Measurement of Intercolumnar Forces between Parallel Guanosine Four-Stranded Helices

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ABSTRACT The deoxyguanosine-5′-monophosphate in aqueous solution self-associates into stable structures, which include hexagonal and cholesteric columnar phases. The structural unit is a four-stranded helix, composed of a stacked array of Hoogsteen-bonded guanosine quartets. We have measured by osmotic stress method the force per unit length versus interaxial distance between helices in the hexagonal phase under various ionic conditions. Two contributions have been recognized: the first one is purely electrostatic, is effective at large distances, and shows a strong dependence on the salt concentration of the solution. The second contribution is short range, dominates at interaxial separations smaller than about 30–32 Å, and rises steeply as the columns approach each other, preventing the coalescence of the helices. This repulsion has an exponential nature and shows a magnitude and a decay length insensitive to the ionic strength of the medium. Because these features are distinctive of the hydration force detected between phospholipid bilayers or between several linear macromolecules (DNA, polysaccharides, collagen), we conclude that the dominant force experienced by deoxyguanosine helices approaching contact is hydration repulsion. The observed decay length of about 0.7 Å has been rationalized to emerge from the coupling between the 3-Å decay length of water solvent and the helically ordered structure of the hydrophilic groups on the opposing surfaces. The present results agree with recent measurements, also showing the dependence of the hydration force decay on the structure of interacting surfaces and confirm the correlations between force and structure.

INTRODUCTION

Guanine, one of the bases of nucleic acid, shows the special and unique ability to self-associate in water into stable structures. Ordered gels and fibers from guanosine have in fact been observed in the past (Gellert et al., 1962; Saenger, 1984). More recently, we reported that deoxyguanosine derivatives exhibit in water an extended polymorphism, which includes the formation of hexagonal and cholesteric phases at high and low concentration, respectively (Mariani et al., 1989, 1993; Bonazzi et al., 1991; Amaral et al., 1992; Ciuchi et al., 1994; Franz et al., 1994). Moreover, naturally single-stranded guanine-rich sequences from telomers and other chromosomal locations have been found to form a family of higher-order structures (the so-called quadriplexes) (Sen and Gilbert, 1991). All of these macromolecular assemblies have a common basic building block, the disk-shaped planar quartet (tetramer) formed by four Hoogsteen-bonded guanosine moieties, shown in Fig. 1 (Saenger, 1984; Fisk et al., 1982; Mariani et al., 1989; Sen and Gilbert, 1991).

Concerning the lyotropic phases, we demonstrated by x-ray scattering experiments and optical microscopy observations that they are columnar, each column composed of tetramers stacked in planes perpendicular to the column axis at the typical distance of 3.4 Å (see Fig. 1) (Mariani et al., 1989; Amaral et al., 1992). In each column, the tetramers are not piled in register, but are rotated with respect to each other to form a four-stranded helix (Mariani et al., 1989; Bonazzi et al., 1991). However, the helix pitch was not clearly measured in x-ray scattering experiments, but according to results obtained in fibers (Gellert et al., 1962) and to circular dichroism calculations (Gottarelli et al., 1990; Bonazzi et al., 1991), a left-handed unit rotation between successive tetramers of 30° should be considered reasonable. An important feature of guanine-containing structures is that these structures specifically bind alkali monovalent cations to form the four-stranded helices. Moreover, the four-stranded helices appear to be differently stabilized, depending on the identity of the cation (Detellier and Laszlo, 1980; Sen and Gilbert, 1991; Franz et al., 1994). This effect has been explained in terms of a size-selective binding of cations within the central cavity of the tetramers, which facilitates the self-assembling process. Nevertheless, the alkali cations have also been observed to affect the polymorphic properties of guanosine analogs (Ciuchi et al., 1994).

The possible biological relevance of this guanine special feature is, as yet, undefined—it has been proposed that the formation of the quadriplex within chromosomes might provide a means for the parallel pairing of the four homologous chromatids during meiosis and the dimerization of the telomeric ends of chromosomes (Sundquist and Klug, 1989; Sen and Gilbert, 1991, 1992), and may have a prebiotic significance in the origin of the genetic code (Detellier and Laszlo, 1980; Sen and Gilbert, 1991). However, distance-dependent interactions involved in all of the mentioned processes have been neglected, but it has been rec-

