Right- and Left-Handed Helixes of Poly[d(A-T)]·Poly[d(A-T)] Investigated by Infrared Spectroscopy

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ABSTRACT: The secondary structures of double-stranded poly[d(A-T)]·poly[d(A-T)] in films have been studied by IR spectroscopy with three different counterions (Na^+, Cs^+, and Ni^{2+}) and a wide variety of water content conditions (relative humidity between 100 and 47%). In addition to the A-, B-, C-, and D-form spectra, a new IR spectrum has been obtained in the presence of nickel ions. The IR spectra of Ni^{2+}–poly[d(A-T)]·poly[d(A-T)] films are analyzed by comparison with previously assigned IR spectra of left-handed poly[d(G-C)]·poly[d(G-C)] and poly[d(A-C)]·poly[d(G-T)], and it is possible to conclude that they reflect a Z-type structure for poly[d(A-T)]·poly[d(A-T)]. The Z conformation has been favored by the high polynucleotide concentration, by the low water content of the films, and by specific interactions of the transition metal ions with the purine bases stabilized in a syn conformation. A structuration of the water hydration molecules around the double-stranded Ni^{2+}–poly[d(A-T)]·poly[d(A-T)] is shown by the presence of a strong sharp water band at 1615 cm^{-1}.

Three double-helical conformations have been observed by X-ray crystal diffraction at atomic resolution in short DNA oligomers: the B, A, and Z family structures (Wang et al., 1979; Dickerson et al., 1981). The interconversion from right-handed B to left-handed Z conformation of poly[d(G-C)]·poly[d(G-C)] has been extensively studied and obtained in numerous experimental conditions [for review, see Rich et al. (1984)]. When A-T base pairs are introduced in the DNA sequence, the induction of Z DNA becomes more difficult. The fact that [d(A-C)]·[d(G-T)] tracts can form Z DNA is supported by immunological and two-dimensional gel electrophoretic studies performed on negatively supercoiled plasmids (Nordheim et al., 1982; Haniford & Pulleyblank, 1983; Nordheim & Rich, 1983): segments of DNA containing A-T base pairs form Z DNA. In the case of the regularly alternating polynucleotide poly[d(A-C)]·poly[d(G-T)], the Z conformation is not observed in the presence of Na^+ counterions (Vorlickova et al., 1982) but is obtained through specific interactions with divalent ions in films (Taillandier et al., 1984) and in solution (Taboury & Taillandier 1985), by covalent modifications of the bases or binding of 2-(acetylamino)fluorene (AAF) (Wells et al., 1982; Mc-Intosh et al., 1983). Recently, several oligonucleotidic structures containing A-T base pairs have been studied by X-ray crystal diffraction [d(m^5C-G-T-A-m^5C-G); Wang et al., 1984], Raman spectroscopy [d(C-G-C-A-T-G-C-G) and d(m^5C-G-T-A-m^5C-G); Benevides et al., 1984], and IR spectroscopy [d(C-am^2A-C-G-T-G) and d(m^5C-G-C-A-m^5C-G-T-G-C-G); Taboury et al., 1984a]. All these studies show that these oligonucleotides containing A-T base pairs can acquire a Z conformation.

Double-stranded poly[d(A-T)]·poly[d(A-T)] is capable of adopting a wide variety of helical conformations. Results obtained by fiber X-ray diffraction (Davies & Baldwin, 1963; Arnott et al., 1974; Arnott & Selsing, 1975; Leslie et al., 1980; Gupta et al., 1980; Drew & Dickerson, 1982; Mahendrasingam et al., 1983), Raman spectroscopy (Small & Peticolas, 1971; Thomas & Peticolas, 1983; Thomas & Benevides, 1985), and infrared dichroism (Pilet et al., 1975; Brahms et al., 1976) have been interpreted in terms of classical right-handed helical conformations.

Modified poly[d(A-T)]·poly[d(A-T)] poly[d(am^2A-T)]·poly[d(am^2A-T)] (Howard et al., 1984) and poly[d(A-s^4T)]·poly[d(A-s^4T)] (Arnott et al., 1980; Jovin et al., 1983) undergo cooperative conformational transitions interpreted in terms of B → Z helical transitions. Nevertheless, at the present time there seems to be no experimental observation of unmodified poly[d(A-T)]·poly[d(A-T)] in Z conformation.

