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Structural forms, stabilities and transitions in double-helical poly(dG-dC) as a function of hydration and NaCl content. An infrared spectroscopic study

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ABSTRACT

The poly(dG-dC) helical duplex forms a modified, B-family structure (B^*) at very high hydration and a normal B structure at slightly lower hydration. The B^* structure is slightly different in sugar-phosphate and base-stacking conformations than the B structure. Increasing the hydration or decreasing the NaCl content stabilizes B^* with respect to B.

Poly(dG-dC) forms the Z structure at low NaCl contents when the hydration is sufficiently reduced. At moderate NaCl content, the B to Z transition is sharp and cooperative for hydration with D_2O. Hydration with H_2O broadens the transition which occurs at lower hydration. This suggests that hydrogen bonding is stronger in the Z structure and helps stabilize Z over B.

IR spectra may be used to quantitatively estimate the fractions of B and Z structures present in a sample. Some new indicator bands are described.

INTRODUCTION

The helical duplex of poly(dG-dC) has been shown to exist in the B and Z structural forms in aqueous solution and in the hydrated solid state by a variety of techniques including infrared spectroscopy (1-3). (For a review of the chemistry and biology of Z DNA, see reference 4.) In aqueous solutions, NaCl promotes the B to Z transition (5). In films and fibers, NaCl and decreasing hydration (or water activity) stabilize the Z structure (4). Several ions (6) and complex ions (7) have also been found which stabilize the Z structure. It was recently found, using IR spectroscopy, that at very high hydrations and with an NaCl to nucleotide ratio (r) greater than one, poly(dG-dC) exists in a modified B-structural form (1).

The concentrations of NaCl and values for the activity of water which stabilize the B and Z structures are germane to the possible existence of regions of Z structure for DNA in vivo. We therefore studied the relative stabilities of the structural forms of poly(dG-dC) as both NaCl content and hydration were varied in order to answer such questions as: What is the

lowest NaCl content which will promote the Z structure as hydration is lowered? What is the corresponding hydration? Are these conditions possible in vivo? Do additional structural modifications exist and what conditions produce them? What are the sources of the free energy of stabilization for these structures? Can infrared spectroscopy be used to quantify these transitions?

A number of previous studies have shown that IR spectroscopy of hydrated films is a sensitive and conclusive method for detecting the B to Z transition in poly(dG-dC) (1-3, 6) through the use of a number of clearly resolved indicator bands. However, these studies have focused on high hydrations and NaCl contents. In this paper we describe the IR spectra of poly(dG-dC) at lower NaCl contents and hydrations than have previously been used and have tried to answer the above questions.

MATERIALS AND METHODS

The helical duplex form of poly(dG-dC) was purchased from Pharmacia P.L. Biochemicals, was dissolved in 0.1M NaCl, precipitated with ethanol and the precipitate was washed extensively with 70% ethanol-H_2O. This removed buffers and salts which may accompany the samples as received. The UV and IR spectra of the washed sample were essentially identical to those previously published (3).

Nonoriented films of poly(dG-dC) were formed on AgCl plates. The mass of the polymer was determined by UV absorbance (ϵ_{260} = 17.9 mg/ml cm) and careful volumetric measurement to insure the accurate calculation of the molar ratio of NaCl to nucleotide (r) added to the polymer. The amount of NaCl in a given sample is indicated by the value of r in parentheses. For example, NaCl(0.56) indicates that the sample contains NaCl at r = 0.56. The details of the preparation of films and procedures for hydration by controlling the ambient relative humidity (rh) have been given in detail (8,9).

Spectra were recorded, stored and manipulated using the Perkin-Elmer model 683 spectrophotometer and 3500 minicomputer.

Two methods were used to measure absorbance values for poly(dG-dC) hydrated with D_2O. In the first method the spectrum of liquid D_2O was adjusted and then subtracted from the spectrum of the sample. The adjustment was accomplished by measuring the difference in abosrbance between 1158 and 1335 cm^{-1} ($x = A_{1158} - A_{1335}$) for a dehydrated (44% rh) sample of poly(dG-dC). It is convenient to let $A_{1335} = 0$ which may be

accomplished by subtracting the measured value of A_{1335} from the entire spectrum. For the polymer at a higher hydration, find $y = A_{1158} - A_{1335} + x$. Find $v = A_{1158} - A_{1335}$ for the reference D_2O spectrum. Calculate the adjustment factor $f = y/v$, multiply the reference spectrum of D_2O by f and subtract this spectrum from that of the hydrated polymer.

This method uses the difference in the absorbance of D_2O between 1158 and 1335 cm^{-1} to adjust the spectrum of D_2O to fit the hydration of the polymer at a given rh. This assumes that the molar absorptivities of the polymer at 1158 and 1335 cm^{-1} do not change with hydration above 44% rh and that the spectrum of D_2O hydrating the polymer is similar to that of pure liquid D_2O. This method was applied to the region between 1158 and 700 cm^{-1} and is based on the fact that the ratios of absorbance differences in a given spectrum are independent of the path length of the sample.

