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Left-Handed Helical Structure of Poly[d(A-C)]·Poly[d(G-T)] Studied by Infrared Spectroscopy†

E. Taillandier,* J. A. Taboury, S. Adam, and J. Liquier

ABSTRACT: Infrared spectroscopic studies demonstrate the ability of poly[d(A-C)]·poly[d(G-T)] to adopt a Z-type conformation. The Z form of the unmodified polynucleotide is induced by Ni^{2+} counterions and not by Na^+. The B → Z equilibrium is shifted at room temperature, in the presence of 1 Ni^{2+}/nucleotide, by an increase in the concentration of poly[d(A-C)]·poly[d(G-T)]. The importance of specific binding of Ni^{2+} ions on the N7 site of purines in the stabilization of the Z form is also discussed.

Left-handed Z DNA is favored in crystals of perfectly alternating $[d(G-C)]_n$ sequences (Wang et al., 1979; Crawford et al., 1980; Drew et al., 1980; Fujii et al., 1982). However, it is predicted from steric considerations that any purine-pyrimidine sequence should be able to adopt a left-handed structure. The $[d(A-C)]_n$·$[d(G-T)]_n$ sequence often occurs in eukaryotic cells (Hamada & Kakunaga, 1982), and possible roles for Z elements in transcriptional activation have been suggested (Rich, 1982). The stabilization of the Z conformation of $[d(A-C)]_n$·$[d(G-T)]_n$ by negative supercoiling has been presented (Nordheim, 1983; Haniford, 1983). It has also been reported by X-ray fiber diffraction that poly(dA-dC)·poly(dG-dT) may occasionally adopt a left-handed helical structure at low humidity (Arnott et al., 1980). However, no UV or CD (circular dichroic) spectra characteristic of the Z conformation have yet been obtained with dilute solutions of the unmodified polymer, even in the presence of very high concentration of various metal ions or with alcoholic solutions (Vorlickova et al., 1982; Zimmer et al., 1982): the negative band at 295 nm of the canonical Z form of poly[d(G-C)]·poly[d(G-C)] has not been observed for poly[d(A-C)]·poly[d(G-T)]. In solution, to promote the Z conformation of poly[d(A-C)]·poly[d(G-T)], in addition to high salt content, covalent modification by (acetylamino)fluorene or high temperature and methylation on the C5 position of cytosine were required (Wells et al., 1982; McIntosh et al., 1983). By using infrared spectroscopy, we show that the unmodified poly[d(A-C)]·poly[d(G-T)] polymer in hydrated films can adopt A, B, or Z conformations depending on the type of counterions and on the degree of hydration. We have found that Ni^{2+} is able to induce in a condensed phase of poly[d(A-C)]·poly[d(G-T)], a Z form similar to that induced in poly[d(G-C)]·poly[d(G-C)], but Na^+ fails to convert poly[d(A-C)]·poly[d(G-T)] into the Z form. An increase in DNA concentration and an increase in cationic binding sites are the two factors that favor the Z conformation.

Materials and Methods

Poly[d(A-C)]·poly[d(G-T)] (lot 719-97) was purchased from P-L Biochemicals. Samples were deposited on ZnSe windows and gently dried so as to give homogeneous films. The desired amount of metal ions is obtained by diffusion of a droplet of the $NiCl_2$ solution followed by slow evaporation. Films are placed in cells with controlled relative humidity (H_2O or D_2O). Hydration of the complexes is determined directly from the IR spectra of the samples. The Perkin-Elmer 180 double-beam spectrophotometer is coupled to a Hewlett-Packard 9825 A calculator, allowing systematic data treatment such as base-line and water-contribution corrections and scaled spectrum subtraction.

