Conditions for the Stability of the B, C, and Z Structural Forms of Poly(dG-dC) in the Presence of Lithium, Potassium, Magnesium, Calcium, and Zinc Cations

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ABSTRACT: The occurrence of alternative structures for the lithium, sodium, and potassium salts of poly(dG-dC) was determined as a function of hydration using IR spectra of nonoriented gels. Poly(dG-dC)·K with added KCl ($r = 0.56$ where r is the moles of KCl per mole of nucleotide residue) gave results essentially identical to the much studied poly(dG-dC)·Na with added NaCl ($r = 0.56$). Both gave a sharp transition from a unique B structure (hereafter designated B*) to the Z structure upon dehydration. Poly(dG-dC)·Li with added LiCl ($r = 0.36$) assumed the B* structure at high hydration but made a broad transition to the C structure as hydration was lowered. We believe this is the first clear evidence of the C structure for poly(dG-dC). No other structures (A, D, or Z) were observed at any hydration in nonoriented gels. Poly(dG-dC)·Na with added $ZnCl_2$ ($r = 0.2$) existed as a mixture of the B* and Z structures in maximally hydrated gels. A broad, incomplete transition to a higher mole fraction of Z structure occurred upon dehydration. Zn^{2+} promotes the Z structure for poly(dG-dC) and appears to bind to guanine residues. Poly(dG-dC)·Na with added $MgCl_2$ or $CaCl_2$ ($r = 0.2$) assumed the normal B* structure at maximum hydration with no hint of Z structure. Slight dehydration produced a very sharp transition to the Z structure. Both Mg^{2+} and Ca^{2+} are strong promoters of the Z structure but do not bind to cytosine or guanine residues.

The alternative structures available to regions of $(dG\text{-}dC)_n$ in DNA have been of interest since the discovery that oligomers and polymers with this sequence could exist in the Z structure [for reviews, see Rich et al. (1984), Rich (1983), and Dickerson et al. (1983)]. Poly(dG-dC) also assumes a special B structure [wrinkled B, hereafter called B*; Arnott et al. (1983) and Loprete and Hartman (1991)] in hydrated gels and the A structure in crystalline, oriented gels (Leslie et al., 1980). The D structure was suggested for polycrystalline samples of poly(dG-dC)·Li, but this could not be confirmed (Leslie et al., 1980). Unique circular dichroism (CD) spectra were found for solutions of poly(dG-dC)·Li (Pohl, 1976). The existence of the C structure was suggested from the CD data (Pohl, 1976). However, an X-ray diffraction study showed that LiCl will not induce the C structure for a fiber of DNA immersed in solution and that the CD spectrum previously assigned to the C structure cannot be used for diagnostic purposes (Zimmerman & Pheiffer, 1980). A later CD study also failed to confirm the existence of the C structure for poly(dG-dC) in LiCl solutions (Behe et al., 1985).

Several cations of physiological or toxicological interest (divalent Mg, Cd, Hg, Ni, and Co ions) are known to stabilize the Z structure over the B structure for poly(dG-dC) under various conditions including hydrated, nonoriented gels (Keller & Hartman, 1987; Keller et al., 1988; Taboury et al., 1984). However, Ag(I) stabilizes the B structure and eliminates the Z structure in poly(dG-dC) despite the presence of NO_3^-, which is a strong Z promoter (Keller et al., 1988).

Zn^{2+} and its complex ions promote the Z structure for poly(dG-dC) in aqueous solutions (Woisard et al., 1985), but Zn^{2+} has not been studied in hydrated gels of poly(dG-dC). Zn^{2+} promotes the C structure in nonoriented gels of poly(dA-dC)·poly(dG-dT)·Na and poly(dA-dT)·Na at hydrations which would give the A structure in the absence of Zn^{2+} (Loprete & Hartman, 1989, 1990).

The Z structure of poly(dG-dC) is stable in aqueous solutions containing K^+, at room temperature (Behe et al., 1985; Preisler, 1987), Mg^{2+} (Behe et al., 1985; Pohl & Jovin, 1972), and Ca^{2+} (Behe & Felsenfeld, 1981).