A second method of correcting for the background absorbance of D_2O is to use the "three-point fattening" program included in the Perkin-Elmer 680 software which constructs a curved baseline through three specified frequencies (we used 1335, 1158 and 908 cm^{-1}) and subtracts this from the spectrum of the polymer. This gave results which were similar to the first method.

An even simpler method would give reasonable answers for IR instruments without computer capability. Tangent to tangent base lines may be constructed between 1335, 1158 and 908 cm^{-1} and absorbance values obtained as differences.

RESULTS

1. B-Family Structures

Spectra were recorded for films of poly(dG-dC) with and without NaCl as a function of hydration using H_2O and D_2O as the hydrating substances. This tactic shifts the interfering bands due to water and replaces NH and NH_2 groups with ND and ND_2 groups. This modifies certain bands of the cytosine and guanine residues and may also influence the stabilities of the alternative structures for poly(dG-dC).

At maximum hydration (i.e., 100% rh) the spectra of the three samples (r = 0, 0.24 and 0.56) were essentially identical for hydration with D_2O (see Figures 1 and 2 curves B*). The spectra of these samples were also quite similar at 100% rh(H_2O) although differences exist between spectra obtained with H_2O and D_2O as the hydrating substance. Our spectra confirm those previously obtained for highly hydrated poly(dG-dC) with

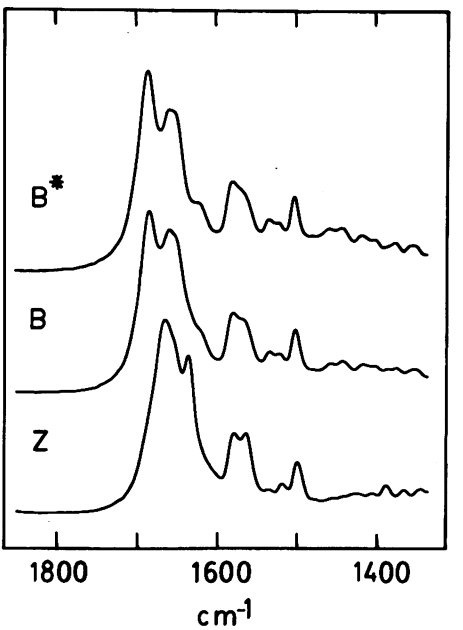

Figure 1. The infrared spectrum (absorbance vs. wavenumber) of poly(dG-dC) with NaCl, r = 0.56 between 1850 and 1340 cm^{-1}. Spectra for the B^* structure (100% rh, D_2O), the B structure (98% rh, D_2O) and the Z structure (94% rh, D_2O) are shown.

higher NaCl content ($r \geq 1$) (1,2). We call this form of poly(dG-dC) the B^* structure.

The spectrum of poly(dG-dC) with NaCl(0.56) obtained at a lower hydration (98% rh, D_2O) has not been previously published and differs from the B^* spectrum (see Figures 1 and 2 curves B). We call this form of poly(dG-dC) the B structure since its spectrum closely resembles the spectrum (below 1350 cm^{-1}) of B-form, calf-thymus DNA (9). In comparing the spectra of B^* and B we note positive and negative changes in absorptivity in bands due to guanine residues (1683(-), 1565(+), 1538(-) and 785 cm^{-1} shoulder(+)) and cytosine residues (1622 cm^{-1}, shoulder (-)) and in unassigned bands (1458(-) and 1387(+) cm^{-1}). (See Table 1 and Figure 3 for quantitative comparisons). These changes in absorptivity suggest slightly different amounts of base-pair overlap in the B^* and B structures (10,11). The lack of changes in frequency for the bands due to the guanine and cytosine carbonyl groups (1683 and 1655 cm^{-1} respectively) suggests that cytosine-guanine base pairs have similar hydrogen-bond

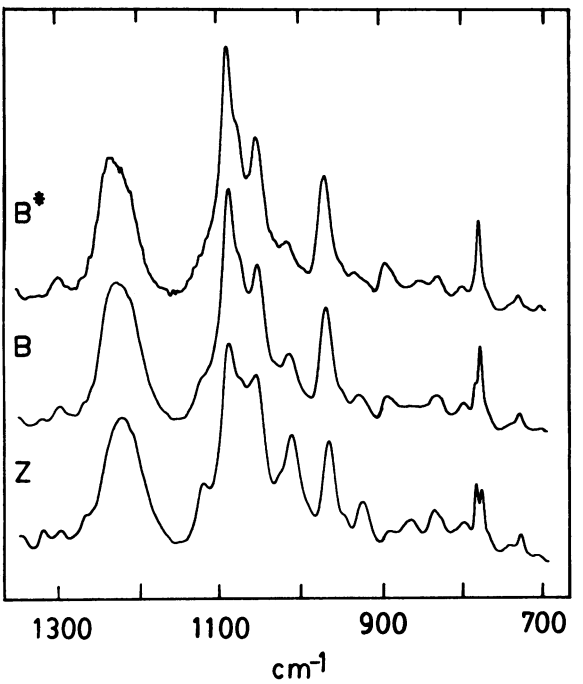

Figure 2. Same as Figure 1 except the 1350 to 700 cm^{-1} region is given.

strengths and base-pairing configurations in the B^* and B structures.