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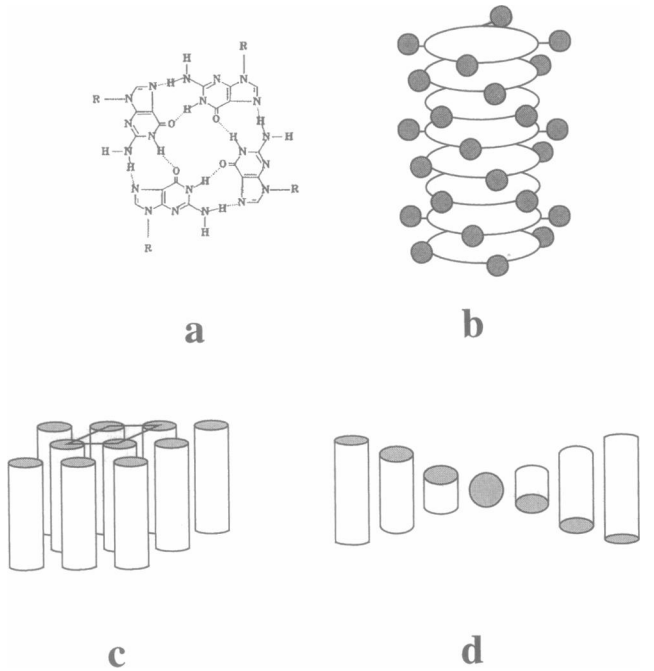

FIGURE 1 Model of formation of the columnar mesophases. (*a*) Tetrameric arrangement of guanine bases bonded in a Hoogsteen mode. The radius of the tetrameric disk is about 12.5 Å (Fisk et al., 1982). (*b*) Structure of the four-stranded helix based on the piling up of discrete tetramers: the disks represent the guanosine tetramers and the grey circles indicate the sugar groups. Note that the stacking is helical (see the text). (*c*) Hexagonal and (*d*) cholesteric arrangements of the four-stranded helices: the helices are represented as cylinders. In the hexagonal phase, the two-dimensional unit cell is indicated.

ognized for a long time that for their ability to create strong and specific associations, the forces between and within biological molecules and aggregates, act as a key to the regulation of cellular activity and functions. The problem of the interactions of DNA with itself and with monovalent cations should be considered a relevant example (Luzzati et al., 1967; Eisenberg, 1976; Rau et al., 1984; Rau and Parsegian, 1992).

Indeed, the contribution of hydration to the energetics of macromolecular assembly in water is well recognized, but only recently it has been underlined that hydration forces should contribute far more than usually thought (Rand, 1992). The water role in macroaggregate interactions has been conveniently measured by using the osmotic stress method (Parsegian et al., 1986). Osmotic stress is the controlled removal of water from the system under investigation: the system is allowed to come to equilibrium with a polymer solution of known osmotic pressure, and the separation between the macroaggregates is measured by x-ray diffraction experiments. Under conditions in which the polymer is excluded from the macroaggregate lattice, the osmotic pressure of the polymer solution is the osmotic stress compressing the lattice. This force of compression is equal and opposite to the macroaggregate repulsion at the observed lattice spacing. In this way, forces in different systems, such as charged or electrically neutral lipid bilayer membranes (Marra and Israelachvili, 1985; Rand et al., 1988, 1990), DNA double helices (Rau et al., 1984; Rau and Parsegian, 1992), stiff polysaccharides (Rau and Parsegian, 1990), and self-assembled proteins (Leikin et al., 1994) have been investigated (see also Israelachvili, 1994). As a result, a repulsive hydration force, which decays exponentially with separation and which for a separation of less than some 10 Å completely overshadows electrostatic repulsion, has been revealed. This repulsion, which is only weakly dependent on ionic strength, has been recognized to derive from the work required to dehydrate the hydrophilic surfaces.

In this paper, we use the osmotic stress method to determine the forces between parallel four-stranded helices of deoxyguanosine-5′-monophosphate (d(pG)). In particular, osmotic stress measurements have been performed on the basis of the observation that d(pG) in solution, in both the presence and the absence of KCl or NaCl, when exposed to a polymer such as polyethyleneglycol (PEG), condenses into a hexagonal phase separate from the polymer solution. PEG is excluded from the d(pG) condensed phase and exerts an osmotic pressure on it, whereas water and small ions are free to equilibrate between the two phases. At equilibrium, the distance between columns, which we measure by x-ray diffraction, is determined by a balance of the expansive forces due to intercolumnar repulsion and the compressive osmotic stress from the bathing PEG solution: from the osmotic pressure, the force between the parallel columns could be derived and the different contributions investigated.

MATERIALS AND METHODS

The method for direct force measurements by osmotic stress has been described by Rand and co-workers in several papers (Parsegian et al., 1986; Rand et al., 1988, 1990; Rau et al., 1984; Rau and Parsegian, 1992), to which the reader should make reference for details. Osmotic pressures Π of PEG solutions were taken from Parsegian et al. (1986) and measured in some cases as indicated by Rau and Parsegian (1992). According to these authors, we observed that salt does not seem to have a significant effect on PEG osmotic pressure, and PEG does not seem to have a significant effect on salt activity.

d(pG) ammonium salt and PEG (15,000–20,000 MW) were of commercial origin (99% purity; Sigma, Milan, Italy). Samples were prepared by equilibrating the d(pG) against PEG-salt solutions of known osmotic pressure in vast excess. Condensed phases were easily obtained in PEG solutions of at least 10 wt%. A maximum polymer concentration of 50 wt% was used. The concentrations of the KCl solutions considered in this work were 0, 0.1, 0.4, 0.5, 1.0, and 2.0 M. Control experiments were also performed by using NaCl solutions at a fixed PEG concentration of 25 wt%.

