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Dynamic Light Scattering in Pretransitional Region of the I Ch Phase Transition of Deoxyguanosine 5′-Monophosphate

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Dynamic light scattering in the pretransitional region of the I→Ch phase transition of deoxyguanosine 5'-monophosphate reveals the presence of two diffusive modes. The slower mode is assigned to the translational motion of large spherical aggregates, similar to those observed in isotropic solutions of DNA and other polyelectrolytes. The faster mode is assigned to the translational motion of stacked arrays of guanosine tetramers and can be described by the polyion diffusion.

INTRODUCTION

Guanosine is the only one of the four DNA nucleotides that is able to self-aggregate into stable ordered structures. The basic aggregation unit is a planar tetramer (see Figure 1(a)) formed by four hydrogen-bonded guanosine molecules[1]. Guanosine tetramers pile on top of one another to form cylindrical stacks (Figure 1(b)). Highly concentrated aqueous solutions of guanosine exhibit a columnar lyotropic liquid crystalline polymorphism[1-3], forming cholesteric (Ch) and hexagonal (H) phases at high and low water content, respectively (Figure 2). The mesophases are induced by orientational

ordering of the cylindrical stacks. The mechanism of the formation of the stacks, which starts to take place already in the isotropic phase, is not yet well understood.

In the ammonium salt of 2'-deoxyguanosine 5'-monophosphate (d(pG)), as reported in our recent paper[4], the formation of the stacks is pronounced only in a concentration region close to the isotropic-cholesteric (I →Ch) phase transition. This pre-transitional region is characterized by the appearance of an additional dynamic mode in the dynamic light scattering (DLS) spectra. In this paper we report on the features regarding dispersion relations of the DLS modes observed in the pre-transitional region and on the effects of added salt on the properties of the modes.

EXPERIMENTAL

The d(pG) in the form of sodium salt was purchased from Sigma (99% purity). The ammonium salt d(pG) was obtained by exchanging the cations using an Amberlite 200 ion exchange resin (Fluka). Solutions of the required concentration were prepared by mixing the resulting material with distilled water or with 0.5M KCl, respectively. The concentrations of the investigated solutions, expressed as weight percent, ranged from 0.1 to 15 wt%. All the
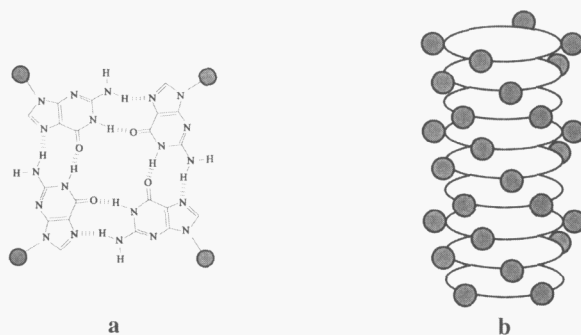

FIGURE 1   The self-aggregation scheme of d(pG) molecules. (a) A tetramer of four hydrogen-bonded guanosine molecules. The gray spheres indicate the sugar-phosphate groups. (b) Stacking of the disk-shaped tetramers.
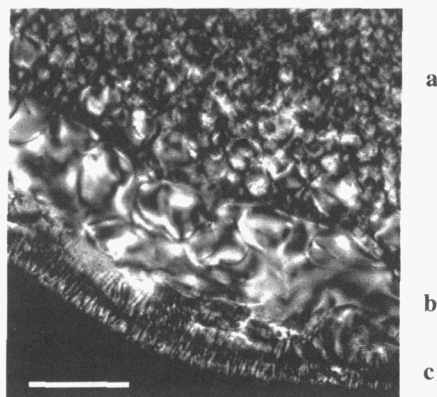
FIGURE 2   Optical microscopy image of a sequence of the liquid crystalline phases as observed during peripheral evaporation of a droplet of the 12.5 wt% d(pG) solution. (a) Coexistence of isotropic and cholesteric phases. (b) Cholesteric phase. (c) Highly ordered hexagonal phase. The bar corresponds to 250 µm.

samples were examined with optical polarizing microscopy and appeared isotropic, except the 12.5 wt% sample, which showed co-existence of the isotropic and cholesteric phases, and the 15 wt% sample that displayed a cholesteric texture.

DLS experiments were performed using an ALV 5000 digital correlator and a He-Ne laser ($\lambda = 632.8$ nm) as a light source. The fluctuations of the scattered light were investigated at various scattering angles and the normalized homodyne intensity autocorrelation function $g^{(2)}(t) = \langle I(0)I(t)\rangle/\langle I\rangle^2$ was measured. In the Gaussian approximation the normalized field correlation function $g^{(1)}(t)$ is related to $g^{(2)}(t)$ by the relation[5]

$$g^{(2)}(t) = 1 + \alpha \left| g^{(1)}(t) \right|^2 , \tag{1}$$

where α is the spatial coherence factor depending on the apparatus. The dynamic response of the sample is expressed by the field correlation function of the form

$$g^{(1)}(t) = \sum_i A_i e^{-t/\tau_i} , \tag{2}$$

where A_i is the amplitude and τ_i is the relaxation time of the i-th dynamic mode. The corresponding diffusion coefficients of the modes may be calculated as $D_i = 1/\tau_i q^2$, where $q = (4\pi n/\lambda)sin(\theta/2)$ is the scattering wave vector, n the solution refractive index, λ the laser wavelength and θ the scattering angle.