Results

Three different infrared spectra have been obtained with poly[d(A-C)]·poly[d(G-T)] films depending on the counterion and the water content. In presence of Ni^{2+} (1Ni^{2+}/nucleotide), a Z-type spectrum is observed. In the case of Na^+ (1 Na^+ in excess/nucleotide), B- or A-type spectra are recorded, depending on the relative humidity (B form above 86% RH; A form between 71 and 58% RH). The Z conformation has never been observed in the case of Na^+ counterion, and no A

† From the Laboratoire de Spectroscopie Biomoléculaire, U.E.R. Santé, Médecine, et Biologie Humaine, 93012 Bobigny Cedex, France.
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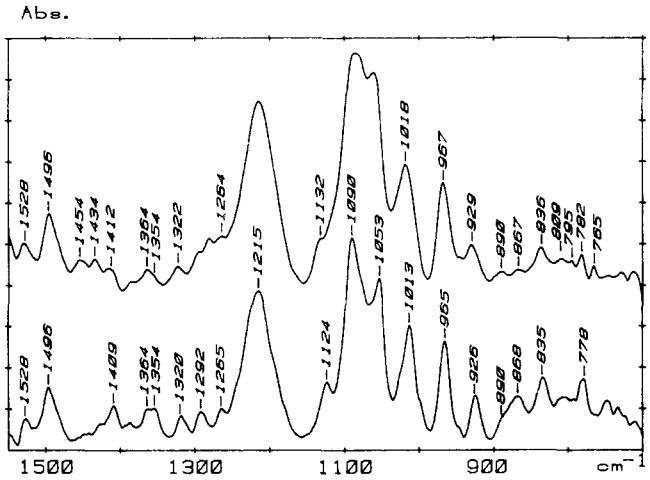

FIGURE 1: Comparison between spectrum of poly[d(A-C)]·poly[d(G-T)] with $NiCl_2$ (top) and spectrum of the Z form of poly[d(G-C)]·poly[d(G-C)] (bottom).

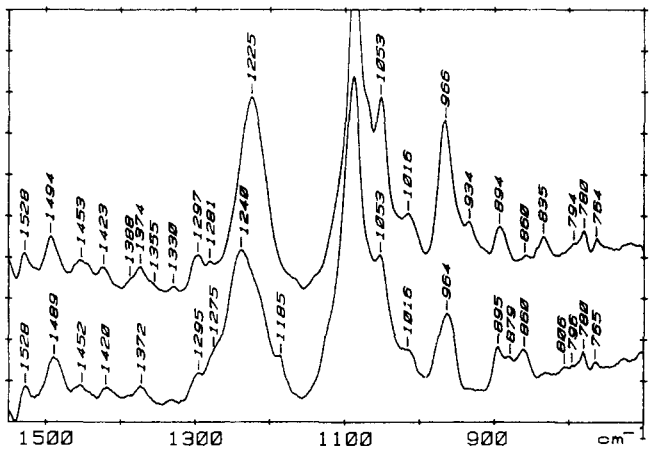

FIGURE 2: Infrared spectra of right-handed forms of poly[d(A-C)]·poly[d(G-T)] with NaCl: (top) B form; (bottom) A form.

geometry has been detected in presence of Ni^{2+}. When double-helical form is not present at high humidities, further dehydration leads neither to the Z form nor to the A form, and the coil → Z and coil → A transitions are never observed.

Left-Handed Conformation of Poly[d(A-C)]·Poly[d(G-T)]. The infrared spectrum of poly[d(A-C)]·poly[d(G-T)]-Ni^{2+} is interpreted as reflecting a left-handed configuration by comparison with the Z spectrum of poly[d(G-C)]·poly[d(G-C)] (Taillandier et al., 1981; Pilet & Leng, 1982; Taboury & Taillandier, 1984; Ghomi et al., 1984; Taboury et al., 1984). These two spectra are presented Figure 1 between 1550 and 700 cm^{-1}. All the distinguishing features of the Z-conformation spectrum are observed for poly[d(A-C)]·poly[d(G-T)]–Ni^{2+}. Thus, we detect absorptions characteristic of the left-handed geometry of poly[d(A-C)]·poly[d(G-T)], around the same wavenumbers as in poly[d(G-C)]·poly[d(G-C)] at 1412, 1354, 1322, 1264, and 929 cm^{-1}, and observe the simultaneous presence of three bands at 867, 836, and 809 cm^{-1}. Moreover, the right-handed → left-handed transition is reflected by changes in the relative intensities and shifts of several bands in the poly[d(G-C)]·poly[d(G-C)]; we observe the same modifications between the spectra of the B form of poly[d(A-C)]·poly[d(G-T)] (Figure 2) and that of poly[d(A-C)]·poly[d(G-T)]-Ni^{2+}. Thus, the intensity of the strong absorption at 1090 cm^{-1} (mainly due to the PO_2^- symmetric stretching vibration) decreases; the bands involving the sugar moiety at 1132 and 1018 cm^{-1} are enhanced; the intensity of

