 1II and 3, top I) and reflects a classical B geometry. The C2′-endo/anti marker bands of dA and dT are detected at 1430, 1327, 1295, and 1281 cm^{-1}. When the relative humidity is decreased, a new IR spectrum is obtained (Figure 1IV and 3, bottom III) different from that of A-form poly(dA-dT) (Figures 1I and 3, bottom I). In fact, we observe the simultaneous presence of IR absorption bands at 1430 and 1327 cm^{-1} on one hand, at 1422 and 1338 cm^{-1} on the other, and at 1297 and 1281 cm^{-1}. The absence of absorption around 1401, 1317, and 1273 cm^{-1} rules out the possibility of the existence of C3′-endo/anti thymidines. The dT nucleosides are thus in C2′-endo/anti geometry, which is confirmed by the presence of the marker bands located at the same wavenumbers as in the B conformation of poly(dA-dT), i.e., 1430, 1327, 1295, and 1281 cm^{-1}. The dA nucleosides are in a C3′-endo/anti geometry as shown by the shifts of the adenosine components from 1430 to 1422 cm^{-1} (=−8 cm^{-1}) similar to that observed for adenosine in poly(dA-dT) under the B → A transition (1425 − 1417 cm^{-1} = −8 cm^{-1}) and from 1327 to 1338 cm^{-1} (=+11 cm^{-1}) (1327 to 1335 cm^{-1} = +8 cm^{-1}) in the case of poly(dA-dT) (see Table I). The same results are obtained in the case of the decadeoxynucleotide d(A_5T_5) as can be judged by Figure 1V and 3, bottom IV. Both sets of bands of C3′-endo/anti dA and C2′-endo/anti dT are found respectively at 1417 and 1338 cm^{-1} and at 1428, 1330, 1295, and 1281 cm^{-1}.

Phosphate and Deoxyribose Vibrations (*1250–1000-cm^{-1} Region*). The antisymmetric phosphate stretching vibration as PO_2^- is observed near 1226 cm^{-1} for the B-family helices (Figure 3 top) and at 1241 cm^{-1} for the A helix (Figure 3 bottom I,II). In the low-RH spectrum of poly(dA)·poly(dT), two components can be observed in this peak, the first one at 1226 cm^{-1} and the second one at higher wavenumbers around 1236 cm^{-1}, possibily reflecting the existence of two different phosphate environments for the dA and dT strands.

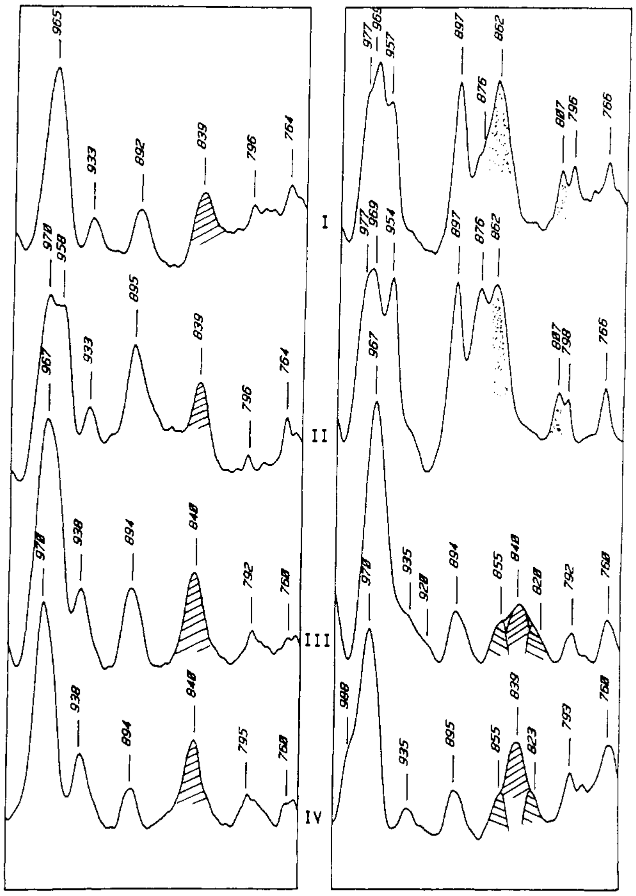

FIGURE 4: IR bands due to the vibrations of the backbone: (left) high-RH spectra; (right) low-RH spectra. The spectra are (I) poly(dA-dT), (II) poly(d[8-^{2}H]A-dT), (III) poly(dA)·poly(dT), and (IV) d(A_5T_5). The characteristic IR marker bands for the A conformation are stippled. Those of the B conformation are hatched up to the right while those of the heterononous conformation are hatched up to the left.