In this work we investigate the secondary structures of poly[d(A-T)]·poly[d(A-T)] by IR spectroscopy. As expected, the helical conformation of poly[d(A-T)]·poly[d(A-T)] depends on the cation species and on their amounts present in the films as well as on the hydration. In addition to the classical IR spectra of right-handed conformations, a completely new IR spectrum of poly[d(A-T)]·poly[d(A-T)] is obtained in the presence of Ni^{2+} and discussed by comparison with IR data of Z helixes of poly[d(G-C)]·poly[d(G-C)] (Taillandier et al., 1981; Pilet & Leng, 1982; Taillandier et al., 1985; Taboury et al., 1985) and of poly[d(A-C)]·poly[d(G-T)] (Taillandier et al., 1984). This leads us to propose that the B → Z transition can be induced in poly[d(A-T)]·poly[d(A-T)] films by the presence of transition metal ions. This result may have interesting biological implications, as A-T-rich regions have been invoked as functional domains in eukaryotic DNA (Moreau et al., 1981, 1982).

Materials and Methods

Poly[d(A-T)]·poly[d(A-T)] was purchased from P-L Biochemicals (lots 658/92 and 317.810), dissolved in 2 M NaCl, pH 7, for 24 h, and ethanol-precipitated after a dialysis against 0.1 M NaCl, pH 7. The precipitate was redissolved in a NaCl solution so as to obtain the desired amount of added Na^+ (in a range varying between 0.5 and 3 Na^+ in excess per nucleotide). DNA concentration was typically 15 mM. The polynucleotide was studied in films prepared by slow evaporation of this solution on a ZnSe plate. Ni^{2+}–poly[d(A-T)]·poly[d(A-T)] samples were obtained by diffusion of a droplet of $NiCl_2$ solution in the film followed by slow evapo-

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ration. Cs^+–poly[d(A-T)]·poly[d(A-T)] samples were prepared by dissolving the polynucleotide in 0.1 M CsCl, pH 7, followed by ethanol precipitation. The precipitate was redissolved in CsCl so as to obtain the desired amount of added Cs^+ ions.

Films were placed in cells with controlled relative humidity (H_2O or D_2O). IR spectra were recorded with a Perkin-Elmer 180 double-beam spectrophotometer coupled to a Hewlett-Packard 9825A calculator. Data treatment has been performed following the procedure previously described (Taboury et al., 1985).

Results

For films estimated to contain one Na^+ per nucleotide, the IR spectra are correlated with a B-type geometry at high relative humidities (i.e., above 93% RH) and with an A-type geometry at low relative humidities (i.e., below 76%).

Transitions between the A and B forms of Na^+–poly[d(A-T)]·poly[d(A-T)] depend on the salt content of the samples. When the Na^+ content is decreased (typically 0.5 Na^+ per nucleotide), the B → A transition is no longer observed. Instead of A-type spectra, slightly altered B-form spectra are recorded at low humidities. Such conditions are those described to be observed by fiber x-ray diffraction of the Na^+–poly[d(A-T)]·poly[d(A-T)] blocked in the D conformation (Davies & Baldwin, 1963; Mahendrasingam et al., 1983). Although the spectra recorded at high and low relative humidity, in presence of Cs^+ ions, are only slightly different, a detailed analysis shows that, in fact, continuous smooth changes occur gradually between the B and C family conformations of Cs^+–poly[d(A-T)]·poly[d(A-T)] (results to be published).

In spite of the marked propensity of poly[d(A-T)]·poly[d(A-T)] to remain in a B genus conformation under conditions where poly[d(G-C)]·poly[d(G-C)] converts to Z DNA, Ni^{2+}–poly[d(A-T)]·poly[d(A-T)] spectra are found to be extremely different when compared to B-form spectra and similar to Z-form spectra. We are going to present in detail different spectral regions sensitive to the B → Z conformational changes.

Phosphodiester Backbone Vibrations. The IR absorption bands between 1000 and 750 cm^{-1} corresponding to sugar–phosphodiester chain vibrations are presented in Figure 1. Spectrum a in Figure 1 (one Na^+ per nucleotide, RH 76%) presents absorption bands at 876, 862, and 807 cm^{-1}, characteristic of an A conformation (C3′-endo/anti geometry).