Several of these studies employed solutions in which the DNA concentration was less than 1% of the concentration in vivo. [The mass of DNA in an *Escherichia coli* divided by the volume of a typical cell is ~9.5 mg/mL or 30 mM in nucleotides, assuming one genome per cell (Keller & Hartman, 1986a).] Other work employed oriented or crystalline samples in which lattice forces may stabilize particular structures (Loprete & Hartman, 1989). We have therefore determined the stabilities of the alternative structures available to the Li, Na, and K salts of poly(dG-dC) and poly(dG-dC)·Na with added $ZnCl_2$, $MgCl_2$, and $CaCl_2$ in the form of hydrated, nonoriented gels in which hydration can be varied and perturbations due to crystal-lattice forces are minimized (Loprete & Hartman, 1989). We used infrared spectra since they provide clear fingerprints of the A, B, C, D, and Z structures of DNA (Loprete & Hartman, 1989, 1990, 1991; Keller & Hartman, 1986a,b; Taboury et al., 1985; Taillandier et al., 1981, and references therein). Particular care has been taken to purify poly(dG-dC) so that the effect of counterions and added salts can be observed without perturbation from extraneous salts or buffers remaining from the synthesis of the polynucleotide. The results presented here will be compared with previous results for other alternating, purine–pyrimidine polynucleotides studied under the same conditions.

MATERIALS AND METHODS

Poly(dG-dC) was purchased from Pharmacia Biochemicals. Residual salts and buffers (which may remain from the polymerization protocol) were removed by extensively washing the polymer with aqueous solutions using Centricon-10

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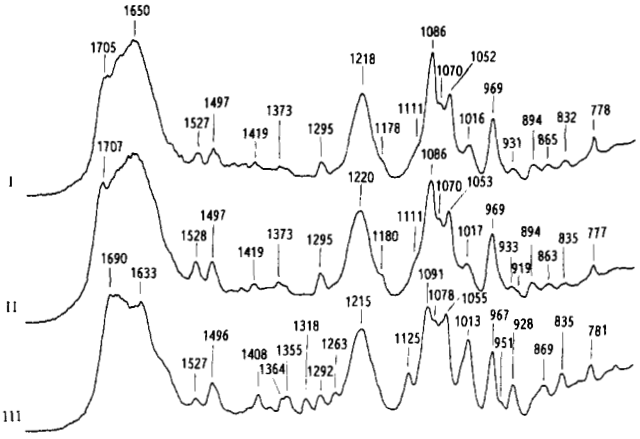

FIGURE 1: Infrared spectra (absorbance in arbitrary units vs frequency in cm^{-1}) of nonoriented gels of poly(dG-dC) hydrated with H_2O: (I) B* structure of poly(dG-dC)·Na with NaCl (r = 0.56), 94% RH. (II) B* structure of poly(dG-dC)·K with KCl (r = 0.56), 94% RH. (III) Z structure of poly(dG-dC)·K with KCl (r = 0.56), 90% RH.

microconcentrators (Amicon Corp.). The Li, Na, and K salts of poly(dG-dC) were prepared by washing poly(dG-dC) with solutions of LiCl, NaCl, and KCl, respectively, using a Centricon-10. These procedures, including removal of excess salt, preparation of samples, control of relative humidity (RH), and methods of recording and manipulating IR spectra with the Perkin-Elmer 683-3600 system have been described (Loprete & Hartman, 1989, 1990; Keller & Hartman, 1986a,b).

RESULTS

Poly(dG-dC)·K and Poly(dG-dC)·Na. Poly(dG-dC)·Na with added NaCl (r = 0–4.0) is known to exist in a B family structure (B*) in highly hydrated, nonoriented gels (Loprete & Hartman, 1991; Keller & Hartman, 1986a; Taboury et al., 1985, and references therein). With NaCl ($r \geq 0.36$), a rapid transition to the Z structure was observed upon dehydration (Keller & Hartman, 1987; Keller et al., 1988). With $r <$ 0.36, mixtures of the B and Z structures were observed with the mole fraction of Z proportional to r. The K salts of poly(dA-dC)·poly(dG-dT) and poly(dA-dT) assumed structures different from the corresponding Na salts (Loprete & Hartman, 1989, 1990).

We wished to compare these results with those obtained for poly(dG-dC)·K since K^+ is the preponderant univalent cation inside living cells. We therefore recorded the IR spectra of nonoriented gels of poly(dG-dC)·K with added KCl (r = 0.56) and poly(dG-dC)·Na with added NaCl (r = 0.56) as a function of hydration. The spectra at all hydrations were quite similar (Figures 1 and 2) except for very small differences below 1300 cm^{-1}. For example, the 1220-cm^{-1} band was slightly broader for poly(dG-dC)·K than for poly(dG-dC)·Na for the B* structures at RH ≥ 94% (Figure 1, spectra I and II). These changes are consistent with minor difference in the phosphodiester conformation between the Na^+ and K^+ samples. The transition from the B* to the Z structure was sharp (between 94 and 90% RH) as the gels were dehydrated with either H_2O (Figure 1, spectra II and III) or D_2O (Figure 2, spectra II and III) as the hydrating agent. Note that all B* indicator bands (labeled with wavenumber values) are present at 94% RH with no absorbance from Z indicator bands. The reverse is true at 90% RH. [The Z indicator bands are labeled with wavenumber values in Figures 1III and 2III.]