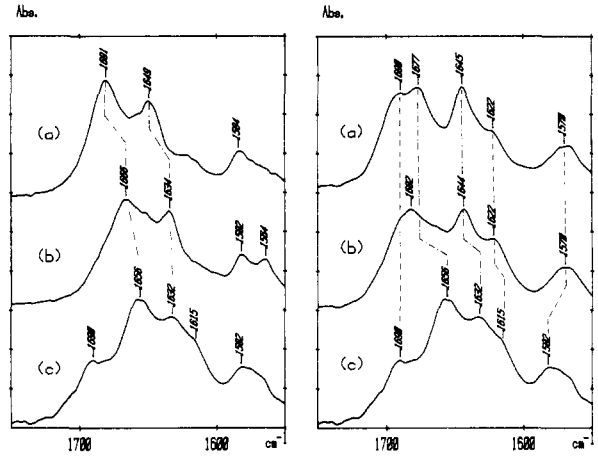

FIGURE 3: Infrared spectra in the base double-bond stretching vibration region of deuterated polynucleotides: (left) (a) poly[d(G-C)]·poly[d(G-C)] in the B form (NaCl) (98% RH), (b) poly[d(G-C)]·poly[d(G-C)] in the Z form (NaCl) (76% RH), and (c) poly[d(A-C)]·poly[d(G-T)] in the Z form ($NiCl_2$) (76% RH); (right) (a) poly[d(A-C)]·poly[d(G-T)] in the B form (NaCl) (98% RH), (b) poly[d(A-C)]·poly[d(G-T)] in the A form (NaCl) (66% RH), and (c) poly[d(A-C)]·poly[d(G-T)] in the Z form ($NiCl_2$) (76% RH).

the deoxyribose band found at 890 cm^{-1} is decreased. In the 1500-1300-cm^{-1} region, the absorption located at 1374 cm^{-1}, involving a dG residue coupled to the glycosidic linkage torsion, is shifted to 1354 cm^{-1}; simultaneously, the 1420-cm^{-1} band is shifted to 1409 cm^{-1}.

Important spectral modifications are detected in the region of the in-plane double-bond vibrations of the bases (between 1800 and 1500 cm^{-1}). Figure 3 presents the IR spectra of both polynucleotides in right-handed and left-handed configurations obtained in D_2O. The bands located at 1681 and 1649 cm^{-1} in poly[d(G-C)]·poly[d(G-C)] are shifted to 1666 and 1634 cm^{-1} under the B → Z transition (Figure 3, left panel, a and b). A similar displacement of the 1677- and 1645-cm^{-1} bands of poly[d(A-C)]·poly[d(G-T)] (Figure 3, right panel, a and c) to 1656 and 1632 cm^{-1} is induced by addition of Ni^{2+}. These two last absorptions can be attributed to G-C base pairs, and their shifts reflect the modification of the base-pair stacking. The two bands located at 1690 and 1622 cm^{-1} in B form not observed in the poly[d(G-C)]·poly[d(G-C)] spectrum are due to A-T base pair contribution.