Many of the absorption bands below 1350 cm^{-1} arise from vibrational modes of the deoxyribose-phosphate chains and are sensitive to changes in the conformation of these chains (10,11). Significant variations between the spectra of the B^* and B structures were seen at 1125, 1015, 925, 895, 853 and 830 cm^{-1} (see Figure 2 and Table 1). We conclude that the B^* and B structures have some differences in conformation for the deoxyribose-phosphate groups. Note however the near constancy of the bands at 1087, 1076 and 1055 cm^{-1}. These change during the B to A transition (9) and the B to Z transition. Therefore, some portion of the sugar-phosphate groups retains the same conformation in the B^* and B structures.

The bands at 1622, 895 and 853 cm^{-1} have maximum absorptivities in the B^* form and are therefore good B^* indicators for spectra measured with D_2O hydration.

Changing the NaCl content in the sample had a profound effect on the B^* to B transition. As r was reduced from 0.56 to 0.24 and then to zero,

TABLE I

Wave number (ν)[a] and normalized absorptivity (A^n)[b] for the structural forms of poly(dG-dC) with NaCl(0.56) and hydrated with D_2O.

B*		B		Z	
ν	A^n	ν	A^n	ν	A^n
1684	15.8	1683	14.3		
				1660	15.3
1655	12.7	1655	12.9		
				1637	12.4
1622	5.1	1618sh	4.7		
1580	4.2	1580	3.9	1580	4.0
1565	3.3	1565	3.4	1564	5.4
1534	1.6	1533	1.1	1537	0.94
1522	1.3	1522	0.98	1520	1.4
1502	3.5	1502	3.1	1501	3.1
1460	1.3	1458	0.67		
1443	1.4	1443	0.92		
1419	0.98	1418	0.76	1428	d
				1408	0.61
1402	0.71	1402	0.61		
		1387	0.45	1390	1.2
1377	0.67	1376	0.57		
				1368	0.86
1355	0.65	1353	0.57		
				1347	0.90
		1322	0.39	1321	1
1300	1.2	1298	1.1	1299	1
				1268	d
1220[c]	c	1220[c]	c	1220[c]	c
		1124sh	2.7	1124	3.6
1087	14	1087	13	1088	12
1076sh	d	1076sh	d	1076sh	d
1065	6.6	1065	7.2	1065	8.9
1053	8.9	1053	8.9	1055	9.7
1016	3.1	1015	3.9	1013	5.9
969	6.7	969	6.4	968	6.2
				950sh	d
933	1.3	929	1.5	927	2.4
895	2.8	895	2.0	895	1.5
				868	1.9
853	1.8				
830	2.0	833	2.1	837	2.6
800	1.3	800	1.6	801	1.9
		786sh	2.7	785	4.0
778	5.0	778	4.6	779	3.9
743	d	742	d	732	d
731	0.8	731	0.9	732	1.1
704	d	704	d	710	d

(a) Wavenumber in cm^{-1} is accurate to $\pm$ 2 cm^{-1}. (b) Absorptivities have been normalized by dividing all absorbance values by the absorbance at 1299 cm^{-1} for the sample hydrated with 94% rh (D_2O). In the 1600 to 1340 cm^{-1} region the broad absorbance band due to D_2O has been partially removed using a tangent to tangent baseline. (c) For this band, ν and A^n can not be accurately measured due to interfering absorbance from D_2O. (d) Absorptivities are omitted due to difficulty in measurement for weak bands and shoulders. Sh means shoulder.