Despite the IR absorption of the hydrating H_2O (ca. 1640 cm^{-1} in Figure 1, spectra I and II), it is clear that the B* indicator band near 1707 cm^{-1} is accompanied by a maximum near 1650 cm^{-1} which has not been previously reported and may correspond to the well-known B* indicator band at 1653 cm^{-1} found with D_2O hydration (Figure 2, spectra I and II).

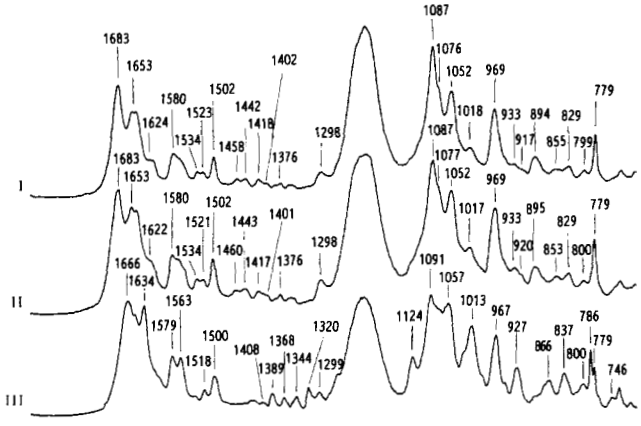

FIGURE 2: Same as Figure 1 except that (III) was recorded at 86% RH and the gels were hydrated with D_2O.

As the gels with either NaCl or KCl were dehydrated to 33% RH and below, the Z structure spectrum was replaced by a spectrum with much broader and less well defined bands which we have assigned to a disordered state. Upon stepwise rehydration, the Z structure and then the B* structure were recovered at the expected values of relative humidity. However, the kinetics of these transitions were often slow and required 2 or more days to be completed. We did not determine whether a true equilibrium could be established at hydrations which would produce appreciable fractions of both B* and Z structures.

We conclude that substitution of K^+ and KCl for Na^+ and NaCl in highly washed samples of poly(dG-dC) has no significant effect on the relatively sharp B* to Z transition observed upon dehydration. In dilute aqueous solutions of poly(dG-dC), a higher concentration of KCl than of NaCl is required to stabilize the Z structure (Preisler, 1987).

Poly(dG-dC)·Li. The lithium salts of calf thymus DNA (ctDNA), poly(dA-dT), and poly(dA-dC)·poly(dG-dT) change from the B structure to the C structure as nonoriented gels of these samples are moderately dehydrated (Loprete & Hartman, 1989, 1990). We therefore determined which structures would occur for poly(dG-dC)·Li upon dehydration. We recorded IR spectra of poly(dG-dC)·Li without (r = 0) and with added LiCl (r = 0.36) and for a control sample of poly(dG-dC)·Na with NaCl (r = 0.56) (see above).

At maximum hydration (100% RH), poly(dG-dC)·Li with LiCl (r = 0.36) gave the B* structure spectrum with all normal B* indicator bands and no Z indicator bands (Figure 3I). At moderate hydration, the spectrum (Figure 3II) closely resembles the spectra of the C structures of ctDNA·Li and poly(dA-dC)·poly(dG-dT)·Li with LiCl (Loprete & Hartman, 1989). This C structure spectrum resembles the normal B* spectrum but with significant modifications. The band at 1222 has a shoulder at 1234 cm^{-1}, giving it a flat-topped appearance. The band at 1087 cm^{-1} is skewed toward high frequency by an undefined band near 1095 cm^{-1}. The bands near 1706, 1070, 969, 935, and 894 cm^{-1} in B* shift respectively to 1702, 1067, 967, 933, and 891 cm^{-1} in the C structure spectrum. These are all clear indications of the C structure (Loprete & Hartman, 1989, 1990). Note the absence of any of the Z indicator bands (compare Figure 3II with Figure 1III). The C structure persisted to lower values of hydration

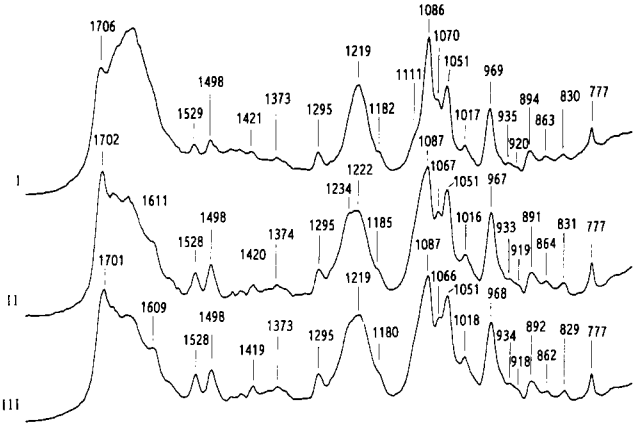

FIGURE 3: IR spectra of poly(dG-dC)·Li hydrated with H_2O: (I) B* structure of poly(dG-dC)·Li with LiCl ($r = 0.36$), 100% RH. (II) C structure of poly(dG-dC)·Li with LiCl ($r = 0.36$), 76% RH. (III) C structure of poly(dG-dC)·Li ($r = 0$), 76% RH.

