

Helix-Specific Interactions Induce Condensation of Guanosine Four-Stranded Helices in Concentrated Salt Solutions

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ABSTRACT Deoxyguanosine-5'-monophosphate in water self-associates into stable structures, which include liquid-crystalline hexagonal and cholesteric phases. The structural unit is a four-stranded helix, composed of stacked Hoogsteen-bonded guanosine quartets. By using the osmotic stress method, we recently measured the force between helices in KCl solutions up to 2 M. In addition to the long-range electrostatic force, a short-range hydration repulsive contribution was recognized. The hydration repulsion is exponential, and shows a decay length independent from the ionic strength of the solution. Here, we report that more concentrated KCl solutions cause condensation of the guanosine helix in a hexagonal phase with constant equilibrium separation of ~ 7 Å between helix surfaces. Long-range attraction, which induces the self-assembly, and short-range repulsion, which prevents the contact between the helices, are implied. By using osmotic stress, the force needed to push helices closer from the spontaneously assumed position has been measured. The attractive force was then estimated as a difference between the net force and the repulsive contribution, revealing an exponential decay length about two times larger than that of the short-range repulsion. The agreement with the helix interaction theory introduced recently by Kornyshev and Leikin (Kornyshev, A. A., and S. Leikin, 1997. Theory of interaction between helical molecules. *J. Phys. Chem.* 107:3656–3674) suggests that the repulsive and attractive forces originate from helix-specific interactions.

INTRODUCTION

Self-recognition and self-assembly processes lead to the formation of complex supramolecular structures starting from simple molecules. An interesting case concerns guanine, which, unique among the bases constituting nucleic acids, is able to form self-assembled four-stranded helical aggregates (Fisk et al., 1982; Mariani et al., 1989; Williamson, 1993). This property plays important biological roles (Sen and Gilbert, 1991; Williamson, 1993) and is related to the particular arrangement of H-bond donor and acceptor groups in guanine (Fig. 1). Four-stranded helices form, in fact, by the regular stacking of tetramers, which result from the assembling of four guanine residues in a Hoogsteen-bonded fashion (Mariani et al., 1989). In homoguanilic deoxyoligonucleotides, helices have also been recognized to lead to columnar liquid crystalline lyotropic phases of hexagonal and cholesteric type (Mariani et al., 1989; Bonazzi et al., 1991; Amaral et al., 1992; Franz et al., 1994; Gottarelli et al., 1997). The corresponding structures are sketched in Fig. 1.

Recently, the role of water in the interactions between four-stranded helices of deoxyguanosine-5'-monophosphate, d(pG), has been measured by using the osmotic stress method (Mariani and Saturni, 1996). The force-per-unit-

length versus interaxial distance between the helices in the hexagonal phase has been obtained under various ionic conditions, i.e., for KCl concentration ranging from 0 to 2 M. Two repulsive exponential contributions were recognized: the first one was electrostatic and it dominated at large distances; the second contribution was short-range and it showed the characteristics of a hydration repulsion, a force that derives from the work required to dehydrate hydrophilic surfaces (Rau et al., 1984; Rau and Parsegian, 1992a; Parsegian and Evans, 1996; see also Bloomfield, 1996). Within the mean-field theory of hydration forces (Leikin and Kornyshev, 1991; Leikin et al., 1994), the very short decay length of the hydration repulsion ($\lambda_r = 0.7$ Å) was suggested to emerge from the coupling between the water correlation length and the pitch of the four-stranded helix (i.e., to be dependent on both surface structure and water properties). Very recently, the short-range repulsion observed between guanosine helices (Mariani and Saturni, 1996) has been discussed as a remarkable example of helix-specific interactions (Kornyshev and Leikin, 1997).

In this paper, we report spontaneous condensation of d(pG) from very concentrated KCl and NaCl solutions. X-ray diffraction revealed that the condensed phases are hexagonal, and that the helices are not condensed to contact. The observed finite equilibrium separation between helices implies a balance between a long-range attraction, which induces the self-assembly, and a short-range repulsion, which prevents the contact. The residual repulsive forces between the helices, as they are brought closer from their spontaneously assumed spacings, have been measured in KCl by the osmotic stress method. The attractive force, estimated as a difference between the net force and the

