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**Interaction of Z form poly(dG-dC).poly(dG-dC) with divalent metal ions: localization of the binding sites by I.R. spectroscopy**

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**ABSTRACT**

The secondary structures of poly(dG-dC).poly(dG-dC) in the presence of alkaline, alkaline earth and first row transition metal ions ( $\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ) are investigated by infrared spectroscopy. The conformational transitions are studied as a function of the hydration of the polynucleotide and counter-ion nature and content. The use of selectively deuterated poly(dG-dC).poly(dG-dC) on the 8-carbon of guanines allows to show that a direct interaction occurs between the N7 site of guanines and the transition metal ions  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$ . In the case of  $\text{Mg}^{2+}$ , for high ion/nucleotide ratios, the interaction occurs essentially at the level of the phosphate groups. This interaction leads to a modification of the left-handed conformation. Based on the IR spectroscopy results, an explanation is proposed for the different efficiencies of these various ions to induce the B  $\rightarrow$  Z transition.

**INTRODUCTION**

In synthetic polynucleotides, the existence of left-handed helices, first suggested by U.V. circular dichroism experiments (1), has been confirmed by X ray crystal diffraction studies (2,3) and characterized by other various techniques :  $^{31}\text{P}$  and  $^1\text{H}$  NMR (4-7), Raman (8,9) and IR spectroscopy (9,10). In native systems, the existence of such conformations has been evidenced by indirect immunological techniques (12-14). This novel conformation is now extensively studied, because of its possible biological importance : hypothesis have been proposed concerning the role played by Z DNA in fundamental processes such as regulation of gene expression (15). Among the various parameters involved in the B  $\leftrightarrow$  Z transition of poly(dG-dC).poly(dG-dC) in solution, the nature and the concentration of various ions has been previously studied by U.V. absorbance and circular dichroism (1,16-19). On the other hand, the important role played in vivo and in vitro by metal ions in processes involving nucleic acids has been investigated for many years (for review, see ref. 20) ; in particular, different binding modes have been proposed : interaction between the ions and the phosphate

groups, either directly or via a water bridge, interactions with electron donor sites of the bases,... (21,22).

The IR spectra of sodium salt poly(dG-dC).poly(dG-dC) have been partly described (10,11,23). In the present work, we have investigated by IR spectroscopy the conformation effect of  $Mg^{2+}$ ,  $Co^{2+}$  and  $Ni^{2+}$  on poly(dG-dC).poly(dG-dC), and the possible binding sites of these ions. The use of a polynucleotide, specifically deuterated on the 8-carbon of guanines (poly(dD8G-dC).poly(dD8G-dC)), allows to localize a structurally important interaction of the transition metal ions with the N7 site of this base.

#### MATERIALS AND METHODS

Poly(dG-dC).poly(dG-dC) (lot 719-76), 2'-deoxyguanosine 5'-phosphate (dGMP) and neutral 2'-deoxycytidine (dC) were purchased from P.L. Biochemicals. Specific deuteration of the guanines was obtained by incubation in  $D_2O$  at 80°C for 3 hours (24-25). Samples were deposited on ZnSe windows and gently dried so as to give homogeneous films. The desired amount of ions is obtained by diffusion of a droplet of the corresponding solution ( $NiCl_2$ ,  $CoBr_2$ ,  $MgCl_2$ , at pH 7), followed by slow evaporation. Films are placed in cells with controlled relative humidity ( $H_2O$  or  $D_2O$ ) (between 32 and 100% RH). The hydration of the complexes is determined directly on the IR spectra of the samples. Ion/nucleotide ratios were varied between 1 and 5. The Perkin-Elmer 180 double-beam spectrophotometer is coupled to a Hewlett-Packard 9825 A calculator allowing systematic data treatment such as baseline and water contribution corrections, scaled spectrum subtraction,...