The d(pG) condensed phases were investigated by x-ray diffraction. Low-angle x-ray diffraction experiments were performed using a Philips PW1830 x-ray generator equipped with a Guinier-type focusing camera operating in vacuum: a bent quartz crystal monochromator was used to select the $CuK\alpha_1$ radiation (λ = 1.54 Å). The diffraction patterns were recorded on a stack of four Kodak DEF-392 films. Scattering data were also recorded on a two-circle diffractometer equipped with a bent-position sensitive detector (CPS120, INEL, Les Ulis, France). A Philips PW1830 was used as x-ray source, run at a power of 1.6 kW with a copper target.

The CuKα_1 line was selected by a Guinier germanium monochromator focused on the detector. The intrinsic resolution of the diffractometer (width of a crystalline peak) was determined to be 1.1 channels. The width of one channel was measured using a LiF single crystal and calculated to be 0.0300(2)°. Samples were mounted in vacuum-tight cells with thin mica windows. The sample cell temperature was controlled at 25°C with an accuracy of 1°C by using a circulating thermostat.

RESULTS

X-ray diffraction experiments

X-ray diffraction profiles indicate that d(pG) condensed phases prepared from solutions containing at least 10 wt% PEG are columnar hexagonal (see Fig. 2). In the low-angle region, the profiles show in fact a series of narrow reflections (from 3 to 5 Bragg peaks) with spacing ratios in the order $1:\sqrt{3}:\sqrt{4}\ldots$ According to our previous works (Mariani et al., 1989; Bonazzi et al., 1991; Franz et al., 1994), these reflections can be indexed considering the two-dimensional hexagonal lattice of p6m symmetry (Luzzati, 1968). It should be noticed that the low-angle x-ray diffraction profiles obtained from phases condensed from more diluted PEG solutions (lower than about 8 wt%) show only a very diffuse reflection. According to our previous results, this finding indicates the presence of the cholesteric phase.

From the Bragg spacings $s_{h,k}$ ($s = (2 \sin \theta)/\lambda$, where 2θ is the scattering angle), the unit cell dimension, a (i.e., the interaxial distance between the four-stranded helices), has been obtained by using

$$a = \frac{2}{\sqrt{3}} \frac{\sqrt{(h^2 + k^2 - hk)}}{s_{h,k}}, \qquad (1)$$

where h and k are the Miller indices of the reflections (Luzzati, 1968). The interaxial spacing decreases both with increasing concentration of the added polymer (see below) and with increasing salt molarity. In Fig. 3, we compare the interaxial distance measured at a fixed PEG concentration (i.e., at the same osmotic pressure) as a function of KCl and NaCl salt concentrations. Inside the experimental error, no differences were detected, indicating a nonspecific effect of monovalent ions on the intercolumnar interactions.

The x-ray diffraction profile also gives experimental evidence on the columnar nature of the d(pG) condensed phases. According to our previous works (Mariani et al., 1989; Bonazzi et al. 1991; Amaral et al., 1992; Franz et al., 1994), a narrow band is in fact observed in the high-angle region at about $s = (3.4\ \text{Å})^{-1}$ (see Fig. 2). This reflection is related to the nature of the order inside the structure elements, which are in fact columns composed of guanosine tetramers stacked perpendicular to the column axis. From the peak position, the distance between the neighboring tetramers has been obtained. As a result, this distance appears to be independent of the osmotic pressure, but is clearly dependent on the salt concentration, as reported in Fig. 4. However, no measurable differences for the presence of Na^+ or K^+ ions in the solution were detected.