One of the most precisely detectable IR absorption bands characteristic of the A conformation in DNAs is located at 1188 cm^{-1} and has been assigned mainly to a deoxyribose motion (Pohle & Fritzsche, 1980). We can verify that in fact in all four top spectra (Figure 3), corresponding to the B helices, this band is not detected whereas in the bottom spectra (Figure 3I,II) it is sharp and intense, reflecting the A geometry deoxyribose. The low-RH spectra of poly(dA)·poly(dT) and d(A_5T_5) also present this absorption but as a shoulder of relatively weak intensity, which may reflect the existence of part of the sugar moieties in A conformation, possibly again those of the dA strand if a heteronomous duplex structure is assumed.

Backbone Conformation (*1000–750-cm^{-1} Region*). Figure 4 (left) shows the IR spectra between 1000 and 750 cm^{-1} of poly(dA)·poly(dT), poly(d[8-^{2}H]A-dT), poly(dA-dT), and d(A_5T_5) films at high RH. The four spectra exhibit the same vibrational mode band located at 839 cm^{-1} for the alternating polymers and 840 cm^{-1} for the nonalternating polymers, which is well-known as an IR marker band of B-type DNA backbone (Brahms et al., 1974; Taillandier et al. 1985). This absorption is observed here at a slightly higher wavenumber than for several DNAs containing 50% G-C and 50% A-T base pairs (840 instead of 835 cm^{-1}). Nevertheless, the IR spectra of these highly hydrated films reflect a B conformation for nonalternating AT sequences as for poly(dA-dT). The Raman marker peak of the B family corresponding to the same vibrational mode of the phosphodiester chains is also detected at 840 cm^{-1} for solutions of DNA polymers containing only AT pairs (Thomas & Benevides, 1985; Small & Peticolas, 1971).

Figure 4 (right I and II) shows the A-form spectra between 1000 and 750 cm^{-1} of poly(dA-dT) and poly(d[8-^{2}H]A-dT) films at low RH. The B → A transition occurs when the water content of the films is decreased. Two characteristic IR absorptions of an A-type backbone are found below 71% RH: a strong band located at 862 cm^{-1} (861 cm^{-1} in D_2O) and a weak narrow band at 807 cm^{-1} (805 cm^{-1} in D_2O), for the C8-deuteriated as well as for the nondeuteriated polymer. These bands are similar to those observed near 860 and 805 cm^{-1} in the IR low-humidity spectra of several native DNAs whose structure is known to be of A type in these conditions (Brahms et al., 1974; Taillandier et al., 1985). Both the 862- and 807-cm^{-1} IR marker bands of a homogeneous A-helix structure containing only A-T base pairs have never been observed in the sepctra of d(A_5T_5) and poly(dA)·poly(dT) films, whatever their water content, as shown in Figure 4 (right III and IV). The d(A_5T_5) films were studied between 100 and 66% RH and those of poly(dA)·poly(dT) between 100 and 32% RH; at lower RH these duplexes are found to be disorganized. Thus, d(A_5T_5) and poly(dA)·poly(dT) do not undergo the B → A transition.

However, the IR absorption profile of d(A_5T_5) and poly(dA)·poly(dT) is significantly affected upon lowering the water content. The marker band of the B-type DNA backbone at 840 cm^{-1} always remains present whatever the RH, but this band is broadened and two new bands appear near 855 and 820 cm^{-1} when the water content is decreased. Their wavenumbers are different from those of the marker bands of a pure A-type backbone. Moreover, the IR profile between 1000 and 750 cm^{-1} differs from that of DNA spectra for which the B and the A forms coexist along the same double helix (Taillandier et al., 1984). In that case, both marker bands of B- and A-type backbones had been observed simultaneously at the same wavenumbers as in pure B and A double helices, i.e., 835 and 860 cm^{-1}. The low-humidity spectra of poly(dA)·poly(dT) and d(A_5T_5) might reflect a heteronomous conformation with a different backbone geometry for both strands. From the IR data of the 1550–1250-cm^{-1} region, which gives the local conformation of the nucleosides, all dT nucleosides are found in C2′-endo/anti conformation and all dA nucleosides in C3′-endo/anti conformation. Thus, taking into account all the IR data, the low-humidity conformations of d(A_5T_5) and poly(dA)·poly(dT) could be considered as a heteronomous conformation with only the dT strand in a B conformation.