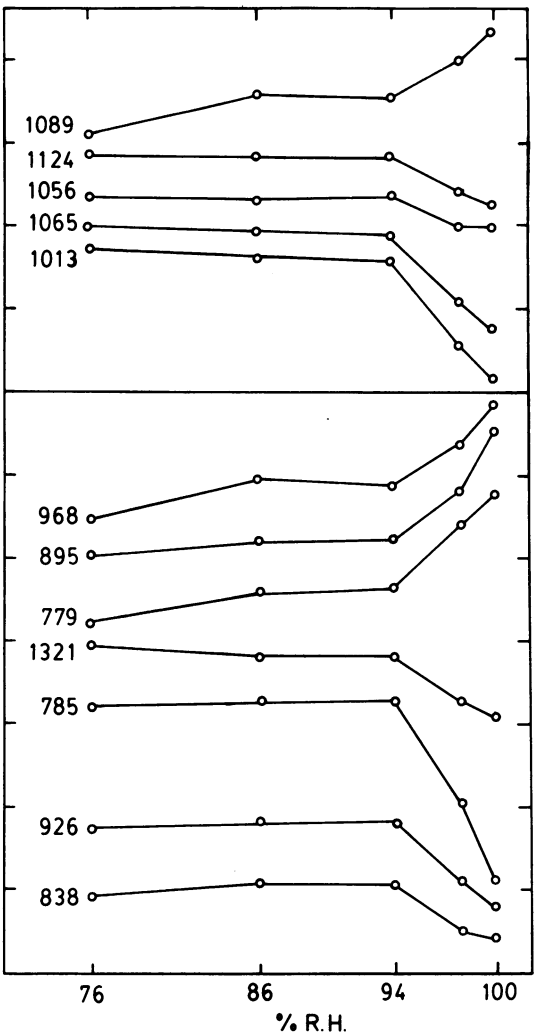

Figure 3. The normalized absorbance as a function of hydration (D_2O) for bands in the spectrum of poly(dG-dC) with NaCl(0.56). The ordinate interval (A^n, see Table 1) is 1.0 in the lower portion and is 2.0 in the upper portion of the figure. Bands are identified by their frequencies at 76% rh.

the midpoint of the transition moved to lower rh values (99, 92 and 80% rh, respectively). These decreasing midpoint values represent much lower hydrations since the absorption isotherm for poly(dG-dC) is elevated by the presence of NaCl (12) as was found for calf-thymus DNA (9). It is clear that NaCl stabilizes the B structure over the B^* structure independent of

hydration, since poly(dG-dC) without NaCl has the B^* structure at 94% rh, but with NaCl(0.24) has the B structure at 94% rh. The latter sample was more highly hydrated than the former but retained the B structure.

As the NaCl content of the sample was reduced, the transition from B^* to B became broader. This may be due a decrease in cooperativity as NaCl is reduced or to metastable states and lack of equilibrium during dehydration and subsequent rehydration (9).

The IR spectra of the B^* structure with different NaCl contents are nearly identical but this is not true for the spectra of the B structure. The bands due mainly to the C6=O of guanine and the C2=O of cytosine (13) (1684 and 1655 cm^{-1}, respectively in B^*) showed only minor changes in absorptivity in going to the B structure with NaCl(0.56). In contrast, these bands were found at 1672 and 1648 cm^{-1} for the B structure at 65% rh(D_2O) with NaCl(0.24) and without NaCl. These frequency shifts suggest a strengthening of the hydrogen bonds between guanine and cytosine or between the C=O groups and the water molecules in the grooves of the helix. The frequency shift for the C6=O band was $12cm^{-1}$ and for the C2=O band was $7cm^{-1}$ so that if hydrogen bonding does indeed explain these shifts (see Discussion), the guanine carbonyl undergoes a greater increase in bond strength. Note that all C6=O groups point into the major groove of the helix and the C2=O groups point into the minor groove in both the B and Z structures (4). These two carbonyl vibrations are known to be coupled (13). Small changes in hydrogen bonding or base-pairing could change the extent of coupling which would further explain the observed shifts.

Other than minor changes in absorptivity as noted above, the bands below 1550 cm^{-1} are the same for the B structure at different NaCl contents. This shows that the sugar-phosphate chain has a similar conformation under these conditions; only the bases were affected by NaCl content.

The existence of the B^* and B structures as separate entities can not be demonstrated for poly(dG-dC) hydrated with H_2O. No distinct IR bands were found for the B^* or B structure. The observed spectral changes are all consistent with a partial transition to the Z structure as hydration is lowered.

2. The B to Z transition and NaCl content.

The transition from the B^* to Z structure has previously been observed for highly hydrated poly(dG-dC) with high NaCl content ($r \geq 1$) using IR spectroscopy (1-3). The existence of the Z structure in vivo would

require a sufficiently high ionic strength and low activity of water. The environment of DNA in cells is likely to have lower ionic strength and also lower water activity than exist in the dilute solutions used for UV and CD studies (5) or in the solid samples used in previous IR studies (1-3). We therefore tried to determine the minimum NaCl content and maximum hydration that would induce formation of the Z structure in poly(dG-dC).

This polymer containing NaCl(0.56) made a nearly complete transition from B at 98% rh(D_2O) to Z at 94% rh (Table 1 and Figures 1 and 2 curves Z). IR bands which characterize the Z structure in samples with NaCl ($r \geq 1$) (1-3) were fully confirmed by our results using samples with NaCl(0.56). However, the bands between 1600 and 1340 cm^{-1} have not been described for hydration with D_2O (although spectra with a compressed frequency scale have appeared, 2). The B and Z indicator bands in this region are as follows (Table 1). The shoulder at 1565 cm^{-1} (B structure) increases in absorptivity and appears as a distinct doublet with the band at 1580 cm^{-1} for Z. The band at 1533 cm^{-1} (B) diminishes in absorptivity and shifts to 1537cm^{-1} for Z. The bands at 1458, 1443, 1418, 1402, 1387, 1376 and 1353 cm^{-1} in the spectrum of the B structure are replaced by bands at 1428, 1408, 1390, 1368 and 1347 cm^{-1} for the Z structure. Although many of these bands are weak (see Table 1), they are clearly visible in expanded spectra (Figure 1) and the patterns produced are quite distinct.