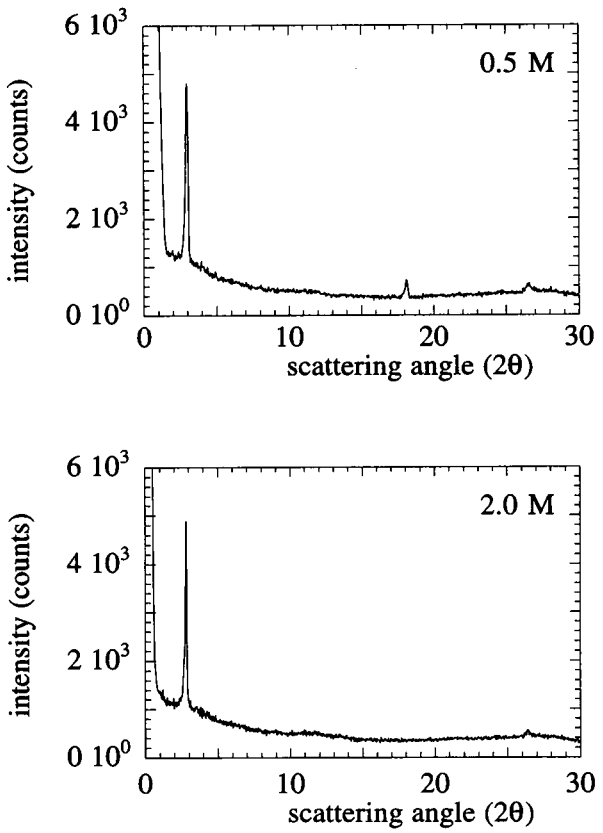

FIGURE 2 X-ray diffraction profiles obtained at 25 wt% PEG from samples with 0.5 and 2.0 M KCl. In the low-angle region, the (10) peak relative to the hexagonal packing of the four-stranded helices is clearly visible (up to five higher order reflections are easily obtained by using the Guinier camera). In the high-angle region, the peak reflecting the stacking of the tetramers is located at a scattering angle 2θ of about 26°, near the maximum of the structure factor of the water. The peak at 2θ of about 18° is due to the mica windows.

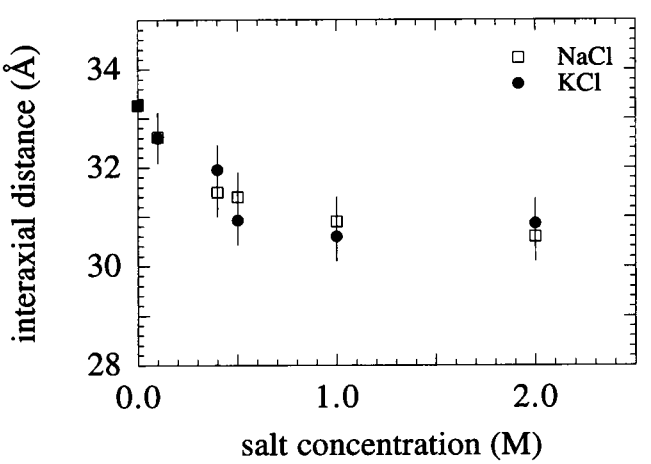

FIGURE 3 Interaxial distance between the guanosine four-stranded helices in the hexagonal phase measured at 25 wt% PEG as a function of KCl and NaCl molarity of the solutions. Note that the interaxial distance corresponds to the two-dimensional hexagonal unit cell dimension (see Fig. 1).

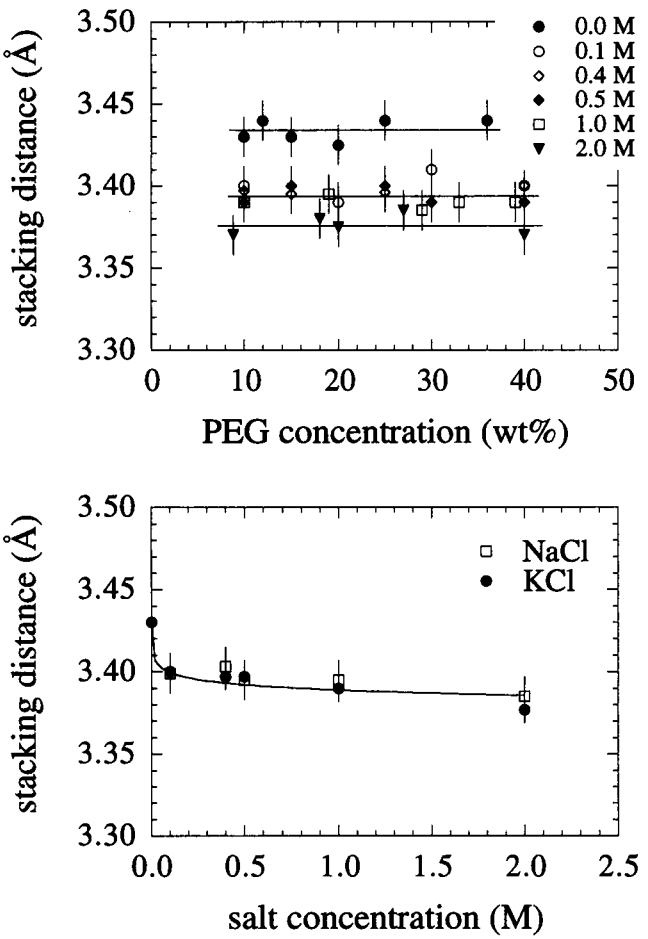

FIGURE 4 (*Top*) Distance between the stacked tetramers as a function of PEG concentration in different KCl solutions (KCl molarities are indicated in the frame). Straight lines through data for 0, 0.5, and 2 M solutions are guides to the eyes. (*Bottom*) Distance between the stacked tetramers as a function of salt molarity. The repeat distance has been measured at 25 wt% PEG in the case of NaCl solutions and averaged from all measurements made at the same ionic strength in the case of KCl solutions. The solid line is a power fit to the KCl data to show the general trend.