Spectrum b of Figure 1 presents a band at 840 cm^{-1} reflecting a B conformation (C2′-endo/anti geometry) (Brahms et al., 1974). With the spectral resolution used in this work, we obseve that the absorption involving the phosphodiester chain vibration located at 966 cm^{-1} in high relative humidity conditions (B form) is split into three components found at 975, 970, and 953 cm^{-1} in low relative humidity conditions (A form). In the case of a double-helical structure, the theory predicts that the dipolar interactions between adjacent groups of the same and opposite strands will induce the splitting of the absorptions involving these groups into three components (Higgs, 1953). With this assumption, the greater distance between the phosphate groups in B form when compared to the distance in A form minimizes the interactions in the B geometry, and on the IR spectrum only one absorption is detected at 966 cm^{-1} (Figure 1b). Figure 1c presents the IR spectrum of a sample containing 0.5 Na^+ per nucleotide, which clearly differs from the A-form spectrum (no bands at 876, 862, and 807 cm^{-1}) but not significantly from the B-form spectrum. The two absorptions located at 894 and 840 cm^{-1} in the B-form spectrum are broadened and slightly shifted to

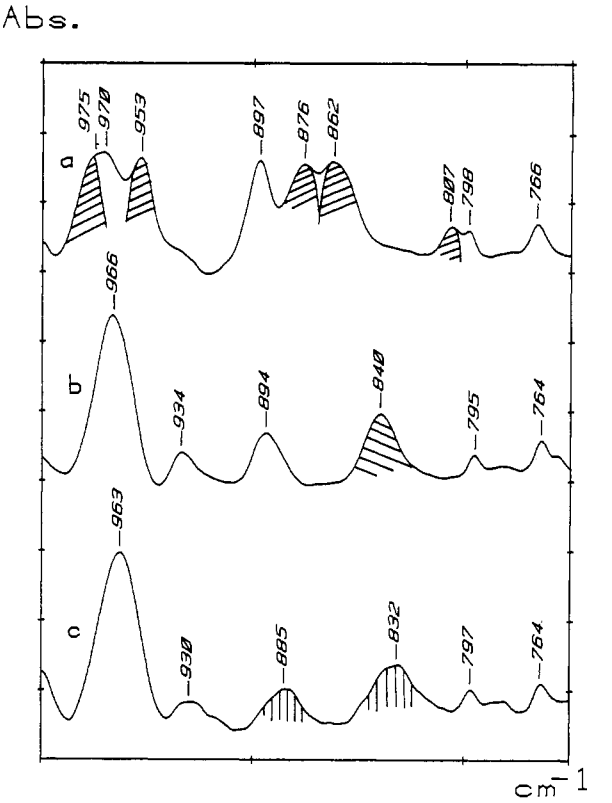

FIGURE 1: Infrared spectra in the phosphodiester chain vibration region of poly[d(A-T)]·poly[d(A-T)] films: (a) 1 Na^+ per nucleotide, RH 71% (A form); (b) 1 Cs^+ per nucleotide, RH 86% (B form); (c) 0.5 Na^+ per nucleotide, RH 71% (D form). Absorptions characteristic of each conformation are hatched.

885 and 832 cm^{-1}, respectively, by decreasing the relative humidity to 71% (D form).

The addition of Ni^{2+} to poly[d(A-T)]·poly[d(A-T)] induces important modifications (Figure 2c). The intensity of the band located around 895 cm^{-1} (Figure 1) characteristic of right-handed helixes is strongly decreased, and the set of absorptions detected in the Ni^{2+}–poly[d(A-T)]·poly[d(A-T)] spectrum (Figure 2c) (928, 864, and 838 cm^{-1}) is similar to the set of bands characteristic of the Z conformation in Na^+–poly[d(G-C)]·poly[d(G-C)] (Figure 2a) and Ni^{2+}–poly[d(A-C)]·poly[d(G-T)] (Figure 2b). The coexistence of both absorptions at 864 and 838 cm^{-1} may reflect the dinucleotidic repeat unit of the structure with differences in the local geometries between the ApT and TpA units, in good agreement with the two ^{31}P NMR peaks observed for other alternating purine–pyrimidine left-handed geometries (Patel et al., 1979; Cohen et al., 1981; Mc-Intosh et al., 1983) and the dinucleotidic repeat unit shown to exist in the Z conformation by X-ray crystal diffraction (Wang et al., 1979).