For the polymer with NaCl(0.24) the B (or B^*) structure was stable to 94% rh(H_2O) as shown by the existence of B indicator bands at 1420, 1375 and 895 cm^{-1} and the lack of Z indicators at 1410 and 1355 cm^{-1} (1,3). By 86% rh(H_2O) the absorptivity of the B indicator bands had decreased and the Z indicator bands increased which shows that a partial transition to the Z structure had occurred. By 84% rh the Z bands showed a significant increase in absorptivity and remained unchanged to 59% rh(H_2O). At 44 and 33% rh(H_2O) these indicator bands demonstrate that the sample exists mainly in the Z structure (these spectra are not shown in Figure 1).

The gradual and incomplete transition from the B to the Z structure for poly(dG-dC) with NaCl(0.24) and H_2O hydration is confirmed both qualitatively and quantitatively by the Z indicator bands at 1321, 1065, 1054, 1017, 930 and 985 cm^{-1} which are mostly due to vibrational modes of the sugar-phosphate chains and also by the guanine band at 1710 cm^{-1} at 94% rh(H_2O) which shifts to 1695 cm^{-1} at lower hydrations (1,3). Since all of these indicator bands change in concert, the molecular subgroups

which they represent undergo conformational changes over the same decrements of hydration.

At least one Z indicator band behaves differently for the polymer with NaCl(0.24). The shoulder at 1112 cm^{-1} (B^* or B indicator with H_2O) at high hydration shifts to 1123 cm^{-1} by 84% rh but does not undergo the normal increase in absorptivity (1,3) upon further dehydration. The assignment of this band is unknown but we conclude that the orientation or interaction of some molecular subgroup is different in the Z structure formed with NaCl at $r \leq 0.24$ and $r \geq 0.56$.

Since the Z form of poly(dG-m^5dC) has been observed in aqueous solutions at very low ionic strength (14,15), we studied the infrared spectra of poly(dG-dC) without NaCl as hydration with H_2O was lowered. Although some broadening and minor shifts were observed for the B indicator bands at 1420 and 1375 cm^{-1}, no significant Z bands or shoulders were found at 1410, 1355 or 1123 cm^{-1} down to 23% rh. However the band at 1703 (94% rh) did shift to 1690 cm^{-1}. An increase in absorptivity occurred for bands at 1017 and 932 cm^{-1} at 33% rh. It is difficult to divide these effects between changes in dehydration and orientation but some pretransitional structural changes may be involved.

For poly(dG-dC) with NaCl(0.24) hydrated with D_2O, Z indicator bands first appeared at 44% rh (i.e., at much lower hydrations than with H_2O). Heating at 37°C for 15 hr significantly increased the mole fraction of Z structure. This may be a second example of the thermally driven conversion of B to Z which was previously discovered for poly(dG-m^5dC) (16), and would indicate that $\Delta H^o < 0$ for the B to Z transition under our conditions. Alternatively, the increase in Z structure at 37° may be due to an increase in rate for a transition. These alternatives are under investigation. Note that earlier studies found $\Delta H^o = 0$ for poly(dG-dC) (5).

3. Quantitative measurements of the B^* to B to Z transitions.

Past workers have given qualitative descriptions of IR spectral bands which indicate the B^* and Z structures (1,3) but have not treated these bands quantitatively. We therefore measured the frequency of band maxima and the absorbance for most of the IR bands as a function of hydration of the polymer.

For spectra recorded with D_2O hydration, the background absorbance due to the hydrating D_2O was subtracted by computer methods (see Materials and Methods), and the absorbance of selected indicator bands was plotted

against rh (Figure 3) for the sample with NaCl(0.56). As rh was lowered from 100 to 98%, most bands increased in absorbance (785, 830-838, 927, 1015, 1065, 1124 and 1321 cm^{-1}) some decreased (778, 853, 895, 968, and 1087 cm^{-1}) while the absorbance at 1053 cm^{-1} remained constant (Table 1 and Figure 2). These changes quantitatively define the B^* to B transition for hydration with D_2O.

As hydration was decreased below 98% rh, most bands continued to change as described above except the bands at 1053 and 865 cm^{-1} (not present in B^* or B) which increased in absorptivity as the Z structure formed. In all cases, the spectral changes were largely completed by 94% rh with only minor changes occurring between 94 and 86% rh(D_2O). The transitions from B^* to B and from B to Z are sharp, well defined and appear cooperative for poly(dG-dC) with NaCl(0.56).