#### RESULTS

##### Z conformation of poly(dD8G-dC).poly(dD8G.dC) in the presence of divalent ions

At high RH (> 76%), and whatever the ions ( $Co^{2+}$ ,  $Ni^{2+}$ ,  $Mg^{2+}$ ), the I.R. spectra reflect a Z type double helix conformation of the polynucleotide. The spectral regions corresponding to the absorptions of the bases, of the sugar-base glycosidic linkage and of the phosphodiester backbone show the characteristic features of the left-handed structure.

In the  $1750\text{ cm}^{-1}$ - $1550\text{ cm}^{-1}$  region, the drastic modification of the base pair stacking which occurs during the B  $\rightarrow$  Z transition induces important shifts of the bands corresponding to the double bond vibrations

Abs.

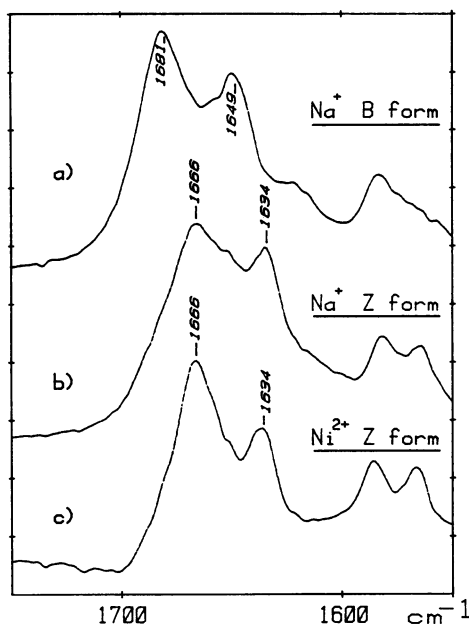


Figure 1. Infrared spectra of poly(dD8G-dC).poly(dD8G-dC) in D<sub>2</sub>O in the base double bond stretching vibration region : a/ Na<sup>+</sup> B form ; b/ Na<sup>+</sup>, Z form ; c/ Ni<sup>2+</sup> (or Co<sup>2+</sup>, or Mg<sup>2+</sup>), 1 M<sup>2+</sup> per nucleotide, Z form.

of the bases. In D<sub>2</sub>O, two bands are observed in the poly(dD8G-dC).poly(dD8G-dC) - Na<sup>+</sup> spectrum, located at 1681 cm<sup>-1</sup> and 1649 cm<sup>-1</sup> in the B conformation (Fig. 1a). They are found respectively at 1666 cm<sup>-1</sup> and 1634 cm<sup>-1</sup> in the Z conformation (Fig. 1b). In the presence of divalent metal ions, the spectrum recorded in D<sub>2</sub>O shows two absorptions at the same latter wavenumbers (Fig. 1c). In the case of spectra recorded in H<sub>2</sub>O, the modification of the base pair stacking in the sodium salt polymer is reflected by a shift of the 1710 cm<sup>-1</sup> band to 1692 cm<sup>-1</sup> (Fig. 2). In the presence of divalent ions, this absorption is also detected at 1692 cm<sup>-1</sup> (results not shown). Thus, the absence of the 1710 cm<sup>-1</sup> band (in H<sub>2</sub>O), and the presence of a doublet at 1666 cm<sup>-1</sup>-1634 cm<sup>-1</sup> (in D<sub>2</sub>O) can be correlated to a Z type base stacking of the poly(dD8G-dC).poly(dD8G-dC) in the presence of divalent metal ions.

In the 1550 cm<sup>-1</sup>-1250 cm<sup>-1</sup> region (Fig. 3), that is to say in the spectral region corresponding mainly to the in plane vibrations of the