This behavior emerges from the interactions between guanosines and these alkali metal ions. Both cations have been found to be able to enter the central cavity of the tetramers and to contribute to the stabilization of the aggregates, keeping together two tetrameric planes via coordination with the oxygenes of the guanine residues (Detellier and Laszlo, 1980). As the tetramer molarity in the solutions used in this work was about 0.05 M, the decrease of the stacking repeat distance already observed at the lowest investigated salt concentrations could be associated with the sudden saturation of the inner binding site. By contrast, the further stabilization of the stacking distance, observed as the salt molarity further increases, is probably due to the progressive screening of the electrostatic repulsion of the negatively charged phosphate groups, brought together in the self-assembled structures, for atmospheric condensation of the counter-ions. An alternative explanation is that the tetramer planes are tilted with respect to the helical axis and that the tilt angle could vary as a function of salt molarity without the real stacking distance changing. However, optical microscopy results (Mariani et al., 1989) and x-ray diffraction experiments on oriented samples (Amaral et al., 1992) evidence that tetramers are stacked perpendicularly to the columnar axis, in both the presence and the absence of KCl.

It should be remarked that the x-ray diffraction measurements here reported reflect a reversible thermodynamic equilibrium condition. In fact, the interaxial distance measured in a sample equilibrated against one set of osmotic pressures and salt concentrations can be reequilibrated against another set of conditions with no apparent hysteresis or dependence on the initial state of the condensed phase. Moreover, to determine whether the PEG acts only through its osmotic pressure and not directly with the d(pG) by partitioning between columns in the condensed regime, we repeated some measurements with 10,000 MW PEG and 8,000 MW PEG: the results are independent of the molecular weight of the polymer and only appear to be dependent on the osmotic pressure.

Osmotic pressure measurements

A plot of the osmotic pressure as a function of the interaxial distance between the columns for condensed d(pG) hexagonal phases in KCl is shown in Fig. 5. At low pressure, interaxial distances show a strong ionic strength dependence. By contrast, at higher pressures, the data appear to be independent of salt concentration and to converge to a common curve.

From the interaxial distance and osmotic pressure, the force between parallel columns has been derived. As reported by Rau et al. (1984) for DNA molecules in hexagonal phase, the osmotic pressure Π could be expressed in terms

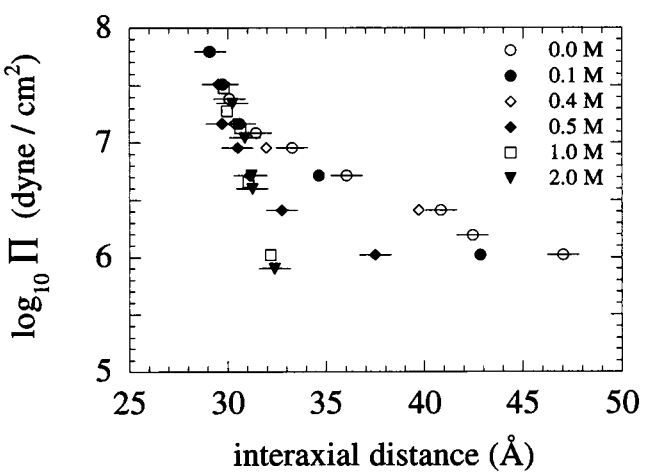

FIGURE 5 Lattice pressure versus interaxial distance between the guanosine four-stranded helices in the hexagonal phase in different KCl solutions. Error bars in interaxial distances are omitted for clarity in some cases but have similar length.

of the energy per unit length (G) per column in the hexagonal lattice by

$$\prod = \frac{dG}{d\sigma} = \left(\frac{dG}{da}/(a\sqrt{3})\right), \quad (2)$$

where σ is the area of the two-dimensional unit cell ($\sigma = a^2\sqrt{3}/2$), and Π is the osmotic pressure in both the condensed phase and in the polymer solution. The force per unit length between the nearest pair, the interaction of which is taken to be pairwise additive, $f(a)$, then results (Rau et al., 1984):

$$f(a) = \prod a/\sqrt{3}. \quad (3)$$

Therefore, from the plot of Fig. 5, the forces between the four- stranded helices could be obtained. Examples referring to some samples are reported in Fig. 6.

DISCUSSION

Interactions between colloidal biological systems originate from van der Waals, electrostatic, hydration, or steric forces (Rand and Parsegian, 1989; Israelachvili, 1994; Parsegian and Rand, 1995). Direct measurements of those interactions have shown that in the crucial last 10 Å of separation, hydration forces are far more important that the electrostatic and van der Waals forces traditionally assumed to be dominant between charged surfaces (Rand et al., 1988, 1990; Rau et al., 1984; Rau and Parsegian, 1992). In particular, at these separations, an exponential force has been described to arise from the spatially varying perturbation of water near the charged surface (hydration force). Such hydration repulsion has the form (Israelachvili, 1994)

$$f(a) = f_0 e^{-a/\lambda}, \quad (4)$$

which shows that force decays exponentially with distance. The hydration force is characterized by a hydration coefficient f_0, which reflects the degree to which the surface orders the boundary water, and by a decay distance λ, which is a property of the water itself and which reflects the way that ordering is propagated through water. In the case of parallel DNA double helices, Rau and co-workers (1984) reported that this strong repulsive force appears to be detectable when the interaxial distance is lower than about 30–35 Å and grows exponentially with a 2.5–3.5 Å characteristic distance as the molecules are brought together.