The out of plane base vibrations can be clearly observed below 800 cm^{-1}: A-T vibrations are detected at 795 and 764 cm^{-1} while the G-C contribution is located at 782 cm^{-1}, for both B and Z conformations. The spectrum of this region recorded in D_2O presents, in the case of poly[d(G-C)]·poly[d(G-C)], a doublet characteristic of the Z conformation at 778-784 cm^{-1}. Unfortunately, in the case of poly[d(A-C)]·poly[d(G-T)], the deuteration shifts a thymine band from 764 to 774 cm^{-1}, which makes impossible a characterization of the handedness of the double helix.

Concerning the absorptions of the A-T base pairs, the effect of the right-hand → left-hand helix transition is clearly detected thanks to a band located at 1434 cm^{-1}, which exists neither in the poly[d(G-C)]·poly[d(G-C)] spectrum nor in the B-form spectrum of poly[d(A-C)]·poly[d(G-T)]. In the double-bond in-plane vibration region of the bases, the B → Z transitions induce a shift of the absorptions involving the A-T pair bands from 1621 and 1570 cm^{-1} in the B form to 1615 and 1582 cm^{-1} in the Z form (Figure 3, right panel, a and c).

Peak by peak survey of the poly[d(A-C)]·poly[d(G-T)]–Ni^{2+} spectrum shows that a structural transition occurs, and

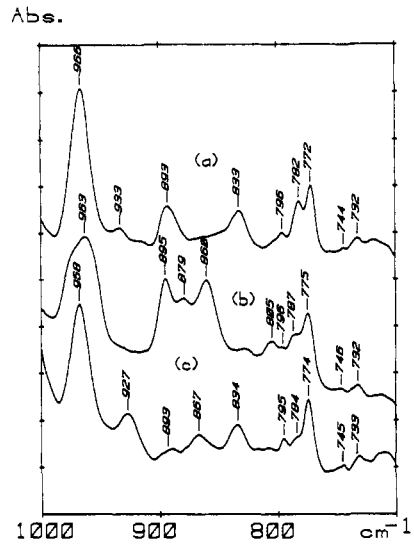

FIGURE 4: Infrared spectra in 1000–700-cm^{-1} region of deuterated poly[d(A-C)]·poly[d(G-T)]: (a) in the B form (98% RH); (b) in the A form (66% RH); (c) in the Z form (76% RH).

the similar spectral shifts of many bands as compared to poly[d(G-C)]·poly[d(G-C)] indicate that the B → Z transition is likely to occur in poly[d(A-C)]·poly[d(G-T)].

Right-Handed B → A Transition of Poly[d(A-C)]·Poly[d(G-T)]–Na$^+$. The IR spectra of poly[d(A-C)]·poly-[d(G-T)] in the A and B conformations (Figure 2) have been identified by comparison with the two spectra usually observed for films of native Na$^+$-DNA with a base composition around 50% A-T and 50% G-C, recorded at 86 and 66% relative humidity. The main features that are characteristic of the B → A transition in nondeuterated samples have been observed for poly(dA-dC)·poly(dG-dT) in the presence of Na$^+$. As shown in Figure 2, the strong band involving the PO_2^- antisymmetric stretching vibration is shifted from 1225 cm^{-1} at high humidities (B form) to 1240 cm^{-1} at low humidities (A form) (Tsuboi, 1970; Pilet & Bahms, 1972). An absorption is observed at 1185 cm^{-1} in the low-humidity spectrum of poly(dA-dC)·poly(dG-dT) and not in the high-humidity spectrum (Pohle & Fritzsche, 1980). Finally, a band at 835 cm^{-1} is found for the B form while the A form is characterized by two bands at 860 and 806 cm^{-1} (Brahms et al., 1974).

The IR spectra show that poly[d(A-C)]·poly[d(G-T)] can adopt the three A, B, and Z geometries, depending on the water and salt content. The type of helix of the polynucleotide can be determined by the different characteristic absorptions previously discussed. The 800-950-cm^{-1} region, which has been classically used to characterize the A and B geometries, is also extremely useful to detect the Z conformation (Figure 4): presence of the 927-cm^{-1} absorption, simultaneous existence of the 867- and 834-cm^{-1} bands with similar intensities, and decrease of the 892-cm^{-1} absorption.