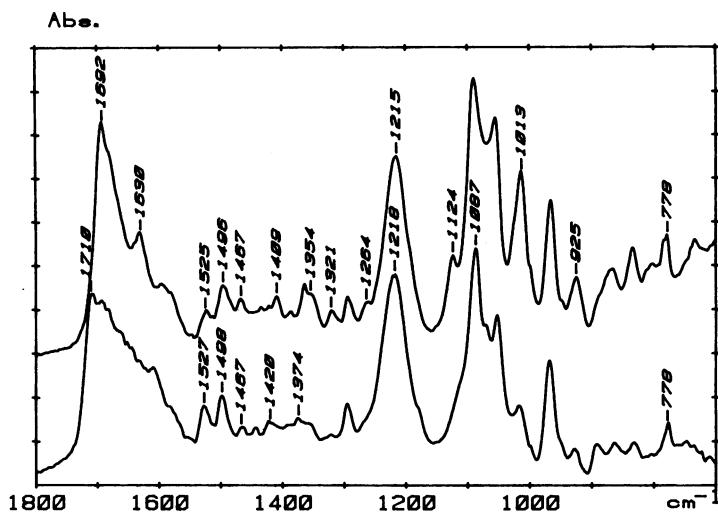


Figure 2. IR spectra of poly(dD8G-dC).poly(dD8G-dC).Na<sup>+</sup> : bottom : B form (98% RH), top : Z form (86% RH).

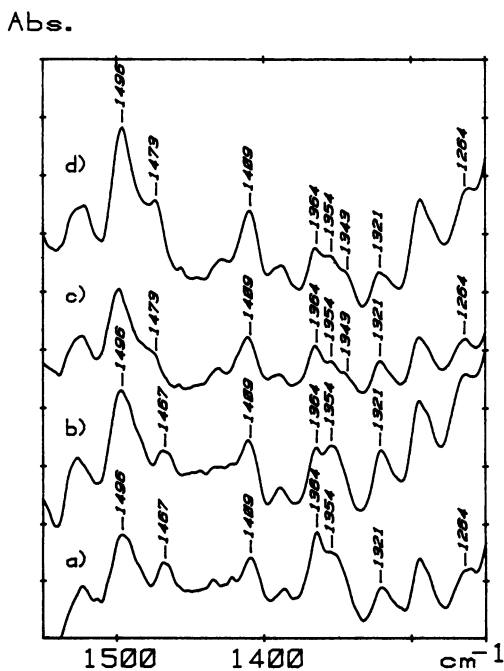


Figure 3. IR spectra of poly(dD8G-dC).poly(dD8G-dC) in the sugar base and glycosidic linkage vibration region. Ion/nucleotide ratio = 1. RH > 76% ; a/ Na<sup>+</sup>, b/ Mg<sup>2+</sup> ; c/ Ni<sup>2+</sup> ; d/ Co<sup>2+</sup>.

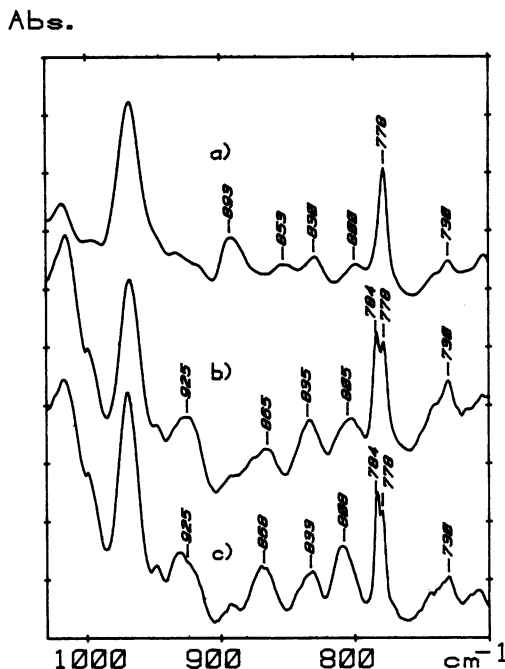


Figure 4. IR spectra of out of plane vibrations of poly(dD8G-dC).poly(dD8G-dC) in  $D_2O$ : a/  $Na^+$ , B form ; b/  $Co^{2+}$  (1 per nucleotide), Z form ; c/  $Ni^{2+}$  (1 per nucleotide), Z form.