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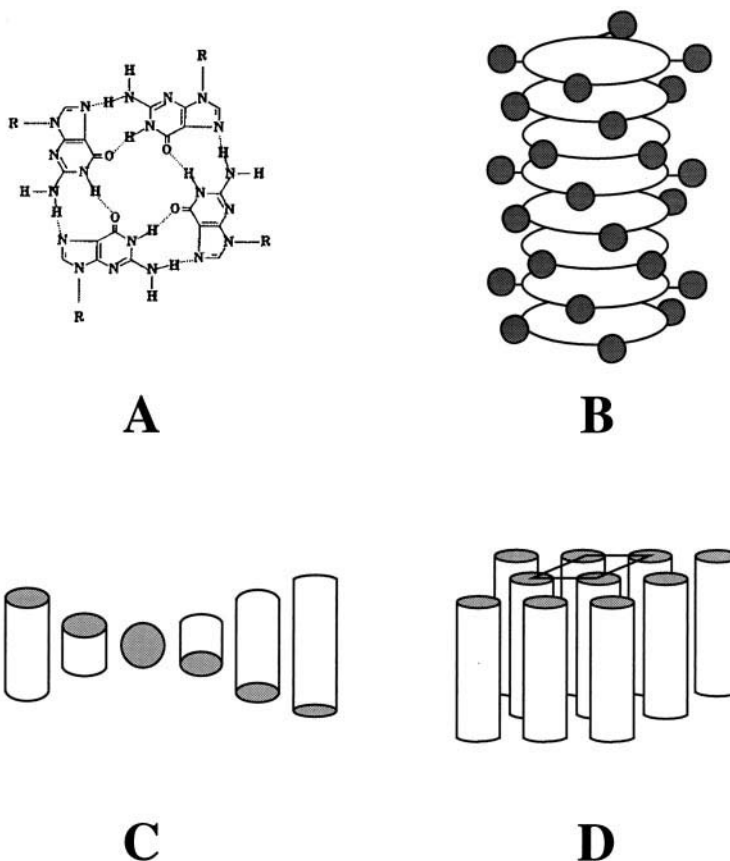


FIGURE 1 Structural models for the guanosine self-assembling. (A) Arrangement of the guanine quartet bonded in a Hoogsteen mode. The radius of tetramer is ~ 12.5 Å (Fisk et al., 1982). (B) Structure of the four-stranded helix: the disks represent the guanosine tetramers and the gray circles indicate the sugar residue. The stacking is helical. (C) Cholesteric and (D) hexagonal arrangements of the four-stranded helices: helices are represented as cylinders. In the hexagonal phase, the 2-dimensional unit cell is indicated.

repulsive contribution, shows an excellent agreement with the predictions for the helix interaction theory developed by Kornyshev and Leikin (1997), suggesting that the helix-helix recognition force originates from helix-specific cross-correlation interactions.

MATERIALS AND METHODS

The method for force measurements by osmotic stress has been described in detail by Parsegian and co-workers in several papers (e.g., Parsegian et al., 1986). Osmotic stress is the controlled removal of water from the system under investigation: in the present case, d(pG) is let to come in equilibrium with a high-molecular-weight polymer (i.e., polyethylene glycol) solution of known osmotic strength and the separation between helices is measured by x-ray diffraction. From the interaxial distance between the helices, a , and the osmotic pressure Π , the force-per-unit length between the parallel helices can be obtained by using (Rau et al., 1984):

$$f(a) = \Pi a / \sqrt{3}$$

The d(pG) ammonium salt, NaCl, KCl, and polyethylene glycol (PEG) 15000–20000 MW were of commercial origin (Sigma, Milano, Italy; 99% purity) and were used without further purification. Samples were prepared as reported by Mariani and Saturni (1996), equilibrating d(pG) against a vast excess of water solutions of salt and/or PEG of known ionic activity and osmotic strength. Osmotic pressures of PEG solutions were taken from Parsegian et al. (1986).

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 $\text{CuK}\alpha_1$ radiation ($\lambda = 1.54$ 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 (INEL CPS120, France). A Philips PW1830 was used as x-ray source, run at a power of 1.6 kW with a copper target. The $\text{CuK}\alpha_1$ 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 $0.0300(2)^\circ$. 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 on salt-condensed phases

X-ray diffraction profiles indicate that d(pG) phases condensed from KCl or NaCl solutions are hexagonal and give experimental evidence on their columnar structure (Fig. 2). In the low-angle region, the profiles show in fact a series of narrow reflections (at least three Bragg peaks were observed in the Guinier camera), whose spacing ratios index in a 2-dimensional hexagonal lattice (Mariani et al., 1989; Bonazzi et al., 1991; Franz et al., 1994). Moreover, a narrow band is observed in the high-angle region at $\sim s = (3.4 \text{ Å})^{-1}$; according to previous results (Mariani et al., 1989; Bonazzi et al., 1991; Amaral et al., 1992; Franz et al.,