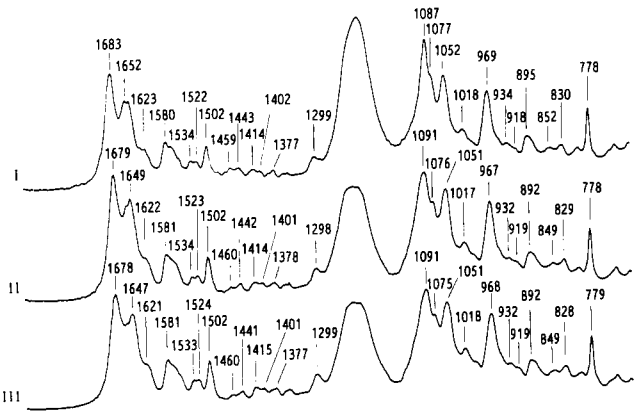

FIGURE 4: Same as Figure 3 except the gels were hydrated with D_2O.

(65% RH). At still lower hydrations a disordered form was observed.

Upon stepwise rehydration, the C and B* structures were recovered with little hysteresis. The spectra at 86% RH were quite similar (but with minor observable differences) in a descending or ascending relative humidity series. This suggests that the mole fraction of B* and C present at 86% RH is slightly different in the descending and ascending series.

Poly(dG-dC)·Li without added LiCl also gave the B* structure at high hydration. At 76% RH, a more fully developed C structure spectrum was observed without LiCl than with LiCl (Figure 3, spectra II and III). Note the clear shoulder at 1230 cm^{-1} and the deeper minimum at 1070 cm^{-1} without LiCl. This spectrum (Figure 3III) looks very similar to the C structure spectrum for poly(dA-dC)·poly(dG-dT)·Li (Loprete & Hartman, 1989).

Substituting D_2O for H_2O as the hydrating agent did not change the structures observed (Figure 4, spectra I and II). The following frequency shifts indicate the change from the B* to the C structure with D_2O hydration: 1683 to 1679, 1652 to 1649, 969 to 967, 934 to 932, 895 to 892, and 852 to 849 cm^{-1}. The relatively sharp band near 1087 shifted to 1091 cm^{-1} and broadened as the C structure formed. The band at 1077 cm^{-1} also became stronger and better resolved.

We conclude that Li^+ stabilizes the C structure for poly(dG-dC) at hydrations which would induce the Z structure for the Na or K salts of poly(dG-dC). Li^+ is unique among the ions we have studied because it alone promotes a particular structure (the C structure) in all base sequences tested including ctDNA.

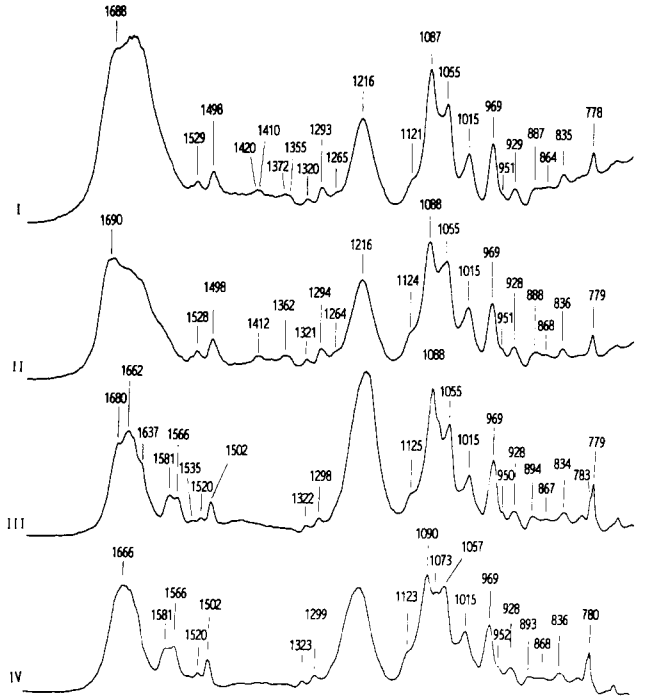

FIGURE 5: IR spectra of poly(dG-dC)·Na with $ZnCl_2$ ($r = 0.2$): (I) mixture of B* and Z structures, 100% RH H_2O. (II) Mostly Z with some B* structure, 86% RH H_2O. (III) B* and Z structures, 100% RH D_2O. (IV) Mostly Z with some B* structure, 76% RH D_2O.