bases, the spectra of the poly(dD8G-dC).poly(dD8G-dC)- $M^{2+}$  are characterized by the presence of the  $1409\text{ cm}^{-1}$ ,  $1321\text{ cm}^{-1}$  and  $1264\text{ cm}^{-1}$  bands, which are also observed for the poly(dD8G-dC).poly(dD8G-dC)- $Na^+$  in the Z form (Fig. 3a). However, a new spectroscopic feature is detected in this region in the case of the interaction with the transition metal ions  $Co^{2+}$  and  $Ni^{2+}$ : we have previously shown that the absorption located at  $1374\text{ cm}^{-1}$  in the B form and  $1354\text{ cm}^{-1}$  in the Z form of poly(dG-dC).poly(dG-dC)- $Na^+$  involves the dG residue in an *anti* and a *syn* conformation respectively (26); this band, which remains at  $1354\text{ cm}^{-1}$  in the case of  $Mg^{2+}$  (Fig. 3b), is split into two components at  $1354\text{ cm}^{-1}$  and  $1343\text{ cm}^{-1}$  in the case of  $Co^{2+}$  or  $Ni^{2+}$  (Fig. 3, c and d). This, and also the effect on the  $1467\text{ cm}^{-1}$  band (which will be discussed in the second part of this work), suggests a strong interaction of the transition metal ions with the guanines.

In the region below  $800\text{ cm}^{-1}$  (spectral region of the out of plane base vibrations) guanine and cytosine contributions are superimposed at  $778\text{ cm}^{-1}$  in the case of the B conformation (Fig. 4a). The spectra of the

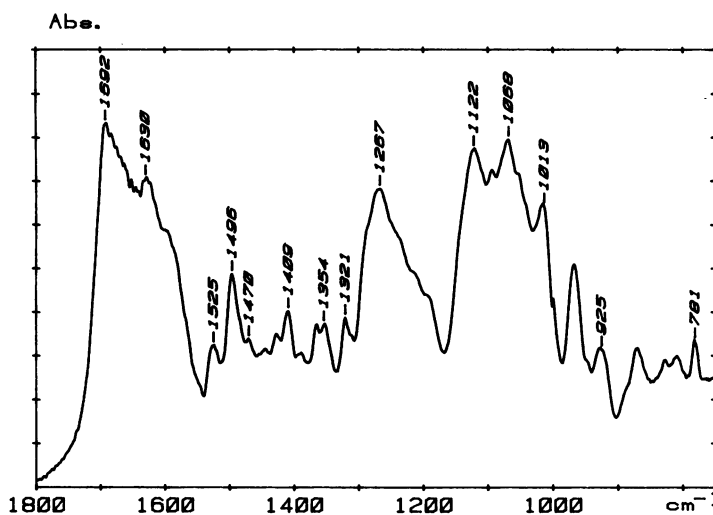


Figure 5. IR spectrum of poly(dD8G-dC).poly(dD8G-dC) in the presence of  $Mg^{2+}$  (5 per nucleotide), RH = 32%).

poly(dD8G-dC).poly(dD8G-dC)- $M^{2+}$  complexes present, in  $D_2O$ , two absorptions located at  $778\text{ cm}^{-1}$  and  $784\text{ cm}^{-1}$  (Fig. 4 b and c). This doublet is characteristic of a Z conformation of the alternating G-C polymer ; thanks to the effect of the C-5-methylation of the cytosine, these two bands are attributed to out of plane vibrations of cytosines and guanines respectively (26,27).

Concerning the deoxyribose and the phosphodiester backbone vibrations, three absorptions of the poly(dD8G-dC).poly(dD8G-dC)- $M^{2+}$  complexes can also be correlated with the Z conformation ; two intense bands at  $925\text{ cm}^{-1}$  and  $1014\text{ cm}^{-1}$ , and a weaker one at  $1124\text{ cm}^{-1}$  (spectra not shown).

All these spectroscopic evidences show that in highly concentrated phases (films above 76% RH), the divalent ions  $Mg^{2+}$ ,  $Co^{2+}$  and  $Ni^{2+}$  stabilize the left-handed conformation of the poly(dD8G-dC).poly(dD8G-dC).