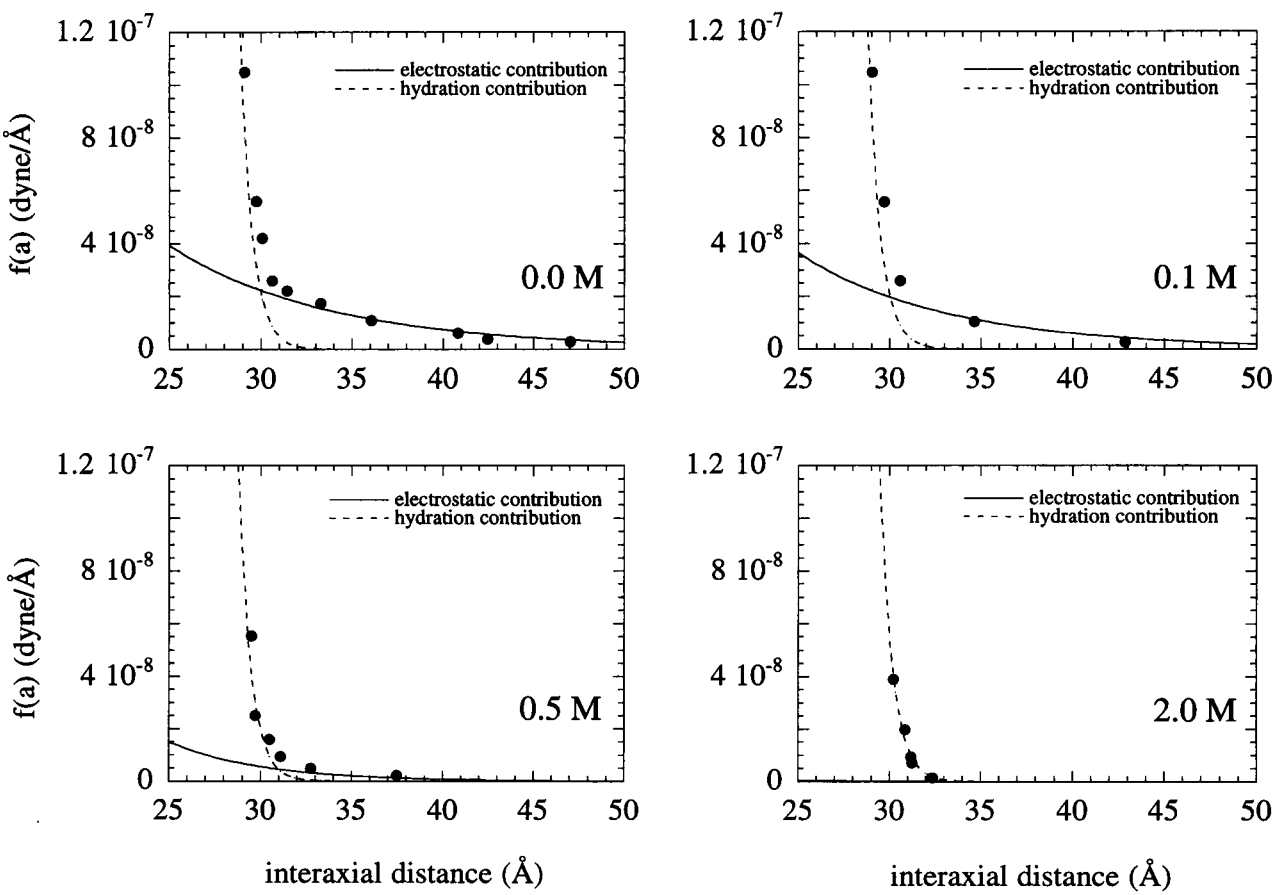

FIGURE 6 Plot of the forces per unit length between the guanosine four-stranded helices in the hexagonal phase ($f(a)$, as defined by Eq. 3) versus interaxial distance, obtained at different ionic strengths. The corresponding KCl molar concentrations are indicated in the frames. In each plot, the contributions for electrostatic and hydration forces, calculated by double fitting procedures using $f(a) = f(a) + F_{el}(a)$ (Eqs. 4 and 5), are shown.

By contrast, at large interaxial distances, force magnitudes and slopes are expected to be ionic strength dependent, as they mainly arise from fluctuation-enhanced electrostatic repulsion (Israelachvili, 1994). In the case of parallel cylindrical particles, as linear polyelectrolyte molecules in solution, the force has the approximate form (Parsegian, 1973; Israelachvili, 1994)

$$F_{el}(a) = F_0 \frac{e^{-ka}}{\sqrt{ka}}, \quad (5)$$

where the force coefficient F_0 is independent of the axis-to-axis distance a, and k is the inverse of the Debye length.