Three IR absorption bands around 967, 933, and 876 cm^{-1} in the spectra of poly(dA)·poly(dT) may involve both vibrations of the DNA backbone and of the bases. The 967-cm^{-1} band is split into three components upon the B → A transition (Figure 4I) and also split into two components by specific C8-deuteriation of adenine (Figure 4, left I and II). Its profile is different for spectra recorded in H_2O or in D_2O, thus showing that it may be sensitive to thymine deuteriation. The pure A form characteristic shape of this band has never been found for poly(dA)·poly(dT) and d(A_5T_5). We must notice that one of the differences between the low-RH spectra of poly(dA)·poly(dT) (Figure 4, right III) and d(A_5T_5) (Figure 4, right IV) is the presence of a shoulder detected at 988 cm^{-1} in the latter case. The ratio of the relative intensities of the 988/970-cm^{-1} bands, 0.2/1, may reflect an unusual conformation at the level of the A-T junction in the oligomer, two out of ten base pairs being involved in this inversion in the homopurine tract. The intensity of the 933-cm^{-1} band increases with the AT content in native DNAs, in B conformation. This band disappears upon the B → A transition (Figure 4I). In agreement with the hypothesis of a heteronomous structure of poly(dA)·poly(dT) and d(A_5T_5), its intensity decreases at low RH (Figure 4, right III and IV) but is still present in both spectra of the nonalternating sequences.

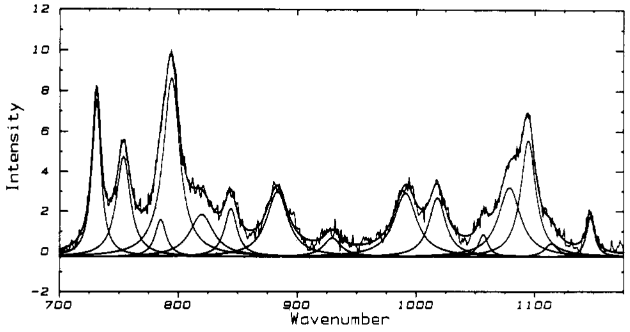

FIGURE 5: Raman spectrum of d(A_5T_5) expanded to exhibit the frequency region 700–1175 cm^{-1}. The spectrum has been resolved into the minimum number of Lorenztian lines, which are shown.

Raman Data

For studies of the polynucleotide and oligonucleotide conformations in solution, we have turned to the Raman effect. Excellent Raman spectra of these materials in solution were obtainable. There are two regions of this spectra that are of interest because of the conformational information which they contain—the 750–1200-cm^{-1} and the 1000–1500-cm^{-1} region. In order to study these regions in detail, we examine each of them in an expanded form in which the spectra have been resolved into the minimum number of Lorentzian line shapes. The method has been described elsewhere (Kubasek et al., 1986). This permits a quantitative analysis of the spectra in terms of the areas of the bands and their heights. Figure 5 shows the expanded Raman spectra of d(A_5T_5) in the 700–1175-cm^{-1} region. The Raman marker bands at 842 cm^{-1} for C2′-endo and the Raman marker band at 816 cm^{-1} for C3′-endo conformations are plainly visible. It must be noted that the frequencies of these bands are unusually high. The 842-cm^{-1} Raman band is almost certainly due to the same vibrational mode as the 840-cm^{-1} band observed in the IR spectrum of d(A_5T_5) noted above. The nominal Raman frequencies for these marker bands are 835 and 809 cm^{-1} for DNAs, but it is not unreasonable that specific sequencies such as d(A_5T_5) may have a slightly different set of torsional angles that gives rise to slightly different marker band frequencies. The d(A_5T_5) molecule is plainly in a predominately B-type conformation judging from the weakness of the "810-cm^{-1}" band and the presence of the "835-cm^{-1}" band with its normal B-DNA intensity. As has been shown recently by Wartell and Harrell (1986) all DNAs possess a weak band at 810 cm^{-1}, and the B form of native DNAs may be considered to have a variety of ring puckers. This conclusion is also consistent with the X-ray-determined conformation of a dodecamer deoxyoligonucleotide in the B form by Dickerson and Drew (1981), who showed only a third of the furnanose rings were in the C2′-endo ring conformation. Figure 6 shows the corresponding expanded Raman spectrum of an aqueous solution of poly(dA)·poly(dT). Again one sees the existence of bands corresponding to two ring puckers as described earlier by Thomas and Peticolas (1983) and confirmed by Wartell and Harrell (1986). In order to evaluate the effect of strand length on the secondary