Decreasing the relative humidity below 58% leads to IR spectra depending on the amount and nature of divalent counter-ion. Fig. 5 presents the IR spectrum of poly(dD8G-dC).poly(dD8G-dC) in the presence of high  $Mg^{2+}$ /nucleotide ratio (above 5), recorded at low relative humidity (32%). Such a spectrum which has never been observed with any of the transition metals studied ( $Co^{2+}$ ,  $Ni^{2+}$ ), has been interpreted as reflecting a new structure in the left-handed family, called  $Z_0$  (10,28). The most striking

changes are observed at the level of the phosphate absorptions. The strong direct interaction of  $Mg^{2+}$  with the  $PO_2^-$  groups is evidenced by a drastic shift of the  $\nu_{as}$  and  $\nu_s$   $PO_2^-$  absorptions from  $1213\text{ cm}^{-1}$  to  $1267\text{ cm}^{-1}$  ( $54\text{ cm}^{-1}$ ) and from  $1088\text{ cm}^{-1}$  to  $1122\text{ cm}^{-1}$  ( $34\text{ cm}^{-1}$ ) respectively. In the case of  $Co^{2+}$  and  $Ni^{2+}$ , for the same high metal ion/nucleotide ratios and in the same extreme dehydration conditions, we have only observed indications of a destabilization of the double helix : presence of a single band around  $1650\text{ cm}^{-1}$  in  $D_2O$  (while two absorption bands are always present in the B and the Z forms in this region), and shift of the  $1297\text{ cm}^{-1}$  cytosine band to  $1282\text{ cm}^{-1}$  (while it is not affected by the presence of  $Co^{2+}$  or  $Ni^{2+}$  when the Z structure is well formed). This last result supports the hypothesis of an interaction between these two ions and the cytosine at very low hydration.

Localization of the divalent metal ions interaction sites

Different nucleophilic sites of the polynucleotide are potential candidates for an interaction with divalent cations : phosphates, carbonyl groups of the bases, N7 site of guanines. The use of selectively deuterated polymers allows us to put forward an eventual interaction with this latter site : the IR spectrum of this deuterated polynucleotide presents an absorption located at  $1467\text{ cm}^{-1}$  which involves the motion of the N7 of guanines and moreover which is not affected by the B  $\rightarrow$  Z conformational transition induced by  $Na^+$  ions. The modifications of the position and the relative intensity of this band will therefore be a probe for specific interactions with the N7 of guanines.

The spectra of the Z conformations of poly(dG-dC).poly(dG-dC) and poly(dD8G-dC).poly(dD8G-dC) in the region of the base absorptions are presented Fig. 6a. The new band in the spectrum of the deuterated compound, observed at  $1467\text{ cm}^{-1}$ , can be attributed by comparison with the spectra of films of dGMP, dD8GMP (Fig. 6c) and dC obtained from neutral pH solutions, and with the computed spectrum corresponding to a 1/1 ratio of dC and dD8GMP presented Fig. 6b. The contributions of the cytosines and of the guanines which are superimposed in the spectrum of the poly(dG-dC).poly(dG-dC) at  $1496\text{ cm}^{-1}$  can be easily distinguished by the deuteration on the C8 of guanines : such a deuteration shifts the dGMP band from  $1482\text{ cm}^{-1}$  to  $1460\text{ cm}^{-1}$  (Fig. 6c). In the case of the polymer, the  $1467\text{ cm}^{-1}$  band can therefore be attributed to a vibration of the selectively deuterated guanine. Raman spectroscopy studies of rGMP have shown that the line located at  $1487\text{ cm}^{-1}$  and attributed to the 8 C-H deformation coupled to the guanine ring brea-

Abs.