The absorption bands at 1065 and 1018 cm^{-1} see their intensities strongly enhanced in the Ni^{2+}–poly[d(A-T)]·poly[d(A-T)] spectra as in the case of the Z-form spectra of the other regular polymers with simple alternation of purine–pyrimidine sequences. Moreover, the antisymmetric stretching phosphate vibration is detected at 1212 cm^{-1} (−15 cm^{-1} with respect to the B geometry, −28 cm^{-1} with respect to the A geometry) while the symmetric stretching vibration can be decomposed into two components at 1094 and 1088 cm^{-1} (Table I), in a similar way as previously described for poly[d(G-C)]·poly[d(G-C)] and poly[d(A-C)]·poly[d(G-T)] (Taillandier et al., 1984; Taboury et al., 1985).

Base, Deoxyribose, and Glycosidic Linkage Vibrations. Figure 3 shows the IR absorption spectra between 1550 and

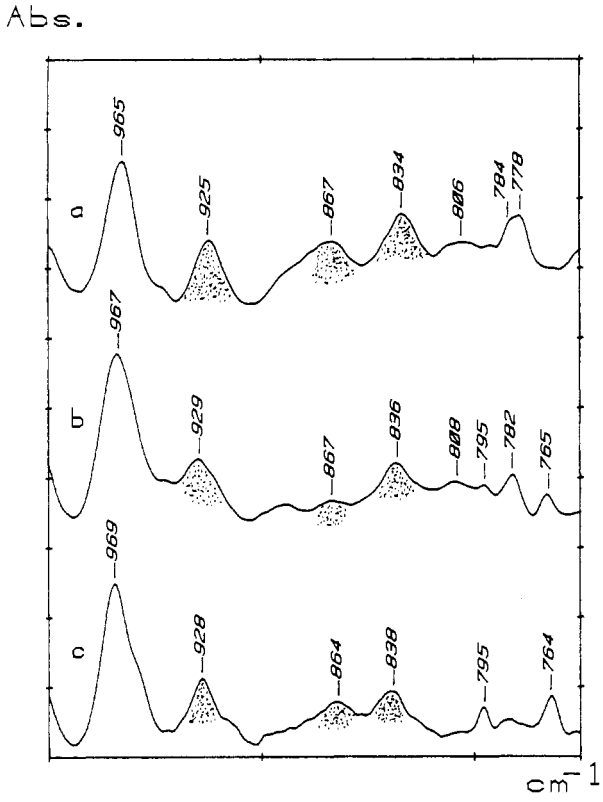

FIGURE 2: Infrared spectra in the phosphodiester chain vibration region of Z conformation of (a) poly[d(G-C)]·poly[d(G-C)], 1 Na^+ per nucleotide, RH 93%, (b) poly[d(A-C)]·poly[d(G-T)], 1 Ni^{2+} per nucleotide, RH 76%, and (c) poly[d(A-T)]·poly[d(A-T)], 2 Ni^{2+} per nucleotide, RH 76%. Absorptions characteristic of the Z conformation are dotted.