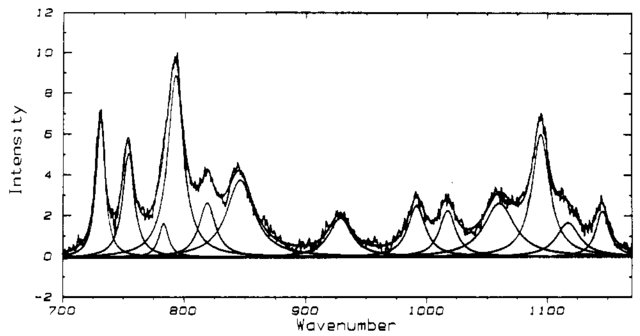

FIGURE 6: Raman spectrum of poly(dA)·poly(dT) expanded to exhibit the frequency region 700–1175 cm^{-1}. The spectrum has been resolved into the minimum number of Lorenztian Raman bands, which are shown.

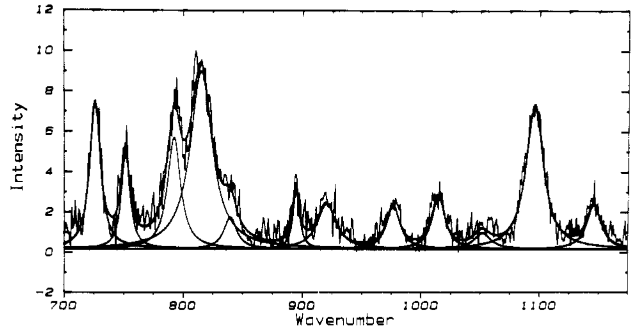

FIGURE 7: Raman spectrum of poly(rA)·poly(dT) in a 25 mM pH 7 phosphate buffer solution at 2 °C. The spectrum is resolved into the minimum number of Lorenztian Raman bands, which are shown in the region 799–1175 cm^{-1}.

Table II: Estimation of Percent Heteronomous Conformation in Various A/T-Containing Deoxypolynucleotides and Deoxyoligonucleotides from Ratios of Raman Band Intensities

	I_{816}/I_{1094} (area)	% hetero-nomous[a]	I_{816}/I_{1094} (height)	% hetero-nomous[a]
poly(dA)·poly(dT)	0.416	32	0.445	45
poly(dA)·p(dT)$_{10}$	0.741	56	0.553	56
d(A_5T_5)$_2$	0.5732	43	0.361	37
poly(rA)·poly(dT)	1.53	100	1.22	100

[a] The percent heteronomous conformation is twice the percent C3′-endo conformation. The percent C3′-endo goes from 16 to 50% in these polymers. This estimate is probably good to ±5%.

structure of the poly(dA)·poly(dT) helix, the Raman spectrum of a double helix with only one chain in high molecular weight, poly(dA)·p(dT)$_{10}$, was also taken (see Table II).

Figure 7 shows the expanded Raman spectrum of poly(rA)·poly(dT) in the range 700–1175 cm^{-1}. This molecule is of interest because it has been suggested to have a fully heteronomous conformation from X-ray diffraction studies (Zimmerman & Pheiffer, 1981). It has been described as being essentially in the B form with the furanose rings on the adenine strand having the C3′-endo form and the furanose rings in the thymine strand having the C2′-endo form. In its Raman spectrum it may be seen that the 812-cm^{-1} band is much stronger in this model DNA than in the poly(dA)·poly(dT) or the poly(dA)·p(dT)$_{10}$ helices. We propose to use this Raman spectrum as a moel for that of a fully heteronomous polynucleotide containing only A and T and to use the intensities of two Raman bands as standards for a 100% heteronomous conformation in order to obtain a quantitative measure of C3′-endo/anti furanose ring conformation in d(A_5T_5) and poly(dA)·poly(dT). The Raman band in Figure 7 which is of interest is the backbone vibration at 813 ± 4 cm^{-1}, which we will take initially as a measure of C3′-endo/anti conformation.