Discussion

Infrared spectroscopy does not impose an upper limit on the concentration of the polynucleotide used. It is thus possible to study solutions with very high concentrations, gels, or hydrated films. In these conditions, poly[d(A-C)]·poly[d(G-T)]–Ni^{2+} adopts a geometry interpreted as a left-handed Z conformation, while UV experiments performed in our laboratory on dilute solutions (in the 10^{-4} M range usually used for UV or CD measurements) fail to induce B → Z transition by progressive addition of Ni^{2+} ions, even with high (up to 10) Ni^{2+} to nucleotide ratios (results not shown). Earlier experiments have illustrated the equilibrium between B and Z DNA: the crystal for the X-ray diffraction studies of the d(C-G)$_3$ hexamer was formed from a low-salt solution. UV measurements on this solution indicated no Z DNA present, but the crystals formed were entirely Z DNA (Rich, 1982). As the concentration is increased, the equilibrium is shifted, and the amount of molecules of poly[d(A-C)]·poly-[d(G-T)]–Ni^{2+} in the Z form also increases. It seems that in numerous UV studies testing various metal ions and alcoholic solutions (Zimmer, 1982; Vorlicokova, 1982; Wells, 1982; McIntosh, 1983) the Z canonical spectra were not obtained because of the too low poly[d(A-C)]·poly[d(G-T)] concentration.

Let us now examine why poly[d(A-C)]·poly[d(G-T)] adopts in the presence of Ni^{2+} a left-handed conformation rather than a right-handed one as in the presence of Na$^+$. The induction of left-handed structures is more easy to obtain with Ni^{2+} than with Na$^+$. The former ion has proved to be more efficient also in the case of poly[d(G-C)]·poly[d(G-C)]. We have observed by infrared spectroscopy that whatever the water content of the hydrated films, poly[d(G-C)]·poly[d(G-C)] adopts a Z structure in the presence of Ni^{2+} (Taboury et al., 1984) as does poly[d(A-C)]·poly[d(G-T)] (present work). In the presence of Na$^+$ (1 Na$^+$ in excess/nucleotide), poly[d(G-C)]·poly[d-(G-C)] undergoes the B → Z transition only if the hydration is lowered from 98 to 86% RH. This difference in efficiencies has also been observed by UV measurements, millimolar amounts of Ni^{2+} being sufficient to induce the Z form of poly[d(G-C)]·poly[d(G-C)] while a high NaCl concentration is necessary to displace in a similar way the B → Z equilibrium. This may be interpreted as due to the hydration of the Ni^{2+} ions, which lowers the general activity of the water molecules and decreases the effective water concentration. However, in the case of poly[d(A-C)]·poly[d(G-T)]–Na$^+$ films, this decrease of water concentration obtained by lowering the relative humidity fails to promote the Z conformation. The decrease of water concentration is sufficient to stabilize the Z conformation of the alternating G-C sequence but not of the polymer containing A-T base pairs. We can therefore assume that the displacement of the B → Z equilibrium involves also another mechanism. Ni^{2+} ions must play another additional role in DNA helical transitions.

The ability to induce the B → Z transition cannot be correlated to the cationic charge of the metal ions. in both cases studied (Na$^+$ and Ni^{2+}), the phosphate negative charges are totally neutralized by the presence of the counterions. however, in the presence of the Ni^{2+}, the Z form is obtained whereas the B → A transition is detected in presence of Na$^+$. Moreover, in both Z and A geometries, the phosphate groups are similarly distant, and it is likely that the decrease of repulsion between charged phosphates due to charge screening is of the same order of magnitude in the B and Z forms. The explanation must then be looked for at the level of a different binding of the ions to the polynucleotide. Alkaline ions, considered as hard metals, are known to interact with the phosphate groups while transition metals or soft metals bind preferentially to a nitrogen site of the purines. We have shown by the use of selectively deuterated poly[d(G-C)]·poly[d(G-C)] that the transition-metal ions Ni^{2+} and Co^{2+} interact directly with the N7 of the guanines and stabilize the Z conformation of the DNA (Taboury et al., 1984). This site-specific binding mechanism of Ni^{2+} allows the adoption by poly[d(A-C)]·poly[d(G-T)] of a Z helix structure, by stabilization of the syn conformation of purines. The discrimination at low humidities betwen Z and A helixes of poly[d(A-C)]·poly[d(G-T)] may be due to this specific interaction between the transition-metal