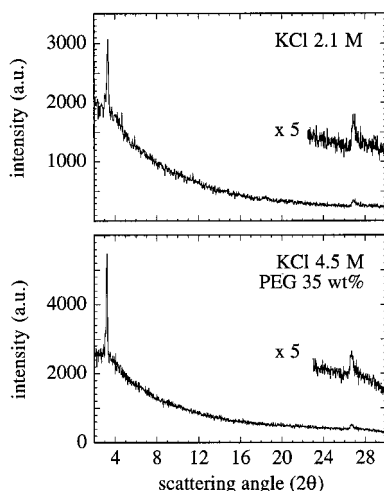


FIGURE 2 X-ray diffraction profiles obtained at 25°C from d(pG) condensed from a KCl 2.1 M solution (*top*) and a PEG 35 wt % solution containing 4.5 M KCl (*bottom*). In the low-angle region, the narrow (10) peak relative to the hexagonal packing of the helices is clearly visible (up to three higher-order reflections are detectable using the Guinier camera). In the high-angle region, the peak reflecting the stacking of the tetramers, located at a scattering angle 2θ of $\sim 26^\circ$, it is also visible.

1994), this reflection is related to the stacking of the tetramers inside the columns.

The unit cell dimension, a , (i.e., the interaxial distance between the four-stranded helices), calculated from the low-angle peak spacings, is reported as a function of the ionic strength of the condensing solution in Fig. 3. Considering that the tetramer diameter is ~ 25 Å (Bonazzi et al., 1991), it appears that in the condensed phases d(pG) helix surfaces are not in contact. Such a finite equilibrium separation implies a balance between attractive forces, bringing the helices together from infinite separation, and repulsive forces, acting at very short distances and preventing the contact between the helices. However, while a “salting-out” effect (i.e., a preferential salt exclusion from the helical surfaces which could create an osmotic stress) can explain the d(pG) condensation in NaCl, the independence of the interaxial separation from the ionic strength of the solution excludes a simple osmotic mechanism for the spontaneous aggregation of d(pG) in KCl.

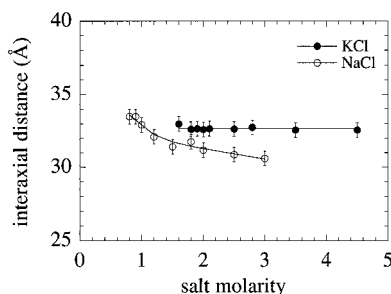


FIGURE 3 Variation of the interaxial distance between d(pG) helices measured in the absence of applied stress as a function of KCl and NaCl concentration of the condensing solution.

From the position of the high-angle band, we also determined the stacking distance between neighboring tetramers. No appreciable differences were detected for different condensing salts or at different salt concentrations.

Osmotic pressure measurements

Osmotic stress measurements have been performed to obtain information on the effective attraction that induces aggregation of d(pG) helices. In order to exclude the possibility that the effect of salt is simply osmotic, forces were measured in KCl solutions. The very high KCl concentration of 4.5 M was considered, but some measurements at 2.0 and 3.0 M KCl were also performed. An x-ray diffraction profile observed from the phase condensed from KCl 4.5 M solution in the presence of PEG is reported in Fig. 2. As before, the spacing ratios of the reflections in the low-angle region show that the phase is hexagonal, while the narrow band observed at $s = (3.4 \text{ Å})^{-1}$ confirms its columnar nature.

A plot of the force-per-unit-length as a function of the interaxial distance between the d(pG) helices in 3 and 4.5 M KCl is shown in Fig. 4. As a comparison, the corresponding data in 0, 0.5, 1.0, and 2.0 M KCl solutions, taken from Mariani and Saturni (1996), are also reported. At low pressure, the interaxial distances show a strong ionic strength dependence, while at higher pressures data are independent of salt concentration and converge to a common curve. A sharp downward turn to the equilibrium spacing of ~ 32 Å in the absence of applied stress is noticeable in the force data for d(pG) in 2.0, 3.0, and 4.5 M solutions.