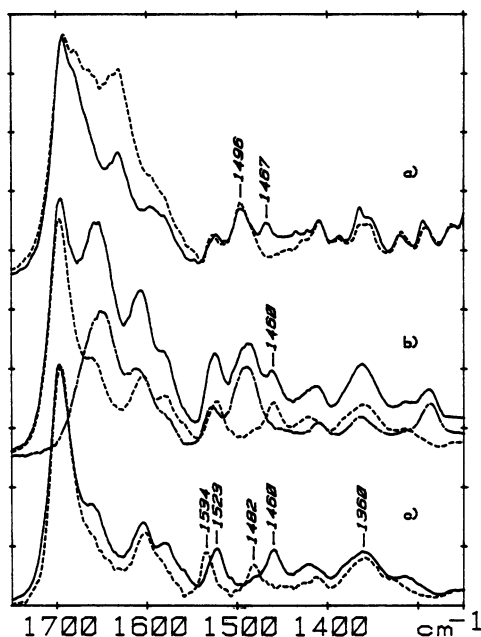


Figure 6. Effect of selective deuteration of guanine : a/ IR spectra of poly(dG-dC).poly(dG-dC) (----), and of poly(dD8G-dC).poly(dD8G-dC) (—); b/ IR spectrum of dD8G (----), dC (— · —), and recalculated spectrum with a 1/1 ratio of dD8G/dC (—); c/ IR spectra of 2'-deoxyguanosine dG(----) and dD8G (—).

thing involving the N7 motion is similarly shifted to  $1464\text{ cm}^{-1}$  by selective deuteration of the C8 site (25). Thus the modifications of the  $1467\text{ cm}^{-1}$  absorption of the IR spectrum of poly(dD8G-dC).poly(dD8G-dC) will reflect specific interactions with the N7 of guanines.

The ions used in our study affect differently this absorption as can be seen Fig. 3. The transition metal ions  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$  shift the  $1467\text{ cm}^{-1}$  band to higher frequencies, the absorption is now detected as a shoulder at  $1473\text{ cm}^{-1}$  (Fig. 3 c and d). At high hydration,  $\text{Mg}^{2+}$  remains effectless on this absorption. However in the conditions corresponding to the existence of the  $Z_0$  conformation, a slight shift of this band to the higher wavenumbers ( $1470\text{ cm}^{-1}$ ) is detected (Fig. 5). In this case we must nevertheless beware of effects not specifically correlated with an interaction on the N7 site and due to the structural modifications of the whole phosphodiester backbone.



Another potentially important interaction site for the metal ions must be expected at the level of the phosphate groups. At high relative humidity the binding of the  $M^{2+}$  ions induces only slight modifications in the stretching vibrations of the  $PO_2^-$ . The  $\nu_s PO_2^-$  absorption band located around  $1087\text{ cm}^{-1}$  is not significantly shifted; however the band becomes broader and its relative intensity is drastically decreased. The  $\nu_{as} PO_2^-$  absorption around  $1215\text{ cm}^{-1}$  in the Z form of poly(dD8G-dC).poly(dD8G-dC)- $Na^+$  is slightly shifted ( $-5\text{ cm}^{-1}$ ) upon addition of divalent ions (spectra not shown). This reflects the absence of specific site binding on the phosphate groups; the ions retain their normal water molecule shell and reduce the effective charge on the polyelectrolyte ("territorial binding"). However, as already discussed, at low relative humidity and high  $M^{2+}/\text{nucl.}$  ratio, a very strong direct interaction with the phosphate groups has been evidenced for  $Mg^{2+}$ .

Concerning the possibility of an interaction with the carbonyl groups of the bases, the spectra presented Fig. 1 (samples in  $D_2O$ ) show that no chelation is detected neither with the transition metal ions  $Co^{2+}$  and  $Ni^{2+}$ , nor with the alkaline-earth  $Mg^{2+}$ , as the doublet at  $1666\text{ cm}^{-1}$ - $1634\text{ cm}^{-1}$  is observed at the same position as with the alkaline ion  $Na^+$ .

## DISCUSSION

We have shown by IR spectroscopy that the  $Na^+$ ,  $Mg^{2+}$ ,  $Co^{2+}$  and  $Ni^{2+}$  stabilize in condensed hydrated phases the poly(dD8G-dC).poly(dD8G-dC) in the Z conformation. The specific deuteration of the 8-carbon of guanines allows to show that depending on the chemical properties of the metal ions (alkaline, alkaline-earth ions and first row transition metal ions), differences exist in the interaction with the N7 site of the guanine.