Poly(dG-dC)·Na with Added $ZnCl_2$. $ZnCl_2$ was found to promote the C structure when added to poly(dA-dT)·Na and poly(dA-dC)·poly(dG-dT)·Na (Loprete & Hartman, 1989, 1990). However, Cd^{2+}, Hg^{2+}, Co^{2+}, and Ni^{2+} are known to promote the Z structure when added to poly(dG-dC)·Na (Keller & Hartman, 1987; Keller et al., 1988; Taboury et al., 1984). We therefore determined the effect of $ZnCl_2$ ($r = 0.2$) on the structures of poly(dG-dC)·Na in nonoriented gels as a function of hydration.

The spectrum of poly(dG-dC)·Na with $ZnCl_2$ ($r = 0.2$) recorded for a gel at maximum hydration (100% RH, Figure 5I) contains indicator bands for both the B* and Z structures and suggests an approximately equal mixture of these structures. Note the about equal absorptivities for the following B* and Z indicator bands (cm^{-1}): 1420 B* and 1410 Z, 1372 B* and 1355 Z, and 895 B* and 885 Z. Bands due to the Z structure at 1320, 1265, 1216, 1121, 1070, 1055, 1015, and 929 cm^{-1} (Keller & Hartman, 1986a; Taboury et al., 1985) are also present but at less than the maximum absorbance expected for complete Z structure (compare spectra II and III in Figure 1 with Figure 5I). A similar result was obtained with D_2O hydration (Figure 5III). The spectra of poly(dG-dC)·Na with either $ZnCl_2$ or $HgCl_2$ ($r = 0.2$) at maximum hydration with D_2O are similar and contain bands for both the B* and Z structures [compare Figure 5III with Figure 1, spectrum c in Keller and Hartman (1987)]. These spectra are also similar to those for poly(dG-dC)·Na with $CdCl_2$ ($r = 0.25$) (Keller et al., 1988).

As hydration was lowered, the samples underwent a broad transition (vs relative humidity) to a higher mole fraction of Z structure (Figure 5, spectra II and IV). The transition from B* to Z structure is broader and is incomplete as hydration is decreased for poly(dG-dC)·Na with $ZnCl_2$ in comparison to poly(dG-dC)·Na with NaCl or poly(dG-dC)·K with KCl. The Z structure spectra of poly(dG-dC)·Na with either $ZnCl_2$ or $HgCl_2$ are quite similar [compare Figure 5IV with Figure 2b in Keller and Hartman (1987)]. These spectra

are also similar to those for poly(dG-dC)·Na with $CdCl_2$ at moderate hydrations (Keller et al., 1988).

Spectra were not recorded for an ascending series of relative humidity values, but rehydration to 100% RH from 44% RH produced the same spectrum (B* plus Z) as obtained at the beginning of the descending series.

Significant differences exist for the guanine bands in the Z structure spectra for poly(dG-dC)·Na with $ZnCl_2$ as compared with the Z structure spectra for poly(dG-dC)·Na and poly(dG-dC)·K without $ZnCl_2$. These differences are seen with D_2O hydration (which removes coupling between NH_2 groups and ring modes by converting NH_2 to ND_2). The guanine bands at 1666, 1579, 1563, and 786 cm^{-1} without $ZnCl_2$ (Figure 2III) have different frequencies, band shapes, and absorptivities when $ZnCl_2$ is present (Figure 5IV). The broadening at 1666 and the decreased absorptivity at 786 cm^{-1} in Figure 5IV are especially striking. The bands due to cytosine (ca. 1622, 1521, 1502, and 779 cm^{-1} in Figure 2II) are not significantly modified by $ZnCl_2$ (Figure 5III).

These changes in the spectrum suggest that Zn^{2+} binds to guanine residues but not to cytosine residues. [For a more detailed analysis of this kind of spectrum, see Keller and Hartman (1987) and Keller et al. (1988), where the same conclusion was reached for Cd^{2+} and Hg^{2+}].

We conclude that $ZnCl_2$, $CdCl_2$, and $HgCl_2$ have similar tendencies to promote the Z structure. Each can induce a significant mole fraction of Z structure (mixed with B* structure) at maximum hydration (100% RH) through binding to guanine residues.

It is noteworthy that Zn^{2+} promotes the C structure in all base sequences studied except poly(dG-dC). The fact that Zn^{2+} induces the Z rather than the C structure in poly(dG-dC) confirms the idea that the base sequence (and not solely the properties of the ion) determines the structure favored in the presence of the ion (Loprete & Hartman, 1989, 1990).