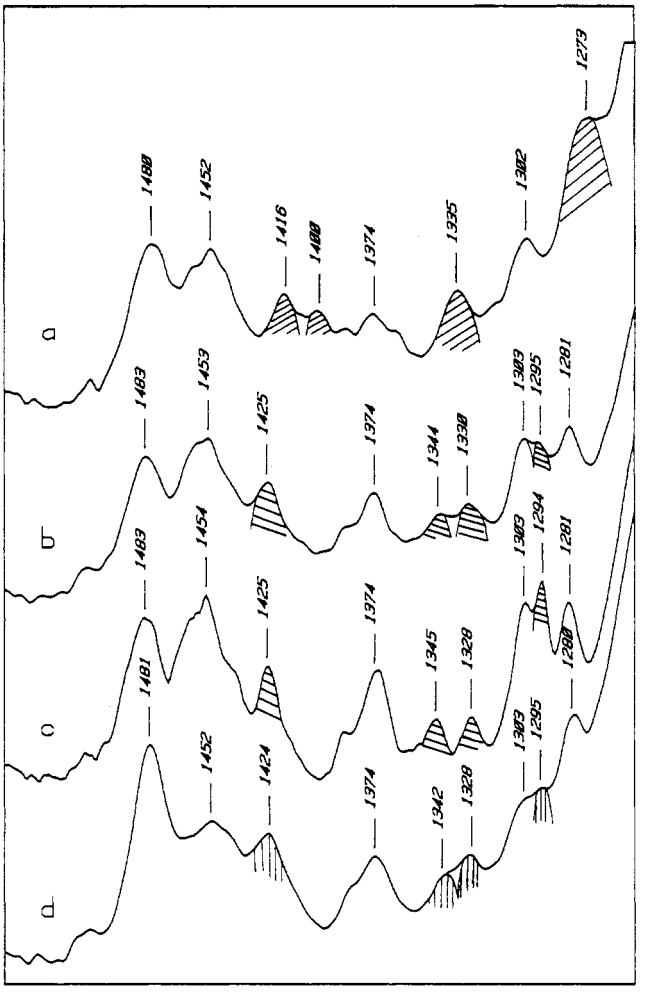

FIGURE 3: Infrared absorption spectra of poly[d(A-T)]·poly[d(A-T)] films in the spectral region of the base, deoxyribose, and glycosidic linkage vibrations: (a) 1 Na^+ per nucleotide, RH 71% (A form); (b) 1 Cs^+ per nucleotide, RH 86% (B form); (c) 1 Cs^+ per nucleotide, RH 58% (C form); (d) 0.5 Na^+ per nucleotide, RH 71% (D form). Absorptions characteristic of each conformation are hatched.

1250 cm^{-1} of poly[d(A-T)]·poly[d(A-T)] with one Na^+ per nucleotide at 71% RH and at 98% RH, one Cs^+ per nucleotide at 71% RH, and 0.5 Na^+ per nucleotide at 71% RH, respectively, conditions in which the A, B, C, and D forms have been characterized. In this spectral region, appreciable coupling should occur between the in-plane adenine vibrations and some of the deoxyribose vibrations. Thus, the wavenumbers of the IR bands may vary, depending upon whether the glycosidic linkage is syn or anti and depending upon whether the furanose ring pucker is C2′-endo or C3′-endo. As expected, the three spectra of the B, C, and D forms (all with C2′-endo/anti conformation) are very similar as far as the positions of the IR bands are concerned and only slightly modified when the relative intensities are considered (hypochromism of the 1452-cm^{-1} band in the D form and hyperchromism of the 1294-cm^{-1} band in the C form). In contrast, the A-form spectrum is different from those of the B genus. Bands around 1425, 1344, and 1330 cm^{-1} appear in the spectra of the B genus (C2′-endo/anti conformation) and at 1416, 1400, and 1335 cm^{-1} in the A-form spectrum (C3′-endo/anti conformation). We must notice the existence in all spectra presented in Figure 3 of an absorption located at 1374 cm^{-1}. This band is characteristic of the purine bound to a sugar in anti conformation (Ghomi et al., 1984). It is observed in the B form of poly[d(G-C)]·poly[d(G-C)] and in A and B forms of poly[d(A-C)]·poly[d(G-T)] (Taillandier et al., 1984).

When Ni^{2+} ions are added to poly[d(A-T)]·poly[d(A-T)], a new spectrum is observed (Figure 4c). The presence of nickel ions induces a shift of the 1425-cm^{-1} band involving the deoxyribose moiety to 1409 cm^{-1} and of the 1374-cm^{-1} absorption involving the C–N linkage to 1357 cm^{-1}, whereas a band around 1320 cm^{-1} is detected. This spectrum is compared with those of Z-form Ni^{2+}–poly[d(A-C)]·poly[d(G-T)] (Figure 4b) and Poly[d(G-C)]·poly[d(G-C)] (Figure 4a). The corresponding wavenumbers and assignments are summarized Table II.

We have previously shown that the C2′-endo/anti–C3′-endo/syn reorientation of the purine nucleoside upon the B → Z transition is responsible for similar effects in poly[d(G-C)]·poly[d(G-C)] and poly[d(A-C)]·poly[d(G-T)] spectra (see table II) (Taillandier et al., 1984; Taboury et al., 1985). In addition, an absorption of the A-T base pairs in Z geometry was found at 1434 cm^{-1} in poly[d(A-C)]·poly[d(G-T)] spectra. It is observed at 1438 cm^{-1} in the case of Ni^{2+}–poly[d(A-T)]·poly[d(A-T)] (Figure 4c).