In the IR spectra recorded with  $Co^{2+}$  and  $Ni^{2+}$  as counter-ions the  $1467\text{ cm}^{-1}$  band involving the N7 motion in the ring breathing coupled to the 8-CD deformation is shifted to a higher frequency. Correlated with this displacement the contribution to the  $1354\text{ cm}^{-1}$  absorption of the dG residu in a *syn* conformation (26) is observed at a lower frequency ( $1343\text{ cm}^{-1}$ ). From these results, it is clear that the 3d7 and 3d8 electronic configuration of  $Co^{2+}$  and  $Ni^{2+}$  have a great affinity for the electron donating site of N7 of guanines. This implies that the perturbation is sufficient to modify the electronic distribution around the N7 and to induce changes in the local force constants. In the case of magnesium ion, two different effects are detected depending on the hydration of the complex. For a high water content, the

1467  $\text{cm}^{-1}$  band remains unshifted, while at low RH it is slightly displaced. X ray diffraction studies (29) have shown that in the d(C-G)<sub>3</sub> crystallized in presence of  $\text{Mg}^{2+}$ , the metal ion is localized close to the N7 site. However as the  $\text{Mg}^{2+}$  is an alkaline-earth metal ion, the electronic perturbation expected on the N7 nucleophile site will be weaker than that due to a transition metal ion. Thus the +3  $\text{cm}^{-1}$  shift of the 1467  $\text{cm}^{-1}$  band at low RH compared to the +6  $\text{cm}^{-1}$  in the case of  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$  can be interpreted as a loss of a water molecule between  $\text{Mg}^{2+}$  and the N7 site while this molecule would be present at higher hydration. We must notice however that the direct chelation of the  $\text{Mg}^{2+}$  to the N7 site, which is not observed at high relative humidity, is not necessary to stabilize the left-handed helix.

Thus we conclude from the different effects of the  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Mg}^{2+}$  on the vibrational mode situated at 1467  $\text{cm}^{-1}$  that the metal-ligand interaction constant is stronger between  $\text{Co}^{2+}$  or  $\text{Ni}^{2+}$  and the N7 of guanines than between  $\text{Mg}^{2+}$  and the same site. This is in good agreement with data previously obtained which proposed a similar order of metal ions binding ability in case of native DNA (30) and an electrostatic site binding of  $\text{Mg}^{2+}$  to phosphate groups (31).

The difference in the spectroscopic effect induced by  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$  compared to the effect of  $\text{Mg}^{2+}$  on the 1467  $\text{cm}^{-1}$  band seems to be well correlated with the efficiency of the metal ions to induce the B to Z transition in solution (1,16,18,32) and allows us to propose an explanation for the particular role of the nucleophile site in the mechanism of this transition. In conditions in which the B conformation in solution is mainly observed, the thermal agitation through the DNA respiration can modify locally the dG conformation into a *syn* geometry without breaking the Watson-Crick base pairing (2,7,33). The lifetime of this configuration may be very short but sufficient to expose, and thus increase the accessibility, of the N7 site of the guanine which will greatly favour the fixation of a metal ion to it. As our IR results show, the  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$  ions interact in a much more drastic way with the guanine than does the  $\text{Mg}^{2+}$ . So it can be assumed that in solution the former ions will stabilize longer the dG residue in a *syn* conformation than  $\text{Mg}^{2+}$ . Therefore a smaller amount of transition metal ions will be needed to induce a cooperative transition to the Z conformation. Thus our IR results give a satisfactory explanation of the quite different concentration of metal ions needed to induce the B to Z transition in solution.

The initiation of a biologically important event by the conformational change of DNA induced by metal-binding, may happen in vivo by local changes in the concentration of other ions, including proteins. Thus regulation may be achieved by small and therefore metabolically controlled, changes in metal concentration.

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