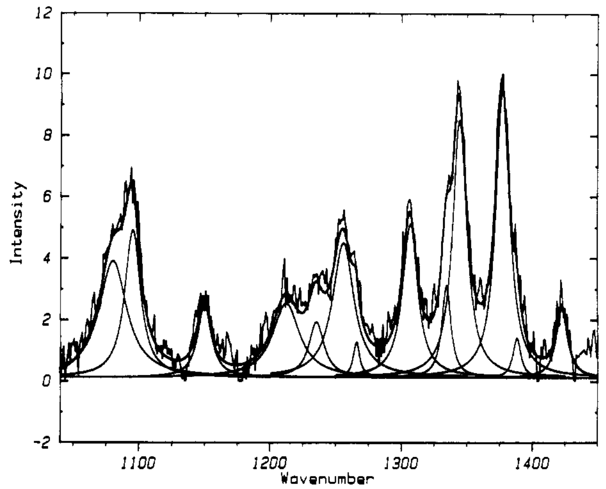

FIGURE 8: Raman spectrum of d(A_5T_5) taken under the conditions of Figure 1 expanded to exhibit the frequency region 1050–1450 cm^{-1}. The spectrum is resolved into the minimum number of Lorenztian bands.

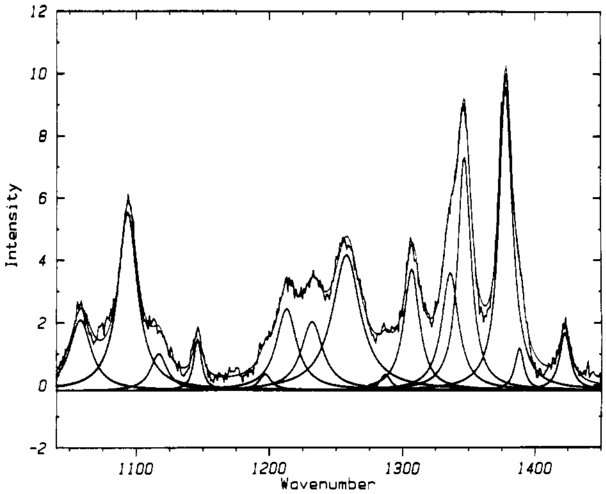

FIGURE 9: Raman spectrum of poly(dA)·poly(dT) taken under the conditions of Figure 1 expanded to exhibit the frequency region 1050–1450 cm^{-1}. The spectrum is resolved into the minimum number of Lorenztian bands.

Figures 8 and 9 show the Raman spectra of poly(dA)·poly(dT) and d(A_5T_5) in aqueous phosphate buffer at pH 7 expanded to show the freqency region of 1050–1350 cm^{-1}. We note that in each of these spectra there are two bands in the region of 1340 cm^{-1} that occur at 1336 and 1343 cm^{-1}. On the other hand, there is only one such band in the Raman spectrum of poly(rA)·poly(dT) shown in Figure 10. We attribute the two bands in this region to the existence of adenine rings attached to furanose rings with both the C3′- and C2′-endo/anti conformations. Following Katahira et al. (1986), we assign the band at 1336 cm^{-1} to the adenine base attached to a furanose ring with the C3′-endo/anti conformation and the 1443-cm^{-1} band to the adenine base attached to a furanose ring in the C2′-endo/anti conformation.

DISCUSSION

The IR spectroscopic results described above plainly show that at high humidities and room temperature both poly(dA)·poly(dT) and poly(dA-dT) are in a B-type double helix. These results are in agreement with the recent NMR studies of d(A_5T_5) in solution (Leupin et al., personal communication) as well as with recent NMR results on poly(dA)·poly(dT)

(Behling & Kearns, 1986; Sarma et al., 1985). As previously shown by IR, films of poly(dA-dT) undergo the B to A transition upon decreasing the humidity of the films in the presence of sodium ions with a sodium content of greater than one Na^+ per phosphate group (Adam et al., 1986). However under the same conditions of water and counterion content, the IR spectrum of poly(dA)·poly(dT) or $d(A_5T_5)$ showed neither a pure A-form DNA spectrum nor a mixture of A- and B-form spectra, reflecting the coexistence of both A and B double helices. Previous measurements of the dichroic ratios of the phosphate double bond stretching vibrations on an oriented sample of poly(dA)·poly(dT) as a function of the RH have yielded values that are different from those expected for an A-type double helix (Pilet et al., 1975). Consequently, we are led to the conclusion that, under conditions of low humidity and salt identical with those in which all native DNAs and poly(dA-dT) go into the A form, the polynucleotide poly(dA)·poly(dT) does not go into an A form. As the water content of the films of poly(dA)·poly(dT) and $d(A_5T_5)$ is decreased, the IR spectrum changes continuously from a B-type IR spectrum to that of an intermediate-type spectrum between those of A-type and B-type conformations. These low-RH spectra suggest a heteronomous conformation in the local structure since they show evidence of thymidine bases in a B-type environment and adenine bases in an A-type environment. In agreement with the model proposed from the fiber X-ray diffraction studies (Arnott et al., 1986), the IR results are consistent with a model of poly(dA)·poly(dT) at low humidity in which the furanose rings in the adenine-containing strand are in the C3′-endo/anti conformation while the furanose rings in the thymine-containing strand are in the C2′-endo/anti conformation.