Poly(dG-dC)·Na with $MgCl_2$. Mg^{2+} is known to promote the Z structure for poly(dG-dC) in aqueous solution (Behe et al., 1985; Pohl & Jovin, 1972; Behe & Felsenfeld, 1981) and in hydrated gels at $r = 4.0$ (Taillandier et al., 1981). We tested nonoriented gels of poly(dG-dC)·Na at a more physiologically reasonably concentration of $MgCl_2$ ($r = 0.2$).

At maximum hydration (100% RH), poly(dG-dC)·Na with $MgCl_2$ ($r = 0.2$) gave a B* structure spectrum which is very similar, if not identical, to the spectrum for poly(dG-dC)·K with KCl (compare Figure 6I with Figure 1II). There is no evidence for Mg^{2+} binding to any specific molecular subgroup which can be identified in the IR spectrum. No hint of the Z structure is observed.

A slight decrease in hydration (98% RH) produced a nearly complete transition to a normal Z structure spectrum (Figure 6II). The transition was completed by 94% RH. Spectra of the B* and Z structures obtained with D_2O hydration (not shown) contained normal (unperturbed) bands due to guanine and cytosine. Figure 6II is quite different from the spectrum obtained for poly(dG-dC)·Na with $MgCl_2$ ($r = 4.0$), which showed major perturbations presumably due to excess $MgCl_2$ (Taillandier et al., 1981).

As these gels were dehydrated to 33% RH and below, changes in the spectra suggested a disordering of the Z structure. Increasing the relative humidity with H_2O from 65% (at which a Z structure spectrum was observed) to 100% gave a Z structure spectrum, but little rehydration had occurred after 3 days at 100% RH. This is an example of hydrational hysteresis which has been observed before but not to such an extreme. Cooling the gel (by placing ice against the external surface of the AgCl window) caused rehydration by condensation and gave the B* structure in ca. 3 min. Samples that were rehydrated to 100 from 76% D_2O did not exhibit this extreme lack of hydration.

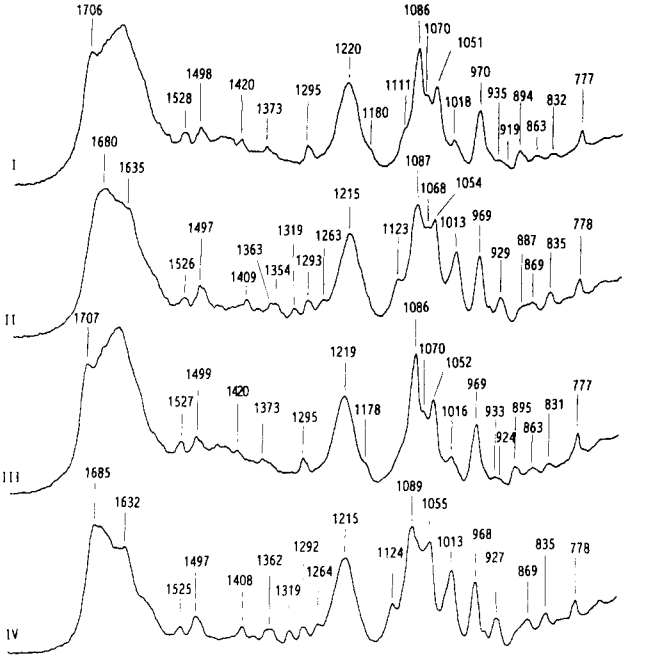

FIGURE 6: IR spectra of poly(dG-dC)·Na with $MgCl_2$ or $CaCl_2$ ($r = 0.2$). Hydration is with H_2O: (I) B* structure with $MgCl_2$, 100% RH. (II) Z structure with $MgCl_2$, 98% RH. (III) B* structure with $CaCl_2$, 100% RH. (IV) Z structure with $CaCl_2$, 94% RH.

We conclude that Mg^{2+} is a strong promoter of the Z structure even at low concentration in gels of poly(dG-dC)·Na.

Poly(dG-dC)·Na with $CaCl_2$. At maximum hydration, poly(dG-dC)·Na with $CaCl_2$ ($r = 0.2$) gave a B* structure (Figure 6III) with no trace of Z structure. Slight dehydration (98% RH) produced nearly complete Z structure and little or no B* structure remained at 94% RH (Figure 6IV). These results are similar to those obtained with $MgCl_2$.

Rehydration to 100 from 65% RH gave a B* spectrum with normal H_2O content but required 4 days to become kinetically stable with little or no Z structure present. A disordered state was observed at 33% RH and below.