ions and the N7 site of the purines.

In vivo, the DNA concentration in nuclei can be favorably compared to the concentration at which the Z form of poly[d(A-C)]·poly[d(G-T)] has been obtained with Ni^{2+} ions. The existence of left-handed DNA sequences that possibly play a role in gene expression (Rich, 1982) may thus be promoted by small amounts of divalent cations such as Ni^{2+}.

Registry No. Poly[d(A-C)]·poly[d(G-T)], 55684-99-6; poly[d(G-C)], 36786-90-0; Ni, 7440-02-0.

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Fast Atom Bombardment Mass Spectrometry of Glycosphingolipids. Glycosphingolipids Containing Neutral Sugars†

Mark E. Hemling, Robert K. Yu, R. Donald Sedgwick,‡ and Kenneth L. Rinehart, Jr.*

ABSTRACT: Natural and synthetic glycosphingolipids containing neutral sugars have been analyzed by positive and negative ion fast atom bombardment mass spectrometry. Basic structural characterization including saccharide size and sequence and ceramide composition is possible on the basis of the fragment ions observed. The degree of fragmentation could be increased by using higher sample concentrations and lower fast atom beam energies. Commercially available synthetic compounds that had been presumed to be pure were shown to contain homologous fatty acids. Mixtures of glycosphingolipids such as those obtained from Gaucher's spleen and from human erythrocytes can be characterized and quantitated.

The growing interest in the diverse biological functions of cell membrane glycosphingolipids (GSL's)[1] as cell surface antigens, cell–cell recognition sites, and receptors for a variety of signal molecules (Fishman & Brady, 1976; Yamakawa & Nagai, 1976; Hakamori, 1981; Ledeen & Yu, 1982) has prompted the constant search for new procedures for their structural analysis. Mass spectrometry (MS) because of its high sensitivity and speed, has always figured prominently in this regard. First applied to GSL's in 1969 by Sweeley & Dawson (1969) and by Samuelsson & Samuelsson (1969), it has gained rapid recognition as a viable tool in furnishing detailed structural information regarding the carbohydrate composition and sequence and, in some instances, the lipophilic constituents. Previous investigations have relied upon either electron ionization (EI) or chemical ionization (CI) for the

† From the School of Chemical Sciences, University of Illinois, Urbana, Illinois 61801 (M.E.H., R.D.S., and K.L.R.), and the Department of Neurology, Yale University School of Medicine, New Haven, Connecticut 06510 (R.K.Y.). *Received January 16, 1984; revised manuscript received May 23, 1984.* This work was supported by grants from the National Institutes of Health (NIH) (GM 27029 to K.L.R. and NS 11853 to R.K.Y.), by instrumentation grants from the National Science Foundation (PCM 81-21494) and the NIH (RR 01575) for the purchase of the ZAB HF mass spectrometer, and by an NIH Traineeship (GM 07283 to M.E.H.).

‡ Present address: Allied Corp., Corporate Research, Morristown, NJ 07960.

[1] Abbreviations: GSL, glycosphingolipid; MS, mass spectrometry; EI, electron ionization; CI, chemical ionization; DI, desorption ionization; FAB, fast atom bombardment; DCI, desorption chemical ionization; FD, field desorption; CTH, ceramide trihexoside; TETA, triethylenetetramine; GC, gas chromatography; TFA, trifluoroacetic acid.

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