In-Plane Double Bond Stretching Vibrations. In H_2O, the A and B helixes present an absorption located above 1700 cm^{-1} (between 1716 and 1702 cm^{-1} depending on the conformation) (Table I). In the presence of nickel ions, a shift to lower wavenumbers takes place; the corresponding absorption is now observed at 1690 cm^{-1}. Similar observations had been made upon the B → Z transition in the spectra of Na^+–poly[d(G-C)]·poly[d(G-C)] and Ni^{2+}–poly[d(A-C)]·poly[d(G-T)] (Taillandier et al., 1981; Pilet & Leng, 1982; Taillandier et al., 1984) and may reflect in these two latter cases the unstacking of the guanines in the Z form compared to the B form in agreement with the data obtained by X-ray diffraction on d(C-G) crystals. This result is therefore consistent with the

Table I: IR Absorption Bands Observed for Different Poly[d(A-T)]·Poly[d(A-T)] Conformations[a]

A form	B form	C form	D form	Z form	assignment
1708, s	1716, s	1712, s	1702, s	1690, m	in-plane double bond stretching of the bases
1690*	1692*		1690*	1695*	
1665*	1665*		1666*	1663*	
1663, s	1663, s	1665, s	1667, s	1660, s	
	1642*			1629*	
1619*	1618*			1615, s	
1605, m	1608, m	1609, ms	1608, m		
1575, w	1580, w		1577, w		
				1495, mw	
1480, m	1483, m	1483, m	1481, m	1483, mw	T, A
1452, m	1453, m	1454, m	1452, m		T
				1438, mw	
	1425, m	1425, m	1424, m		A
1416, mw					
				1408, mw	A
1400, mw					
1387, sh	1387, sh	1389, sh	1387, sh		
1374, mw	1374, mw	1374, mw	1374, mw		A
1364, w					
				1357, w	A
	1344, mw	1345, mw	1342, mw		A
1335, mw					
	1330, mw	1328, mw	1328, mw	1326, w	
1302, mw	1303, mw	1303, mw	1303, mw		A
	1295, mw	1294, mw	1295, mw		
1273, sh	1281, mw	1281, mw	1280, mw	1278, mw	T
1241, s	1226, s	1226, s	1230, s	1212, s	ν as PO_2^-
1188, m	1167, w	1165, w	1168, w		
1125, sh					
1089, s	1089, s	1089, s	1092, s	1094–1088, s	ν as PO_2^-
		1065 s	1065, s	1065, s	
1052, m	1052, m	1048, s			
			1022, m		
1010, w	1014, w	1014, w	1014, w	1018, m	deoxyribose
975, mw					
970, mw	966, m	962, m	963, m	969, mw	
953, mw					deoxyribose and phosphodiester backbone
	934, mw	930, mw			
				928, mw	
897, mw					
876, w	894, mw	887, mw	885, mw		
862, mw					
				864, w	
	840, mw	834, mw	832, m	838, w	
807, w		795, w	797, w		out of plane base vibrations
798, w	795, w	781, w	781, w	795, w	
766, w	764, w	764, w	764, w	764, w	

[a] S = strong; m = medium; sh = shoulder; w = weak; (*) = in D_2O; T = thymine; A = adenine.

Table II: Comparison of Characteristic IR Absorptions of Three Alternating Purine–Pyrimidine Polynucleotides in B and Z Geometries

poly[d(G-C)]		poly[d(A-C)-d(G-T)]		poly[d(A-T)]		
B	Z	B	Z	B	Z	assignment
			1434		1438	dA
1420		1423		1425		deoxyribose C–N linkage
	1409		1412		1408	
1374		1374		1374		deoxypurine anti
	1354		1354		1357	deoxypurine syn
	1318		1322	1330	1326	deoxypurine

assumption that Ni^{2+}–poly[d(A-T)]·poly[d(A-T)] adopts a Z-type conformation.

On the IR spectra of Ni^{2+}–poly[d(A-T)]·poly[d(A-T)], we observe a band located at 1615 cm^{-1}. The sharpness and intensity of this band, together with the fact that it is shifted to 1200 cm^{-1} by deuteration of the sample, show that it can be assigned to the scissoring vibration of the water molecules coordinated to the Ni^{2+} ions. This band reflects the presence of a highly organized water structure around the Ni^{2+}–poly[d(A-T)]·poly[d(A-T)].