The forces between d(pG) four-stranded helices at KCl concentrations not higher than 2.0 M were previously characterized (Mariani and Saturni, 1996). Two exponential, repulsive contributions were recognized. Being strongly sensitive to the ionic strength of the solution and showing decay lengths exactly matching the expected Debye lengths, the first contribution was determined to be electrostatic. The second contribution dominated at interaxial separations

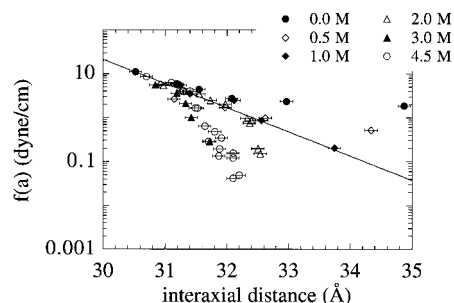


FIGURE 4 Force-distance curves for d(pG) helices obtained at different KCl concentrations at 25°C. Data for 0.0, 0.5, 1.0, and 2.0 M are taken from Mariani and Saturni (1996). The continuous line, with a 0.7 Å exponential decay rate, is the best-fit curve to the 1.0 M data. At all KCl concentrations, forces seem to converge to this exponential line at high pressure.

smaller than ~ 33 Å and it increased exponentially as the helices approached each other, preventing the surfaces from coming into adhesive contact. Since the exponential decay length of this force was insensitive to salt concentration, we concluded that this contribution is a hydration repulsive force (Rau et al., 1984; Rau and Parsegian, 1992a; Israelachvili, 1994). The very small value of the hydration force decay length ($\lambda_r = 0.7$ Å) was rationalized to emerge from the coupling between the 3 Å decay length of water and the helically ordered structure of the hydrophilic groups on the apposing surfaces (Kornyshev and Leikin, 1989; Leikin et al., 1994; Mariani and Saturni, 1996). Data for d(pG) helices in 3.0 and 4.5 M KCl are substantially different. Here, in the absence of the applied stress, there is a stable, finite center-to-center separation of ~ 32 Å, i.e., ~ 7 Å between the helix surfaces. This equilibrium separation, appreciably greater than the diameter of the four-stranded helices, represents the balance between attractive and repulsive forces. As the exponential repulsion observed at short helix separations was detected to be insensitive to the ionic strength of the solution, we estimate the attractive force by subtracting the repulsion from the net force. The resulting attractive force is reported in Fig. 5. While the accuracy of this subtraction is not high, it does give a good qualitative estimate of the attraction contribution. Assuming that the force is exponential, we find that it has a decay length $\lambda_a = 1.32 \pm 0.19$ Å, about two times larger than that of the short-range repulsion. The same value fits well the few data obtained at the 3.0 M KCl concentration.

DISCUSSION

In the present experimental conditions, interactions between guanosine helices can be summarized as follows:

1. The helix-helix interaction at large distances is dominated by a direct electrostatic double layer repulsion between charged rods, an exponential force with Debye screening length λ_D (Mariani and Saturni, 1996). Using equations reported by Brenner and Parsegian (1974), the density charge per unit surface of the rods can be calculated from the previously reported force amplitude. As-

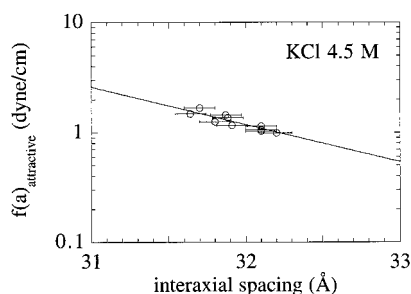


FIGURE 5 Plot of the attractive force-per-unit-length ($f(a)_{\text{attractive}}$, calculated as reported in the text) between guanosine helices versus interaxial distance at 4.5 M KCl concentration. The continuous line, with a 1.32 Å exponential decay rate, is the best-fit curve to the data.

suming that at the pH of the PEG solutions (~ 6) there is only one charge per phosphate residue, data indicate that up to 1 M KCl, there are $\sim 12\%$ of helical charges not balanced by counter-charges.

2. The repulsion at small separation is also exponential; it has a constant decay length of 0.7 Å, independent from the ionic strength of the solution. This force shows the characteristics of a hydration repulsion, and has been associated with the energetic cost of hydrogen-bond network rearrangement in the separating water layer on mutual approach of the helices (Parsegian and Evans, 1996).
3. Attraction dominates at midrange separations for KCl concentrations larger than 1 M, i.e., a higher degree of surface charge neutralization causes spontaneous association of the four-stranded helices. The attraction force can be fitted by an exponential, and it shows a decay length of 1.3 Å, about two times the decay length of the short-range repulsion.