RESULTS

As a function of concentration, the DLS autocorrelation functions of the isotropic ammonium d(pG) solutions show two different regimes. For concentrations below $c = 5$ wt% only one, relatively slow dynamic mode is observed with diffusion coefficients in the interval of $D = 0.4 - 9.0 \cdot 10^{-9}$ cm^2/s. In the pre-transitional region, from $c = 5$ wt% to $c = 10$ wt%, in addition to the slow mode, also a faster relaxation mode appears. In salt-free solutions, the relaxation rate of the fast mode $1/\tau_{fast}$ is about two orders of magnitude larger than the relaxation rate of the slow mode $1/\tau_{slow}$ (Figure 3 and Figure 4). The dispersion relation of the fast mode $1/\tau_{fast}(q)$ exhibits a usual quadratic dependence with a gap at $q = 0$. The dispersion relation of the slow mode $1/\tau_{slow}(q)$, on the contrary, exhibits a systematic deviation from the quadratic dependence, which suggests on the significant presence of the q^4 terms in the dispersion relation. The corresponding apparent diffusion coefficients of the modes show opposite dependencies with respect to concentration. The diffusion coefficient of the slow mode decreases with increasing concentration, while the diffusion coefficient of the fast mode increases with increasing concentration (Figure 5).

The addition of the KCl salt results in a dramatic slowing down of the slow mode and in much less pronounced slowing down of the fast mode (Figure 6). The dispersion properties of the modes, however, remain very similar as in the salt-free samples, only the gap of the fast mode vanishes (Figure 4). Concerning dependence on the concentration, the diffusion coefficient of the slow mode again decreases with increasing concentration, but the diffusion coefficient of the fast mode strikingly changes its fashion
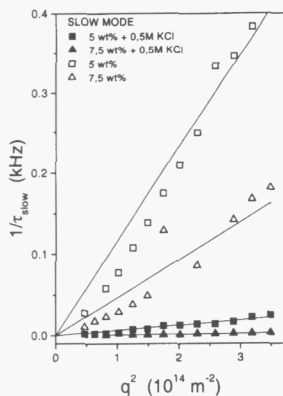
FIGURE 3   Plot of inverse relaxation time versus q^2 for the slow mode.
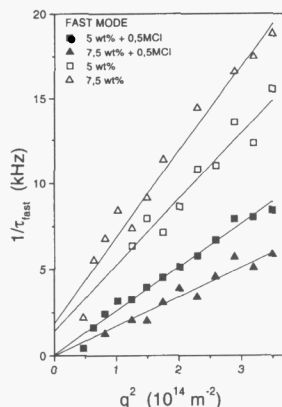

FIGURE 4   Plot of inverse relaxation time versus q^2 for the fast mode.

(Figure 5). While in salt-free solutions D_{fast} increases with increasing concentration, solutions with added salt show a decrease of D_{fast} with increased congestion. The diffusion coefficients of various solutions together with the corresponding hydrodynamic radii are summarized in Table 1. The hydrodynamic radius was calculated as $r_H = kT/6\pi\eta D$, η being the viscosity of water and $T = 298$ K the temperature of the solution.

TABLE 1   Diffusion coefficients and corresponding hydrodynamic radii for various ammonium d(pG) solutions at 25^{0}C.

c (wt%)	D_{slow} (cm^2/s)	r_{slow} (μm)	D_{fast} (cm^2/s)	r_{fast} (nm)
		salt free		
5	11.6×10^{-9}	0.19	3.9×10^{-7}	5.7
6.25	7.8	0.28	4.4	5.0
7.5	4.7	0.47	5.0	4.3
8.75	2.2	0.97	5.7	3.8
10	0.6	3.7	5.9	3.7
		0.5M KCl		
5	0.62×10^{-9}	3.5	2.5×10^{-7}	8.6
7.5	0.07	30.7	1.7	12.8

DISCUSSION

d(pG) molecules in water are negatively charged: depending on the pH of solution, each d(pG) tetramer shows a maximum of 8 elementary charges. Tetramers form cylindrical stacks, which can be then regarded as rigid, highly charged polyions. Despite many investigations focused onto the dynamical properties of polyionic systems, there is still no consensus on some fundamental aspects of their behavior. In particular, DLS measurements of DNA solutions and other polyelectrolytes usually reveal two relaxation modes: a fast mode, attributed to coupled polyion - small ion diffusion, and a slow mode, whose widespread puzzling nature is not yet resolved[6-11].