Discussion

In aqueous and fibrous poly[d(A-T)]·poly[d(A-T)], a large number of different and occasionally contradictory structures have been proposed [see Gupta et al. (1983) for discussion]. However, at present, no spectroscopic evidence of the existence

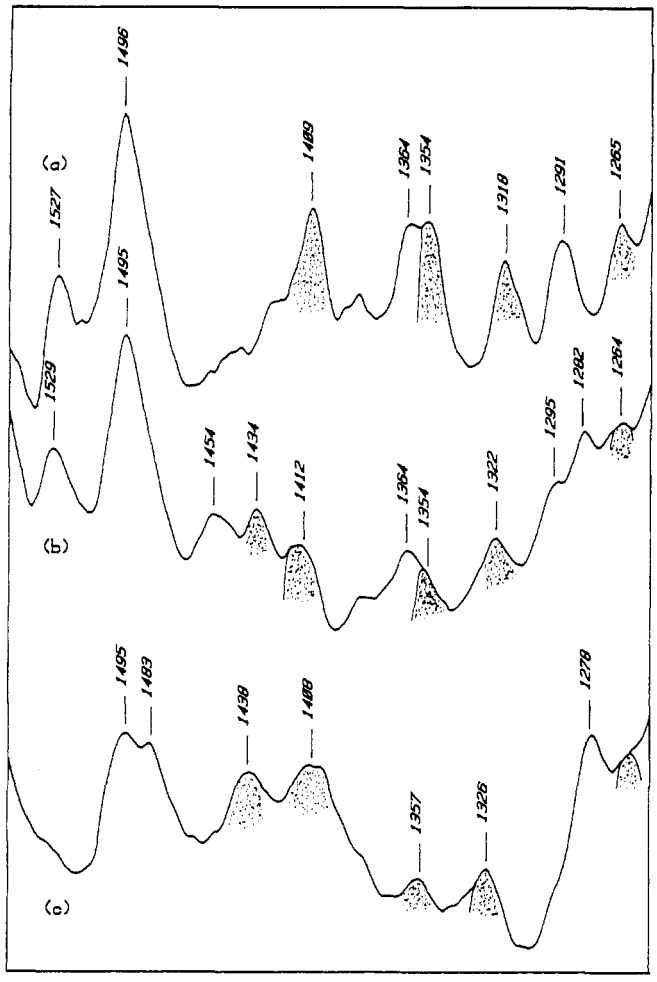

FIGURE 4: Infrared absorption spectra in the region of the base, deoxyribose, and glycosidic linkage vibrations of Z conformation of (a) poly[d(G-C)]·poly[d(G-C)], 1 Na^+ per nucleotide, RH 93%, (b) poly[d(A-C)]·poly[d(G-T)], 1 Ni^{2+} per nucleotide, RH 76%, and (c) poly[d(A-T)]·poly[d(A-T)], 2 Ni^{2+} per nucleotide, RH 76%.

of Z poly[d(A-T)]·poly[d(A-T)] has been obtained (Rich et al., 1985). In this work, besides A-, B-, C-, and D-form IR spectra, we present a new spectrum obtained by addition of Ni^{2+} ions to the polynucleotide. The similar spectral shifts and changes of relative intensities of many bands as compared to poly[d(G-C)]·poly[d(G-C)] and poly[d(A-C)]·poly[d(G-T)] indicate that the B → Z transition is likely to occur in poly-[d(A-T)]·poly[d(A-T)].