The finding that extended sequences of A's and T's in an oligonucleotide tend to prevent it from going into the A form may be a general phenomenon. It is interesting to note that X-ray diffraction studies have shown that the deoxyoligomer d(GGTATACC), which contains the central sequence TATA, is in the A form in crystals (Kennard et al., 1981) while the oligomer d(CGCGAATTCGCG), which contains the central sequence AATT, goes into the B form (Wing et al., 1981). Finally, it should be noted that calculations of base stacking energies by the method of Ornstein et al. (1981) predict that the B form of the sequence AATT is more stable than that of TATA (W. L. Peticolas and Y. Wang, unpublished results).

The conformationally sensitive backbone vibrational bands in the 800–900-cm^{-1} region are stronger in the Raman than in the IR although they occur at the same frequency. This fact and the fact that water is opaque in this frequency region to IR radiation require the use of the Raman effect to study the conformation of $d(A_5T_5)$ and poly(dA)·poly(dT) in aqueous solution. The use of the Raman band at 813 ± 4 cm^{-1} to determine the amount of C3′-endo furanose ring pucker in DNA is well-known (Erfurth et al., 1972, 1975). Thus as discussed above, we have used poly(rA)·poly(dT) as a model for a fully heteronomous conformation. Table II shows the results of the measurements of the peak heights and the peak areas for the Raman conformational marker band at 813 ± 4 cm^{-1} in a number of AT-containing DNAs. We will assume that the conformation described by Zimmerman and Pheiffer (1981) is the conformation of poly(rA)·poly(dT) in solution. By this we mean that it is essentially a B-genus DNA with the exception of the furanose rings of the adenine strand that are in the C3′-endo conformation. We take the area of the peak height of the 813 ± 4 cm^{-1} marker band to estimate the fraction of heteronomous sequence in a DNA using the area of the 1098-cm^{-1} band (the phosphate dioxysymmetric stretch) as an internal standard. Table II shows the results with the percentage of the double helix in the heteronomous form noted in the table. It must be stated that this measurement gives the average fraction of the heteronomous forms on the basis of the Raman time scale, which is very fast. Note that only half of the heteronomous conformation involves a furanose ring with the C3′-endo ring pucker. Thus if the percentage of the heteronomous conformation is 30% as indicated in Table II for poly(dA)·poly(dT), then the percentage of furanose rings is only 15%. This very low percentage of C3′-endo furanose conformation may be the reason that the marker band at 809 cm^{-1} for C3′-endo that is normally very weak in the IR is not found in the IR spectrum of films of poly(dA)·poly(dT) at high humidity—the concentration of the C3′-endo ring puckers is too low. Also, it may be that the A-genus IR marker band at 860 cm^{-1} that is missing in the low-humidity spectrum of $d(A_5T_5)$ and poly(dA)·poly(dT) may be a mode that is not directly sensitive to the C3′-endo ring pucker but rather to another structural parameter of the canonical A-genus DNA helix. The A-genus DNA is characterized by a number of differences in torsional angles other than furanose ring pucker; in addition, the base stacking interactions are distinctly different in the two forms. As may be emphasized, the heteronomous form should probably be considered as belonging to the B genus but with a C3′-endo ring pucker for the adenine chain (Zimmerman & Pheiffer, 1983).

Table II also shows that poly(dA)·$p(dT)_{10}$ has a higher percentage of heteronomous structure than poly(dA)·poly(dT), which we think is due to mainly two factors. First, although the complex was made with stoichiometric equivalent bases of adenine and thymine, there may still be single-stranded adenine regions that tend to adopt C3′-endo conformation. On the other hand, the complex made by a high molecular weight polymer and equal moles of oligomers would be expected to be more conformationally flexible. The result also indicates that it is the poly(dT) strand that stays preferentially in C2′-endo in the heteronomous structure.