In our d(pG) solutions, as revealed from ^{31}P NMR results[4], the stacking of tetramers is pronounced for concentrations higher than 5 wt%. Such concentrations coincide with the presence of the fast mode in the DLS response; therefore, we relate the fast mode with the dynamical properties of the tetrameric stacks. Accordingly, the observed features of the fast mode have been tentatively analyzed using a phenomenological theory for the polyion
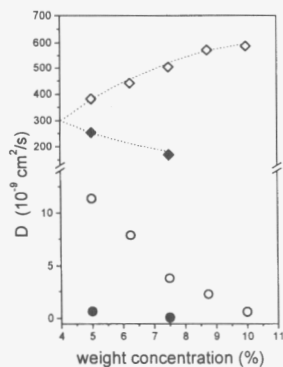

FIGURE 5 Diffusion coefficients as a function of the d(pG) concentration: salt free solutions - slow mode (○), fast mode (◇); solutions with 0.5M KCl - slow mode (●), fast mode (◆).
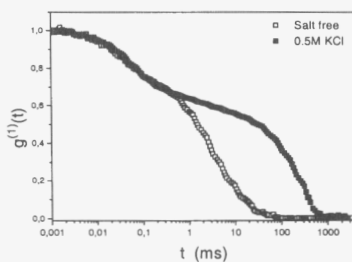
FIGURE 6 Autocorrelation functions of the 7.5 wt% d(pG) solutions with 0.5M KCl and with no added salt.

motion[5,10]. The diffusion coefficient of the fast mode has been expanded into a power series with respect to the concentration of the cylindrical stacks c_c:

$$D = D_0 [1 + k_D c_c + ...],$$ (3)

where D_0 is the mutual diffusion coefficient at $c_c = 0$, and k_D is the diffusion second virial coefficient. The value of D_0 for charged rigid rods can be calculated using the Tirado-Garcia de la Torre relation[12,13]

$$D_0 = \frac{k_B T}{3\pi\eta L_{eff}} [\ln(L_{eff}/d_{eff}) + v],$$ (4)

where η is the solvent viscosity and $v = 0.312 + 0.565(d_{eff}/L_{eff}) - 0.100(d_{eff}/L_{eff})^2$, with L_{eff} being the effective length and d_{eff} the effective diameter of the rods. The L_{eff} and d_{eff} are determined as the sum of the corresponding tetramer dimension and of the screening length of the small-ion field[10,11].

Extrapolation of the observed dependence $D_{fast}(c)$ to the concentration $c = 4$ wt% (Figure 5), which we assume to coincide with the concentration at which the cylindrical stacking proceeds, i.e. with $c_c = 0$, gives the value $D_0 \approx 300 \cdot 10^{-9}$ cm^2/s. Taking into account this result and the value $d_{eff} = 2.9$ nm for the effective diameter of the cylinders, the Equation (4) gives $L = 42$ nm for the length of the stacks. This value is similar (only 4 times larger) to the cylinder length recalculated from X-ray scattering experiments in the cholesteric phase[3]. Several effects can be claimed to explain the observed discrepancy. First, the rigidity of the stacked columns has been inferred by the absence of long range fluctuation enhanced electrostatic repulsion[14], but remains to be proved. Second, the cylinders should be polydisperse in length: in the case of DLS scattering, this results in a signal originating predominantly from scatterers with larger sizes and consequently the measured apparent diffusion coefficient appears lower than the average value expected from Equation (4). Third, the cylinder length is expected to be dependent on concentration[3]: this idea is supported by the results obtained in the presence of KCl, which is known to facilitate the stacking processes. With added salt, the diffusion constant of the fast mode decreases with increasing concentration, a behavior which is very unusual for similar fixed-size polyions[10] but that can indicate an increasing value of L_{eff} in Equation (4).

Therefore, it appears that the variable length of polyions brings complications to the calculation of the virial coefficient k_D and other terms in Equations (4) and (5): to allow for more quantitative analysis of the DLS experiments on d(pG) solutions, a separate information on the length of the tetrameric stacks is hence required. Our investigation, however, shows that a description of the pretransitional phase of the d(pG) as a polyionic system is at least qualitatively relevant.

CONCLUSION

Dynamic light scattering in ammonium d(pG) solutions reveals the presence of two diffusive modes in the pre-transitional region of the I-Ch phase transition. As practically no depolarized scattering is observed, we assign the dominant slow mode to the translational motion of large spherical aggregates, similar to those reported for the isotropic solutions of DNA and other polyelectrolytes[6-11]. The faster mode is assigned to the motion of the self-assembled cylindrical stacks. The dynamic properties of the highly charged stacks are analogous to the diffusion features of rigid polyions, which are coupled to the motion of the small ions.

Acknowledgments

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