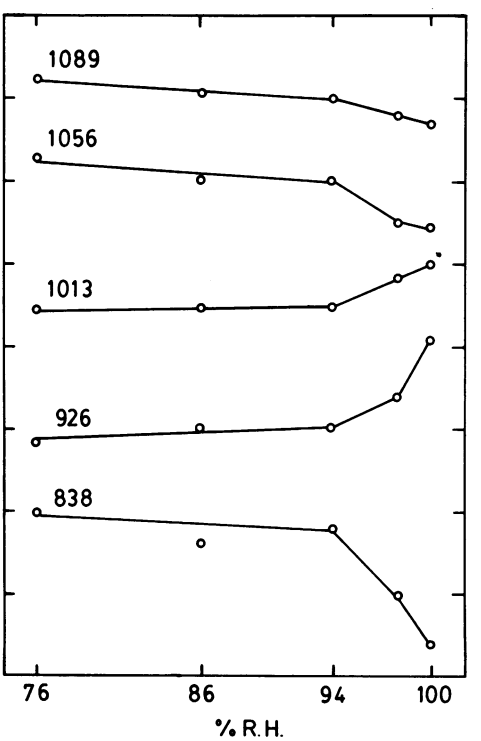

Figure 4. The frequency as a function of hydration (D_2O) for bands in the spectrum of poly(dG-dC) with NaCl(0.56). The curves are placed arbitrarily and are identified by their frequencies at 76% rh. The ordinate interval is 5 cm^{-1}.

A plot similar to Figure 3 was constructed for this sample hydrated with H_2O. The absorbance of the Z indicator bands (1321, 1053, 1017, and 930 cm^{-1}) increased but over a much broader range of rh than with D_2O hydration. The B to Z transition, as measured by these absorbance curves, was completed at 76% rh(H_2O) as compared with 94% rh(D_2O). We conclude that the B to Z transition is either less cooperative or kinetically limited for hydration with H_2O. The midpoint of the transition is at higher rh with D_2O which suggests that hydrogen bonds make a larger contribution to the stabilization energy of the Z structure than the B structure of poly(dG-dC) with NaCl(0.56).

These bands can be used to measure the fraction of B and Z structures present at a given rh for samples which are a mixture of these structural forms. The band at 1015 cm^{-1} is well suited for this purpose since it has a clearly measurable absorbance for each structure. This value can be self-normalized by dividing the absorbance at 1015 cm^{-1} for any hydration by the value measured at for the pure B structure. This ratio (R_{1015}) will change from one for the B form to 1.55 for the Z form with NaCl(0.56) and D_2O hydration. If we assume a linear dependence of the mole fraction(f) of Z on the value of R_{1015}, then $f=(R_{1015}-1)/0.55$. Similar equations may be constructed for other Z indicator bands, and for the B^* to B transition.

Some of the bands indicated by their nominal frequency values in the above discussion gave small but significant shifts in frequency as the transitions occurred (See Table 1 and Figure 4). The shift in the 830 cm^{-1} band from B^* to Z (which has been previously noted) (1,3), was particularly clear but other smaller shifts were still significant and measurable. These frequency shifts (which may be easier to measure than absorbance values) may also be used to estimate the mole fraction of Z in a sample containing B and Z. For the band which shifts from 933 in B to 937cm^{-1} in Z, $f = (v - 833)/5$ where v is the wavenumber of maximum absorption.

The variation of frequency of a given band with hydration in all cases is similar to the variation of absorptivity as shown in Figure 3 although sometimes an increase in absorbance may be accompanied by a decrease in frequency. Such shifts in frequency are caused by changes in the conformation of the absorbing groups which are too small to produce two resolved bands (one from the B and the other from the Z structure). Distinct and resolved bands for the B and Z structures also exist (1683,

1655 and 1600, 1637cm^{-1} and between 1500 and 1340cm^{-1} and 868 cm^{-1}, see Table 1) which show that the B to Z transition as observed in hydrated films is a two state phenomenon. Intermediate or transitional states do not exist for most of the subunits of the polymer. From both Figures 3 and 4, we conclude that all of the molecular subgroups involved in the normal modes of vibration producing these bands have synchronously completed the B* to B and B to Z transitions at 98 and 94% rh(D_2O) respectively.

DISCUSSION

The spectra given above clearly define two structures for highly hydrated poly(dG-dC). IR spectra of the more highly hydrated form (which we call B*) have been observed previously (1-3). Although these samples were said "not to belong to a classical B form family..." (1), subsequent authors have continued to refer to this highly hydrated form as B (3,6). We suggest that B* is a B-family structure since only minor changes in the absorptivities or frequencies of the corresponding IR bands take place upon slight dehydration. The B* indicator bands at 1622(sh) and 853 cm^{-1} disappear, the band at 830 cm^{-1} shifts to higher frequency and the band at 895 cm^{-1} weakens as B* changes to B. The spectrum of the slightly dehydrated polymer is quite similar below 1350 cm^{-1} to that of B-form, calf-thymus DNA so we have assigned this as the B structure of poly(dG-dC). This difference in nomenclature should be noted when reading past work.