In the present case, theoretical correlations with experimental data have been obtained, neglecting van der Waals contributions, that are expected to be fairly weak, especially at high salt concentrations (Rau et al., 1984; Israelachvili, 1994). Therefore, decay constants λ and $1/k$ and hydration and electrostatic force coefficients f_0 and F_0 have been calculated by double-fitting Eqs. 4 and 5 on the force data plotted as a function of interaxial separation. Correlation coefficients as high as 0.995 have been obtained. Examples of the fitting results are reported in Fig. 6, where both electrostatic and hydration contributions are indicated. At all ionic strengths, the repulsive hydration force appears to be dominant at separations smaller than about 30 Å and to rise steeply as the two four-stranded helices approach each other, preventing the surfaces from coming into adhesive contact. Considering the radius of the tetrameric disk, at high pressure the shortest distance between the surfaces of two neighboring four-stranded helices is about 3 Å. Moreover, the effectiveness of the salt in reducing the magnitude of the electrostatic repulsion is remarkable. The screening effect determines a larger and larger difference between the electrostatic and hydration contributions; indeed, for salt concentrations higher than 0.5 M, a fitting procedure based only on Eq. 4 appears largely accurate. These results are quantitatively illustrated in the lower frame of Fig. 7, where the magnitude of hydration and electrostatic forces, extrapolated at 25 Å separation (i.e., for guanosine four-stranded helices at close contact), is plotted as a function of salt concentration.

In the upper frame of Fig. 7, electrostatic and hydration force decay constants are also reported. According to the theory, only the electrostatic decay shows a strong ionic-strength dependence. Moreover, the superposition of the observed electrostatic decay values on the curve representing Debye lengths calculated by the classical equation for 1:1 electrolytes (Israelachvili, 1994),

$$1/k = (3.04/\sqrt{[KCl]})\ \text{Å}, \quad (6)$$

where [KCl] indicates the salt molar concentration, confirms the nature of the force. By contrast, the hydration force decay is insensitive to salt concentration, in complete agreement with previous results on phospholipid bilayers, stiff polysaccharides, self-assembled proteins, and DNA helices (Rand et al., 1988, 1990; Rau et al., 1984; Rau and Parsegian, 1990, 1992; Leikin et al., 1994). Therefore, only at low ionic strengths, the slowly decaying electrostatic force emerges from behind the precipitously changing hydration force.

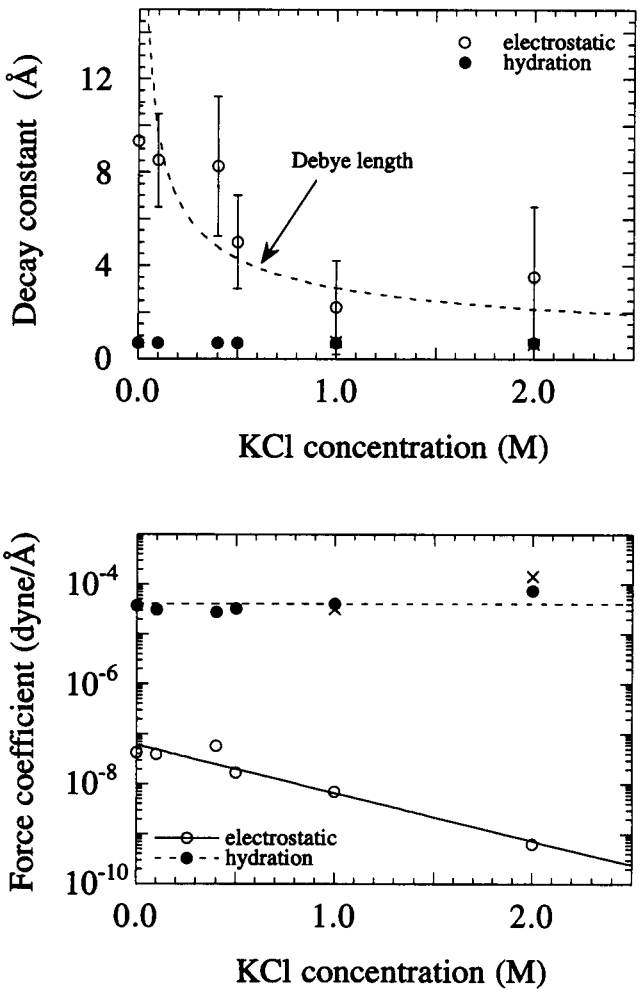

FIGURE 7 Best-fit coefficients relative to the electrostatic and hydration contributions to the force per unit length between the guanosine four-stranded helices in the hexagonal phase as a function of KCl concentration. When they are not shown, the error bars are smaller than the dimensions of the symbols. (*Bottom*) Magnitude of hydration and electrostatic forces at 25 Å separation (f(25 Å) and F_{el}(25 Å); see Eqs. 4 and 5). Note that the ordinate scale is logarithmic. The lines through the data are only guides to the eyes. (*Top*) Hydration and electrostatic force decay lengths (λ and $1/k$; see Eqs. 4 and 5). For comparison, calculated Debye lengths (see Eq. 6) are also reported. In both graphs, crosses indicate the hydration force magnitude at 25 Å separation and decay length as obtained by fitting experimental data, neglecting electrostatic contributions (i.e., by singly using the Eq. 4). As reported in the text, this gives good correlation coefficients only for ionic strengths as high as 1 M.