In addition to using the Raman marker band arising from the backbone vibration, we have also analyzed the 1340-cm^{-1} band of adenine in these materials. The presence of two bands in this region in poly(dA)·poly(dT) and only one band in this region in poly(rA)·poly(dT) and A-form poly(dA-dT) supports our models for the conformations of these polymers in solution. If all of the adenines in poly(rA)·poly(dT) are attached to furanose rings with a C3′-endo/anti conformation, only one band is expected and only one band is observed since all of the adenine bases are in the same environment. On the other hand, the existence of two bands, one at 1336 cm^{-1} and one at 1343 cm^{-1} in poly(dA)·poly(dT), is very strong evidence that there is some degree of heterogeneity in the poly(dA)·poly(dT) conformation in aqueous solution on the Raman time scale since this band shifts frequency on going from a B-type to an A-type DNA (Kitahira et al., 1986). Using the intensity of the adenine band as described above, we obtain 30% for the percentage of heteronomous conformation in both poly(dA)·poly(dT) and $d(A_5T_5)$. This figure of 30% from the intensity of the adenine vibration is in good agreement with the value obtained from the intensity of the sugar–phosphate band at 813 ± 4 cm^{-1} as shown in Table II. However, 30% is lower than the percentage obtained from the intensity of the backbone vibration for $d(A_5T_5)$, which varies from 37 to 43% depending on whether the height or the area of the backbone vibration is used (see Table II). This difference could be due to the fact that part of the intensity of the band

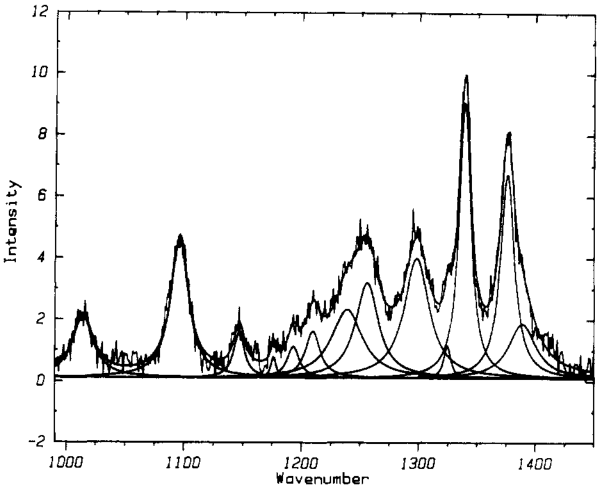

FIGURE 10: Raman spectrum of poly(rA)·poly(dT) taken under the conditions of Figure 9 expanded to exhibit the frequency region 1050–1450 cm^{-1}. The spectrum is resolved into the minimum number of Lorenztian bands.

at 813 ± 4 cm^{-1} is due to a C2′-endo ring pucker with an anomalously high value of the β torsional angle as has recently been suggested by Katahira et al. (1986). These authors also attempted to estimate the degree of C3′-endo furanose ring pucker, and they obtained an estimate of 10% for the fraction of adenosine residues with furanose rings in the C3′-endo/anti conformation. However, it must be noted that these authors did not use a quantitative analysis of the band areas or height but made only a semiqualitative estimate from a visual examination of the appearance of a shoulder at 1336 cm^{-1} on the band at 1443 cm^{-1}. Our curve resoluton shows that the band at 1336 cm^{-1} is more important, and hence, it appears curve resolution gives a better quantitative estimate of the degree of conformational heterogeneity in these polymers.

These results appear to be in disagreement with some recent theoretical results. In order to obtain their assignments of some of the band intensity at 813 ± 4 cm^{-1} to a combination of C2′ endo with a high value for the β torsional angle, Katahira et al. (1986) have used X-ray-determined conformations of mononucleotide cations with double negative charges for conformational studies. Their results on these mononucleotide doubly charged cations are quite reasonable, but the attempt to extend these conclusions to dinucleotides or polynucleotides with a single charge per residue and a different arrangement of orders is, in our view, perilous, because the normal modes of the isolated doubly charged mononucleotides and the singly charged mononucleotides that occur in oligonucleotide chains are not necessary the same. It also may be noted that the results in this work are at variance with theoretical energy minimum calculations of Rao and Kollman (1985). The assignment of the C3′-endo ring pucker in this paper to the adenine-containing strand in the heteronomous conformation does not agree with their theoretical calculation, which puts the C3′-endo ring pucker on the thymine-containing strand.