We conclude that Ca^{2+} is a strong promoter of the Z structure which induces a sharp transition from the B* to the Z structure as the water activity in the gel of poly(dG-dC)·Na is lowered. Ca^{2+} does not bind to DNA subgroups at $r = 0.2$.

DISCUSSION

Dependence of Structural Stability on Preparative Methods. We restudied poly(dG-dC)·Na to provide a control for poly(dG-dC)·K and to assess the effects of new preparative methods on the stability of the alternative structures. The present results for poly(dG-dC)·Na differ in several ways from past work (Keller & Hartman, 1986a): (1) The B* to Z transition occurred between 94 and 90% RH in present work and between 100 and 94% RH in past work (Keller & Hartman, 1986a). (2) The present samples formed a higher fraction of Z structure as relative humidity was reduced. (3) The B* to Z transition appears to be sharper (i.e., occurs over a smaller decrement of hydration) than in past work. (4) The existence of a B structure different from the B* structure in gels hydrated with D_2O was not confirmed.

These differences are likely due to the current use of more effective methods of cleaning and desalting samples of poly-

(dG-dC). Impurities remaining from the synthesis of the polymer might include Mg^{2+} and phosphate buffer. It is possible that past samples (Keller & Hartman, 1986a) contained traces of these as well as some NaCl which may not have been removed by simply washing precipitated poly(dG-dC) with 70 or 80% ethanol. Residual NaCl would increase the true r value above the nominal r value calculated from the NaCl added to the gel. Phosphate buffer would also provide excess Na^+ (or K^+), further increasing r. Higher values of r for NaCl or KCl and the presence of Mg^{2+} could have stabilized the Z structure at higher hydrations in past work so that samples with excess Na^+, K^+, or Mg^{2+} would require higher hydration to retain the B* structure. The presence of these ions could explain why previous samples of poly(dG-dC) formed nearly complete Z structure at higher hydrations (94% RH) than the present samples (90% RH). Points 2 and 3 may be aspects of the same phenomenon caused by an unknown impurity, which diminished the formation of the Z structure.

Although the differences between past and present results are relatively small, it is important to note that the extent of purification of polynucleotides may affect the relative stabilities of the alternative structures.

Effect of Cations on the Structures of Poly(dG-dC) and the Anomalous Behavior of Li^+. We have shown that poly(dG-dC)·K with added KCl assumes the B* structure at high hydrations and makes a moderately sharp transition to the Z structure as hydration is lowered. The spectra and ranges of stability are essentially identical to those found for poly(dG-dC)·Na with NaCl. Samples of poly(dG-dC)·Na containing either $CaCl_2$ or $MgCl_2$ gave extremely sharp transitions from the B* to the Z structure as relative humidity was lowered from 100 to 98% (a small decrement from maximum hydration). Both Ca^{2+} and Mg^{2+} stabilize the Z structure at higher hydrations to a considerably greater degree than K^+ or Na^+, but these four cations are otherwise qualitatively similar.

In contradistinction with K^+, Na^+, Ca^{2+}, and Mg^{2+}, poly(dG-dC)·Li with or without added LiCl assumed the C structure at moderate hydrations.

We believe that the IR spectra given here provide the first clear evidence for the existence of C structure in poly(dG-dC) and show that lattice forces in oriented samples are not needed to stabilize this C structure. The observation of C structure for poly(dG-dC)·Li further demonstrates that Li^+ is unique in promoting a single structure (i.e., the C structure) at moderate hydrations in all base sequences tested (Loprete & Hartman, 1989, 1990).

When the C structure is stabilized with poly(dG-dC), it is clear that Li^+ behaves differently from its alkali-metal congeners Na^+ and K^+. This is expected and reasonable in light of the well-known anomaly in properties between Li and its congeners. However, the properties of Li are usually quite similar to those of Mg and to a lesser extent Ca, each of which strongly promotes the Z structure when added to poly(dG-dC)·Na as the chloride. Therefore, on the basis of the known similarities between Li^+ and Mg^{2+}, it is surprising that Li^+ stabilizes the C rather than the Z structure.

The difficulty in providing an explanation of the C-stabilizing property of Li^+ deserves a brief discussion. The alkali-metal and alkali-earth ions studied here are all hard-metal acids (Pearson, 1963) with inert-gas electronic configurations, and they form very weak, if any, monodentate complexes. We found no IR evidence for coordinate bonding of Li^+, Na^+, K^+, Mg^{2+}, or Ca^{2+} to subgroups in DNA. There is no easy explanation from coordination chemistry for the anomalies reported here between Li and Na and Mg ions.