The average value of the hydration force decay measured in our system is 0.69 ± 0.01 Å. It is interesting to compare this value with those determined for other systems. The present value is in fact lower than the decay distance of 2.5–3.5 Å obtained by Rau and co-workers for DNA (1984), to which the present samples are most closely related. Marcelja's theories based on the polarization of the first

water shells induced by surfaces or surface charged groups, predict an exponential hydration repulsion of unspecified decay length, which should be a property of water itself and not of the perturbing surface (Israelachvili, 1994). A value of the decay length around 2.5–3 Å, which corresponds to the size of a water molecule, has been considered to be reasonable, whereas a variation from this value has been ascribed in flexible structures to entropic or steric contributions to the short-range force (Israelachvili, 1994).

However, four-stranded d(pG) aggregates should have a rather rigid and stiff structure (Franz et al., 1994); then the picture would seem more consistent with a dependence of the repulsive exponential short-range force decay on the structure of the interacting surfaces (Kornyshev and Leikin, 1989). Neglecting the case of disordered surface groups, from theoretical analysis the decay length of a residual repulsion at close separations has been shown to emerge from the coupling between the natural correlation length of the water solvent (λ_w, estimated at 3–5 Å) and the periodically ordered structure of the hydrophilic groups on the opposing surfaces (Kornyshev and Leikin, 1989; Leikin et al., 1993, 1994). When the distance between periodically ordered groups on planar surfaces is comparable to λ_w, the expected decay length λ^* can be calculated by using (Leikin et al., 1994)

$$\lambda^* = \frac{1}{2\sqrt{(1/\lambda_w)^2 + (2\pi/p)^2}}, \qquad (7)$$

where p is the surface repeat spacing. Because of the surface curvature, the expected decay length for interacting cylinders will be slightly smaller. Accordingly, recent measurements of forces between collagen triple helices showing exponential repulsion, with a decay distance of 0.65 Å, have been rationalized by considering the coupling between the 3-Å decay length of water and the 9.6-Å pitch of the collagen helix. In very good agreement with the experimental value, the corresponding decay length predicted by Eq. 7 was found to be 0.7 Å (Leikin et al., 1994).

In the present system, the axial periodicity of hydrophilic groups facing one another on opposing helical surfaces can be derived from fiber crystallographic data (Gellert et al., 1962) and from circular dichroism calculations (Gottarelli et al., 1990; Bonazzi et al., 1991); in each column there is a 12-layer repeat, with 3.4 Å per step. The total repeat distance is then 40.8 Å. Tetramers have a fourfold symmetry (Fig. 1), so that the periodicity on the helical surface is 10.2 Å. Using this value and $\lambda_w = 3$ Å, we find a decay distance λ^* of 0.71 Å, very close to the measured values. The exponential perturbation in water structure surrounding the four-stranded helices then appears to be dominated by the surface periodicity of polar and charged groups. Although we do not exclude other interpretations, from these results it emerges that at small separations four-stranded guanosine helices show forces fully consistent with hydrational force expectations. Therefore, the present system offers further validation of the suggestion of strong connections between force and structure (Kornyshev and Leikin, 1989; Leikin et al., 1993, 1994).

A final comment about the salt effect is in order. Our results indicate that alkali cations in solution affect guanine-containing structures via specific and nonspecific interactions. First, the inclusion of alkali cations, which selectively bind to the central cavity of the tetramers, influences the intracolumnar order and results in a strong reduction of the stacking repeat distance. Second, changes in the ionic strength of the solutions modify intracolumnar and intercolumnar interactions. This nonspecific effect is revealed as the progressive neutralization of the electrostatic repulsion of the negatively charged phosphate groups, which induces a further reduction of the tetramer stacking distance. Moreover, the electrostatic contribution to the intercolumnar repulsive forces also decreases, so that the helices approach each other. At small separations, a strong and rapidly increasing hydration force, connected with the periodically ordered structure of the hydrophilic groups on the opposing surfaces, emerges. As discussed above, this exponential force prevents the adhesion of the four-stranded helices. The biological relevance of these effects is straightforward, if the packing of DNA in cells and viruses and the biological function of quadriplex DNA structures and of higher-order structures of RNA are considered (Sen and Gilbert, 1992).

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