Finally it may be stated that IR spectroscopy indicates that both poly(dA)·poly(dT) and d(A_5T_5) exhibit a fully heteronomous conformation in films at low humidities were poly(dA-dT) is in the fully A-type conformation. This is the first experimental evidence that appears in direct agreement with the X-ray diffraction studies on fibers of poly(dA)·poly(dT). In aqueous solution, Raman bands due to adenosine vibrations indicate that about 30% of the furanose rings attached to adenine are in the C3′-endo/anti conformation. This corresponds to 15% of the total number of deoxyribose rings in the polymer or oligomer, and it may be that these conformations are in a constant state of change with Raman results giving only the averge on the Raman time scale.

REFERENCES

Adem, S., Liquier, J., Taboury, J. A., & Taillandier, E. (1986) *Biochemistry 25*, 3220–3225.

Arnott, S., Chandrasekaren, R., Hall, I. H., & Puigjamer, L. C. (1983) *Nucleic Acids Res.*, *11*, 4141–4155.

Arnott, S., Chandrasekaran, R., Millane, R. P., & Park, H. S. (1986) *J. Mol. Biol. 188*, 631–640.

Behling, R. W., & Kearns, D. R. (1986) *Biochemistry 25*, 3335–3346.

Brahms, S., Brahms, J., & Pilet, J. (1974) *Isr. J. Chem. 12*, 153–163.

Chazin, W. J., Wuthrich, K., Hyberts, S., Rance, M., Denny, W. A., & Leupin, W. D. (1986) *J. Mol. Biol. 190*, 439–453.

Denny, W. A., Leupin, W., & Kearns, D. R. (1982) *Helv. Chim. Acta 65*, 2372–2393.

Dickerson, R. E., & Drew, H. R. (1981) *J. Mol. Biol. 149*, 761–786.

Erfurth, S. C., Kiser, R. J., & Peticolas, W. L. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 938–941.

Erfurth, S. C., Bond, P. J., & Peticolas, W. L. (1975) *Biopolymers 14*, 1245–1257.

Jolles, B., Laigle, A., Chinsky, L., & Turpin, P. Y. (1985) *Nucleic Acids Res. 13*, 2075–2085.

Katahira, M., Nishimura, Y., Tsuboi, M., Sato, T., Mitsur, Y., & Iitaka, Y. (1986) *Biochim. Biphys. Acta* (in press).

Kennard, O., Cruse, W. B. T., Nachmon, J., Prage, T., Shakked, Z., & Rabinovich, D. (1981) *J. Biomol. Struct. Dyn. 3*, 623–647.

Kubasek, W. L., Wang, Y., Thomas, G. A., Patapoff, T. W., Schoenwaelder, K. H., van der Sande, J. H., & Peticolas, W. L. (1986) *Biochemistry 25*, 7440–7445.

Nichimura, Y., Torigoe, C., & Tsuboi, M. (1986) *Nucleic Acids Res. 14*, 2737–2748.

Ornstein, R. L., Rein, R., Breen, D. L., & MacElroy, R. D. (1978) *Biopolymers 17*, 2341–2360.

Pilet, J., Blicharski, J., & Brahms, J. (1975) *Biochemistry 14*, 1869–1876.

Pohle, W., & Fritzsche, M. (1980) *Nucleic Acids Res. 8*, 2527–2535.

Rao, S. N., & Kollman, P. (1985) *J. Am. Chem. Soc. 107*, 1611–1617.

Sarma, M. H., Gupta, G., & Sarma, R. H. (1985) *J. Biomol. Struct. Dyn. 2*, 1057–1084.

Schmidt, E. R. (1984) *J. Mol. Biol. 178*, 1–15.

Small, E. W., & Peticolas, W. L. (1971) *Biopolymers 10*, 69–88, 1377–1416.

Taboury, J. A., Liquier, J., & Taillandier, E. (1985) *Can. J. Chem. 63*, 1904–1909.

Taillandier, E., Fort, L., Liquier, J., Coupez, M., & Sautiere, P. (1984) *Biochemistry 23*, 2644–2650.

Taillandier, E., Liquier, J., & Taboury, J. A. (1985) *Adv. Infrared Raman Spectrosc. 12*, 64–114.

Thomas, G. A., & Peticolas, W. L. (1983) *J. Am. Chem. Soc 105*, 993–996.

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

Wartell, R. M., & Harrell, J. T. (1986) *Biochemistry 25*, 2664–2671.

Wing, R., Drew, H., Takano, T., Broka, V., Tanaka, S., Itakura, K., & Dickerson, R. E. (1981) *Nature* (*London*) *287*, 755–758.

Zimmerman, S. B., & Pheiffer, B. H. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 78–82.