The electrostatic properties of these ions can be illustrated by comparing the normalized electric-field strength (E') at the ionic radius (defined as q/R^2 for the ion divided by q/R^2 for K^+, where q is the ionic charge and R is the ionic radius). This gives the following electrostatic series (E' values are listed after the ion): K^+ (1.0) < Na^+ (1.96) < Ca^{2+} (3.61) < Li^+ (4.91) < Mg^{2+} (8.37).

On the basis of this series, Li^+ should have properties intermediate to Ca^{2+} and Mg^{2+}. Therefore, poly(dG-dC)·Li would be expected to undergo a very sharp B* to Z transition at high hydrations (between 100 and 98% RH) rather than the more gradual transition at lower hydrations observed with K^+ and Na^+. Instead, the C structure is observed with Li^+.

Viewed in this context, Li^+ is unexpectedly different from the alkali-earth ions tested. The general properties of these ions do not account for the unique stabilization of the C structure by Li^+.

Poly(dG-dC)·Na with $ZnCl_2$. Although $E' = 6.46$ for Zn^{2+} (i.e., Zn^{2+} falls between Li^+ and Mg^{2+} in the electrostatic series given above), it is a borderline, soft-metal acid (Pearson, 1963) which accepts coordinate bonds and forms complex ions more readily than the alkali-metal or alkali-earth ions. We have shown that Zn^{2+} interacts with poly(dG-dC) similarly to its congeners Cd^{2+} and Hg^{2+}. Zn^{2+} binds to guanine residues and promotes a Z structure which exists in equilibrium with a B-type structure even at maximum hydration (100% RH). Dehydration produces a broad transition to a more complete Z structure. The transition is much broader than observed with Na^+ or K^+ and especially with Ca^{2+} and Mg^{2+}. It should be noted that Zn^{2+} stabilizes the C structure with other DNAs and does not follow Li^+ in stabilizing a single structure.

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REFERENCES

Arnott, S., Chandrasekaran, R., Puigjaner, L. C., Walker, J. K., Hall, I. H., & Birdsall, D. L. (1983) *Nucleic Acids Res. 11*, 1457–1474.

Behe, M., & Felsenfeld, G. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 1619–1623.

Behe, M. J., Felsenfeld, G., Szu, S. C., & Charney, E. (1985) *Biopolymers 24*, 289–300.

Dickerson, R. E., Drew, H. R., Conner, B. N., Kopka, M. L., & Pjura, P. E. (1983) *Cold Spring Harbor Symp. Quant. Biol. 47*, 13–24.

Keller, P. B., & Hartman, K. A. (1986a) *Nucleic Acids Res. 14*, 8167–8182.

Keller, P. B., & Hartman, K. A. (1986b) *Spectrochim. Acta 42A*, 299–306.

Keller, P. B., & Hartman, K. A. (1987) *J. Biomol. Struct. Dyn. 4*, 1013–1026.

Keller, P. B., Loprete, D. M., & Hartman, K. A. (1988) *J. Biomol. Struct. Dyn. 5*, 1221–1229.

Leslie, A. G. W., Arnott, S., Chandrasekaran, R., & Ratliff, R. L. (1980) *J. Mol. Biol. 143*, 49–72.

Loprete, D. M., & Hartman, K. A. (1989) *J. Biomol. Struct. Dyn. 7*, 347–362.

Loprete, D. M., & Hartman, K. A. (1990) *Biopolymers 30*, 753–761.

Loprete, D. M., & Hartman, K. A. (1991) *Biochem. Biophys. Res. Commun. 174*, 1313–1317.
Pearson, R. G. (1963) *J. Am. Chem. Soc. 85*, 3533–3539.
Pohl, F. M. (1976) *Nature 260*, 365–366.
Pohl, F. M., & Jovin, T. M. (1972) *J. Mol. Biol. 67*, 375–396.
Preisler, R. S. (1987) *Biochem. Biophys. Res. Commun. 148*, 609–616.
Rich, A. (1983) *Cold Spring Harbor Symp. Quant. Biol. 47*, 1–12.
Rich, A., Nordheim, A., & Wang, A. H. J. (1984) *Annu. Rev. Biochem. 53*, 791–846.
Taboury, J. A., Bourtayre, P., Liquier, J., & Taillandier, E. (1984) *Nucleic Acids Res. 12*, 4247–4258.
Taboury, J. A., Liquier, J., & Taillandier, E. (1985) *Can. J. Chem. 63*, 1904–1909.
Taillandier, E., Taboury, J., Liquier, L., Sautiere, P., & Couppez, M. (1981) *Biochimie 63*, 895–898.
Woisard, A., Fazakerley, G. V., & Guschlbauer, W. (1985) *J. Biomol. Struct. Dyn. 2*, 1205–1220.
Zimmerman, S. B., & Pheiffer, B. H. (1980) *J. Mol. Biol. 142*, 315–330.