The observed behavior coincides with the one predicted for the specific interactions between molecules with helical symmetry (Kornyshev and Leikin, 1997). This theory incorporates the helical structure and symmetries into the expressions for the helix electrostatic and/or hydration interactions: in particular, the character of the distance force variation and the balance between the repulsion and attraction depend on the helical symmetry and on the other details of the surface charge distribution on the interacting molecules. Within the framework of this theory, the short-range repulsive force, which is the helix-specific component of an image-charge electrostatic repulsion from the inner core of the opposing helix (or its hydration force analog), is demonstrated to originate from the helical regularity of charged (in the case of electrostatic force) or solvated (in the case of hydration force) residues and to depend on the helical pitch, H , and on the number of helical strands, N_s . Moreover, helix-specific attractions are predicted to originate from nonrandom alignment of opposing helices, so that negatively charged phosphates on one helix face off-phosphate-bound counterions on the opposing helix. At a critical charge neutralization fraction, the attractive force may become stronger than the repulsion and then cause spontaneous aggregation.

The short-range repulsion between multi-stranded helices with identical, equally spaced strands (like d(pG) helices, $N_s = 4$) is described by a single exponential with the characteristic length:

$$\lambda_{r*} = \lambda / (2[1 + 4\pi^2 N_s^2 (\lambda/H)^2]^{1/2}),$$

where λ is either the Debye length for the electrostatic force or the characteristic water correlation length for the hydration force. The expected attraction between such helices is also exponential and has the characteristic length:

$$\lambda_{a*} = \lambda / [1 + 4\pi^2 N_s^2 (\lambda/H)^2]^{1/2},$$

exactly twice the decay length of the short-range repulsion (Kornyshev and Leikin, 1997).

These equations could be used to compare the theoretical decay lengths with those determined experimentally. From previous x-ray diffraction experiments on fibers (Gellert et al., 1962) and from circular dichroism calculations (Bonazzi et al., 1991), the pitch of the d(pG) helices has been estimated to be $H = 40.8 \text{ \AA}$. Using 3–4 \AA as the limiting water correlation lengths, and assuming that the pitch is insensitive to interaxial helix distances and to the salt concentration, decay lengths of 0.7 \AA (0.71–0.75 \AA) and 1.4 \AA (1.42–1.5 \AA) can be calculated, in good agreement with the measured values.

The recognition forces between guanosine helices may therefore be explained inside the hydration force scheme as helix-specific interactions. However, image-charge repulsion may contribute as well; in fact, the charge pattern is defined by the phosphate, it has the same symmetry as the hydration pattern, and the expected electrostatic attractive and repulsive force decay lengths, calculated using $\lambda = \lambda_D$, are approximately the same as for the hydration interactions. It should be noted that the calculated decay lengths are determined almost exclusively by the periodicity of the four-strand helix and are only weakly sensitive to the value of λ .

A last point should be stressed: attractive forces can provide a new way to think about a previously detected temperature dependence of the interaxial spacing between d(pG) helices in gravimetrically prepared samples (Franz et al., 1994). In the hexagonal phase, helices were observed to move closer with increasing temperature (see Fig. 4 in Franz et al., 1994); at water concentration of 50 wt %, the distance between the helix surfaces was observed to change from $\sim 14 \text{ \AA}$ at 25°C down to 8 \AA at 70°C. A similar reversible dependence was also observed in other guanosine derivatives (see, for example, Gottarelli et al., 1997). According to the hydration force scheme, the ordering of water near a surface creates a structurally important difference in the entropy of this water compared to that of the bathing medium. Temperature favors the release of structured water (Rau and Parsegian, 1992b; Leikin et al., 1991); as a result, the entropically favored net attraction should result in a tighter lattice, as experimentally observed. Another possibility, e.g., is that temperature favors the redistribution of bound counterions, so that helices can adopt the most energetically favorable counterion patterns increasing the net attraction.

Data presented here give strong evidence for the dominating role of symmetry in the close interaction between helical molecules, but they should be considered only a further qualitative advance in the understanding of the forces between guanosine four-stranded helices. In fact, several points remain to be fully investigated and further experiments will be necessary to analyze the nature of the interaction forces and to discriminate between different mechanisms of the temperature-induced assembly. Moreover, the role of the different counterions and of the net

charge of the phosphate residues (i.e., the role of the pH of the solution) on the interactions between guanosine helices still remains to be understood. The biological relevance of these experiments is straightforward, particularly in the understanding of the physicochemical properties of the guanine quartet containing higher-order structures (quadruplexes) that are formed by telomers and related sequences, and of their structural sensitivity to sodium, ammonium, and potassium ions (Williamson, 1993; Miura et al., 1995; Chen, 1997).

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