Variations from the classical B structure for DNA are well known within a single oligonucleotide as a function of base sequence (microheterogeneity) (17) and for different polynucleotides (18,19) and in DNA from organisms (20). However, different B-family structures have not previously been reported for poly(dG-dC) in fibers or films.

The lithium salt of poly(dG-dC) was observed in the B structure by X-ray diffraction using a fiber hydrated at 81% rh (18). This would presumably be the "wrinkled B" structure which has a dinucleotide repeat and other variations from the structure of "smooth B DNA" (19). This suggests the possibility that our B* structure could be "wrinkled B" and our B structure could be "smooth B" although we can not conclusively demonstrate this correspondence.

One fiber of poly(dG-dC) was found to be in the A structure at 92% rh and would not change to the B structure (18). The Z structure was also observed for fibers of this sample of poly(dG-dC) with NaCl at 43% rh (18). This observation again agrees with our IR results. However, we did not

observe the A structure for poly(dG-dC) under any of our conditions of hydration or NaCl content. The lithium salt of this polymer may be needed to obtain the A structure.

The free-energy differences which affect the equilibrium between two DNA structures involve changes in the strengths of hydrogen bonds, hydrophobic bonds and electrostatic interactions (21). NaCl will decrease electrostatic repulsions which favors the structure in which the PO_2^- groups are closer together. NaCl in aqueous solution strengthens hydrophobic bonds (22) which favors the structure with the larger base overlap.

Increasing the hydration of poly(dG-dC) favors B^* over B at any content of NaCl, which suggests that hydrophobic forces stabilize B^*. However, only minor differences in base stacking are suggested from the spectra. The similar aggregate strength of hydrogen bonding in B^* and B was noted above. Increasing NaCl stabilizes the B structure which suggests that electrostatic repulsion is more destabilizing in B than in B^*.

Stabilization of Z by NaCl suggests greater electrostatic repulsions in Z as compared to B (4). However, in aqueous solutions at very low ionic strengths the Z form of poly(dG-m^5dC) is stable (14, 15) which suggests that electrostatic and hydrophobic forces are not major factors in stabilizing Z with respect to B.

The infrared spectra of poly(dG-dC) with NaCl(0.56), at higher salt contents and in solution (1-3, 16) show that the bands which arise mainly from the guanine C6=O and the cytosine C2=O stretching motions (at 1683 and 1655 cm^{-1} for the B structure) move to lower frequencies (1665 and 1635 cm^{-1}) for the Z structure with D_2O hydration. We conclude that hydrogen bonds to the C6=O and C2=O groups are stronger in the Z structure than in the B structure and this conclusion is supported by the occurrence of the transition from B to Z at higher hydrations with D_2O than with H_2O. Stronger interbase hydrogen bonds would be expected in the Z structure which has less propeller twist (0^o) than does the B structure (16^o) (17). Hydrogen bonds become stronger as the axis of the N-H (or N-D) bond approaches intersection with the oxygen atom. The Z structure may be further stabilized by hydrogen bonding between sites on the bases and the water molecules hydrating the major and minor grooves of the helix. Evidence does exist for some strongly bound water molecules in the Z structure (24).

An alternative explanation for the shifts in C=O frequencies involves

dipole coupling of nearest neighbor C=O groups which could occur within one base pair (13) or between bases one step apart in the helix (10). Coupling constants obtained empirically for poly(dG-dC) range from -11.5 to +9.8 cm^{-1} (25,26).

If dipole coupling is a valid explanation of the C=O frequencies in poly(dG-dC), our interpretation in terms of hydrogen bonding is weakened. We, therefore, calculated values for D_{11} (using the method of Krimm and Abe, 27) from the geometry of the base pairs (4,24) and the integrated intensity for the C2=O band of cytosine (ca. 20,800 liter $mole^{-1}$ cm^{-2}) which is approximately the same for the C6=O band of guanine (10). The values obtained (the largest was 2.5 cm^{-1} for cytosine in the CpG sequence) are too small to account for the observed frequency shifts upon helix formation or during the B to Z transition. Since the coupling is proportional to R^{-3} (R is the distance between dipole centers) the empirical values (13,25,26) for C2=O coupling with C6=O in one base pair are unlikely to arise from dipole coupling alone. Note that dipole coupling does not occur for the stacked, single-strand form (23) of poly(rC) which gives the same carbonyl frequency as monomer (28).

Hydrogen bonding is probably the major cause of these shifts. We stress that an N-H...O=C hydrogen bond between bases may be weaker than hydrogen bonds between C=O and water for the monomer in solution. This would explain the higher frequency for C6=O in the polymer duplex as compared with the monomer without dipole coupling.