Different reasons can be given to explain the detection of the left-handed Z geometry of Ni^{2+}–poly[d(A-T)]·poly[d(A-T)]. We have previously shown by using selectively deuterated poly[d(G-C)]·poly[(G-C)] on the C8 site of guanines that divalent transition metal ions such as Co^{2+} and Ni^{2+} interacting specifically with the N7 site of the base tend to favor the syn conformation of the purine and, hence, induce the Z structure of poly[d(G-C)]·poly[d(G-C)] (Taboury et al., 1984b). This specific interaction is responsible for the high efficiency of these ions to induce the Z conformation of the polynucleotide: the B → Z transition of poly[d(G-C)]·poly[d(G-C)] in solutions is obtained at submillimolar $NiCl_2$ concentrations (typically 0.4 mM) (Van de Sande et al., 1982; Liquier et al., 1984). The same interaction between the transition metal ions and the purines can be proposed in the case of poly[d(A-T)]·poly[d(A-T)] stabilizing the syn conformation of the purines and favoring the existence of a Z helix. We must notice that when the Z form of poly[d(A-T)]·poly[d(A-T)] is observed, a high structuration of the hydrating water molecules is detected around the Ni^{2+} ions (sharp band found at 1615 cm^{-1}) possibly reflecting that occurring around the DNA. It has been proposed that the relative instability of the A-T base pairs in Z DNA as compared to G-C base pairs might be due to a greater difficulty to order the water molecules at the level of the A-T base pairs (Wang et al., 1984). The presence of nickel might contribute to the organization of the water shell around the DNA. The high polynucleotide concentration, which can be used in the IR studies (and which is closer to the "in vivo" DNA concentrations found in the cell nuclei than those used for UV or CD experiments), also favors the induction of the Z conformation, as shown by the earlier studies concerning the Z conformation of poly[d(A-C)]·poly[d(G-T)] (Taillandier et al., 1984; Taboury & Taillandier, 1985), d(m^5C-G-A-m^5C-G-T-G-C-G), and d(C-am^2A-C-G-T-G) (Taboury et al., 1984a): in dilute solutions (CD measurements), the Z conformation is not obtained for these short oligonucleotides. In more concentration NMR solutions, the Z proportion induced by high Na^+ content is only 20–25%, whereas the Z conformation is stabilized in low-humidity films. Finally, it seems that high DNA concentration, by increasing the formation of specific interactions on the N7 site of the purines, contributes to the stabilization of the syn conformation of the dA residues and favors the left-handed Z-type structure observed for Ni^{2+}–poly[d(A-T)]·poly[d(A-T)].

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DNA Fragmentation and Cytotoxicity from Increased Cellular Deoxyuridylate†

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ABSTRACT: Previous results from this laboratory have shown that thymidylate deprivation results in dramatic elevation of intracellular dUTP and incorporation of dUMP into DNA. The goal of the present studies was to determine whether the latter changes may play a part in the associated cytotoxicity ("thymineless death"), which is ordinarily assumed to be a direct result of reduced intracellular dTTP. The approach used here was to increase intracellular dUTP without allowing dTTP to diminish and observe the effects on cell viability. dUMP pools were expanded by exposure of cells to deoxyuridine [in cell growth medium containing hypoxanthine, methotrexate, and thymidine (HAT medium)], resulting in accumulation of dUTP to levels that approached those of dTTP, which were at, or higher than, the levels in untreated cells. In conjunction with this the cells became nonviable, and newly synthesized DNA was fragmented, both of which occur with thymidylate depletion and, we assume, result from the active process of excision repair at the many uracil-containing sites in DNA. The results indicate that, although the relative importance of low dTTP remains unknown, elevated dUTP can account for the cytotoxicity caused by thymidine starvation. Most of the "dTTP" measured by the DNA polymerase assay in cells treated with methotrexate (MTX) (plus purine supplement) was, in fact, dUTP, which may explain some previous observations of only modest depression of dTTP in cells treated with MTX or similarly acting drugs.

Limitation of thymidine, caused either indirectly through the action of the dihydrofolate reductase inhibitor MTX[1] (with purine supplementation) or directly by inhibition of thymidylate synthetase by FdUrd, is accompanied by rapid loss of cell viability. These drug-induced equivalents of "thymineless death" have ordinarily been assumed to result from insufficient intracellular concentrations of dTTP although the mechanism

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[1] Abbreviations: dThd, thymidine; dUrd, deoxyuridine; dUTPase, deoxyuridine triphosphatase; EDTA, ethylenediaminetetraacetic acid; FdUrd, 5-fluorodeoxyuridine; HAT, cell growth medium containing hypoxanthine, methotrexate, and thymidine; HPLC, high-pressure liquid chromatography; Hx, hypoxanthine; MTX, methotrexate; P_i, inorganic phosphate; POPOP, 1,4-bis[2-(5-phenyloxazolyl)]benzene; PPO, 2,5-diphenyloxazole; Tris·HCl, tris(hydroxymethyl)aminomethane hydrochloride; Ura, uracil.

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