We have shown that the B to Z transition will occur for poly(dG-dC) at a relatively low NaCl content (r = 0.24) if the activity of H_2O (which equals the rh for an ideal solution) is sufficiently lowered. Equivalent conditions may occur in living cells. The concentrations of some relevant species in an E. coli cell are (K^+) = 0.2M, (Na^+) and (Mg^{2+}) = 50 mM. Charged polyamines, such as spermine, are also present and are known to favor the Z structure (4). For a typical E. coli bacterium (a rod 0.55 x 10^{-4} cm in diameter and 2 x 10^{-4} cm long), the average concentration of the nucleotides in DNA would be ca. 30 mM (assuming 5000 genes per bacterium each coding for a protein of 30,000 mole wt.). Proteins bound to DNA could increase the local ionic strength with charged amino-acid residues and could lower the activity of water by surrounding DNA with hydrophobic amino-acid residues. The formation of Z DNA by small regions of correct base sequence is certainly not excluded in normal living cells.

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REFERENCES

1. Taillandier, E., Taboury, J., Liquier, J., Sautiere, P. and Couppez, M. (1981) Biochemie 63, 895-898.
2. Pilet, J. and Leng, M. (1982) Proc. Natl. Acad. Sci. USA 79, 26-30.
3. Taboury, J.A., Liquier,J. and Taillandier, E. (1985) Can J. Chem. 63, 1904-1909.
4. Rich, A., Nordheim, A. and Wang, A. H.-J. (1984) Ann. Rev. Biochem. 53, 791-846.
5. Pohl, F.M. and Jovin, T.M. (1972) J. Mol. Biol. 67, 375-396.
6. Taboury, J.A., Bourtayre,P., Liquier, J. and Taillandier, E. (1984) Nucleic Acids Res. 12, 4247-4258.
7. Behe, M. and Felsenfeld, G. (1981) Proc. Natl. Acad. Sci. USA 78, 1619-1623. Woisard, A., Fazakerley, G.V. and Guschlbauer, W. (1985) J. Biomol. Struct. Dyn. 2, 1205-1220.
8. DiRico, D.E. Jr., Keller, P.B. and Hartman, K.A. (1985) Nucleic Acids Res. 13, 251-260.
9. Keller, P.B. and Hartman, K.A. (1986) Spectrochim. Acta 42A, 299-306.
10. Tsuboi, M. (1974) in Basic Principles in Nucleic Acid Chemistry Vol. 1, Tso, P.O.P., Ed., Academic Press, 399-425.
11. Tsuboi, M., Takahashi, S. and Harada, I. (1973) in Physico-Chemical Properties of Nucleic Acids Vol. 2, Duchesne, J., Ed., Academic press, 92-145.
12. Keller, P.B. and Hartman, K.A. in preparation.
13. Howard, F.B., Frazier, J. and Miles, H.T. (1969) Proc. Natl. Acad. Sci. USA 64, 451-458.
14. Feuerstein, B.G., Marton, L.J., Keniry, M.A., Wade, D.L. and Shafer, R.H. (1985) Nucleic Acids Res. 13, 4133-4141.
15. Behe, J.M. (1986) Biopolymers 25, 519-523.
16. Roy, K.B. and Miles, H.T. (1983) Biochem. Biophys. Res. Commun. 115, 100-105.
17. Dickerson, R.E., Drew, H.R., Conner, B.N. , Wing, R.M., Fratini, A.V. and Kopka, M.L. (1982) Science 216, 475-485.
18. Leslie, A.G.W., Arnott, S., Chandrasekaran, R. and Ratliff, R.L. (1980) J. Mol. Biol. 143, 49-72.
19. Arnott, S., Chandrasekaran, R., Puigjaner, L.C., Walker, J.K., Hall, I.H.and Birdsall, D.L. (1983) Nucleic Acids Res. 11, 1457-1474.
20. Dutta, S., Parrack, P.K. and Sasisekharan, V. (1984) FEBS Letters 176, 110-114.
21. Felsenfeld, G. and Miles, H.T. (1967) Ann. Revs. Biochem. 36(II), 407-448.
22. Kauzmann, W. (1959) Advances in Protein Chemistry 14, 1-63.
23. Bloomfield, V.A., Crothers, D.M. and Tinoco, I. (1974) Physical Chemistry of Nucleic Acids, Harper & Row, 95-102.
24. Wang, A. H.-J., Quigley, G.J., Kolpak, F.J., van der Marel, G., van Boom, J.H. and Rich, A. (1981) Science 211, 171-176.
25. Tsuboi, M. (1985) in Spectroscopy of Biological Molecules, Alix, A.J.P., Bernard L. and Manfait, M., eds., Wiley, 101-107.
26. Semenov, M.A. and Bolbuch, T.V. (1984) Studia Biophys. 102, 215-220.
27. Krimm, S. and Abe, Y. (1972) Proc. Nat. Acad. Sci. USA 69, 2788-2792.
28. Miles, H.T. (1961) Proc. Nat. Acad. Sci. 47, 791-802.