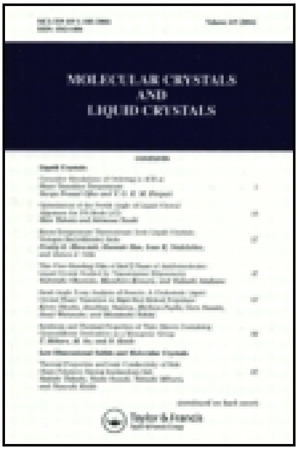

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Melting of Self-Assembled Columnar Aggregates Formed in Aqueous Solutions of Deoxy- and Guanosine 5′-Monophosphate

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Temperature dependence of the self-assembling properties of aqueous solutions of deoxy- and guanosine 5′-monophospate (dGMP and GMP) in the pretransitional region of the I-Ch phase transition was investigated by dynamic light scattering (DLS) and small-angle X-ray scattering (SAXS). The dGMP molecules start to assemble at lower concentrations and form considerably longer aggregates than GMP molecules. Conversely, the aggregate melting curves of both derivatives are very similar and show three characteristic regions: a low temperature interaction-controlled region, a narrow region of intense aggregate dissociation, and a high temperature region of dissociated species.

Keywords: guanosine; lyotropic liquid crystals; polyelectrolytes; self-assembly; scattering methods

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INTRODUCTION

Guanosine molecules (Fig. 1a) in aqueous solutions form complex self-assembled columnar structures. The basic aggregation unit is a planar tetramer (Fig. 1b) formed by four hydrogen-bonded guanosine molecules [1]. Vertical stacking of tetramers induces the formation of long

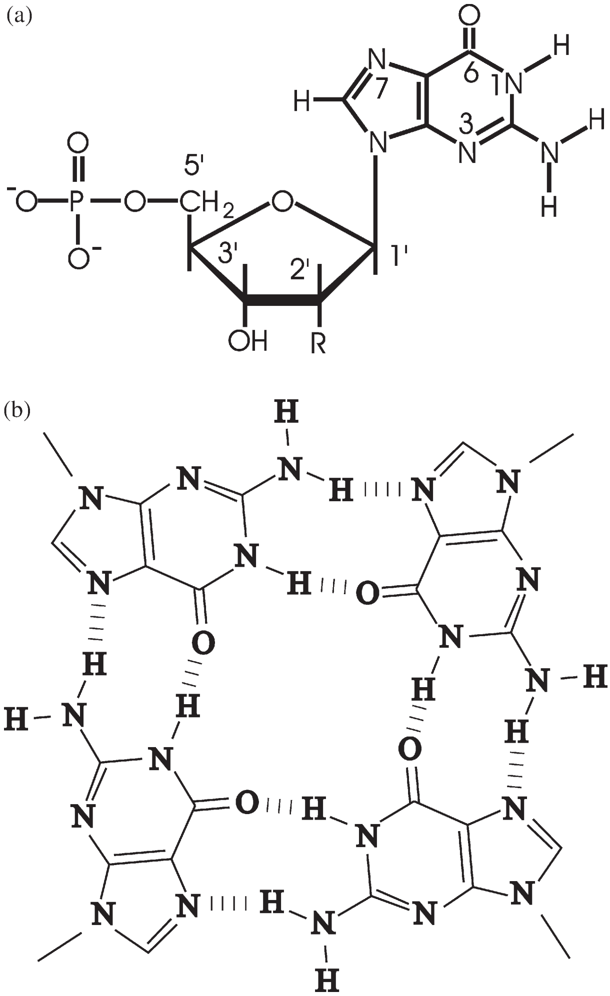

FIGURE 1 (a) The structure of deoxyguanosine 5′-monophosphate (R = H) and guanosine 5′-monophosphate (R = OH). (b) A tetramer (the sugar-phosphate groups are omitted for clarity). (c) Stacking of tetramers induces formation of columnar aggregates with a diameter of 26 Å.

(c)

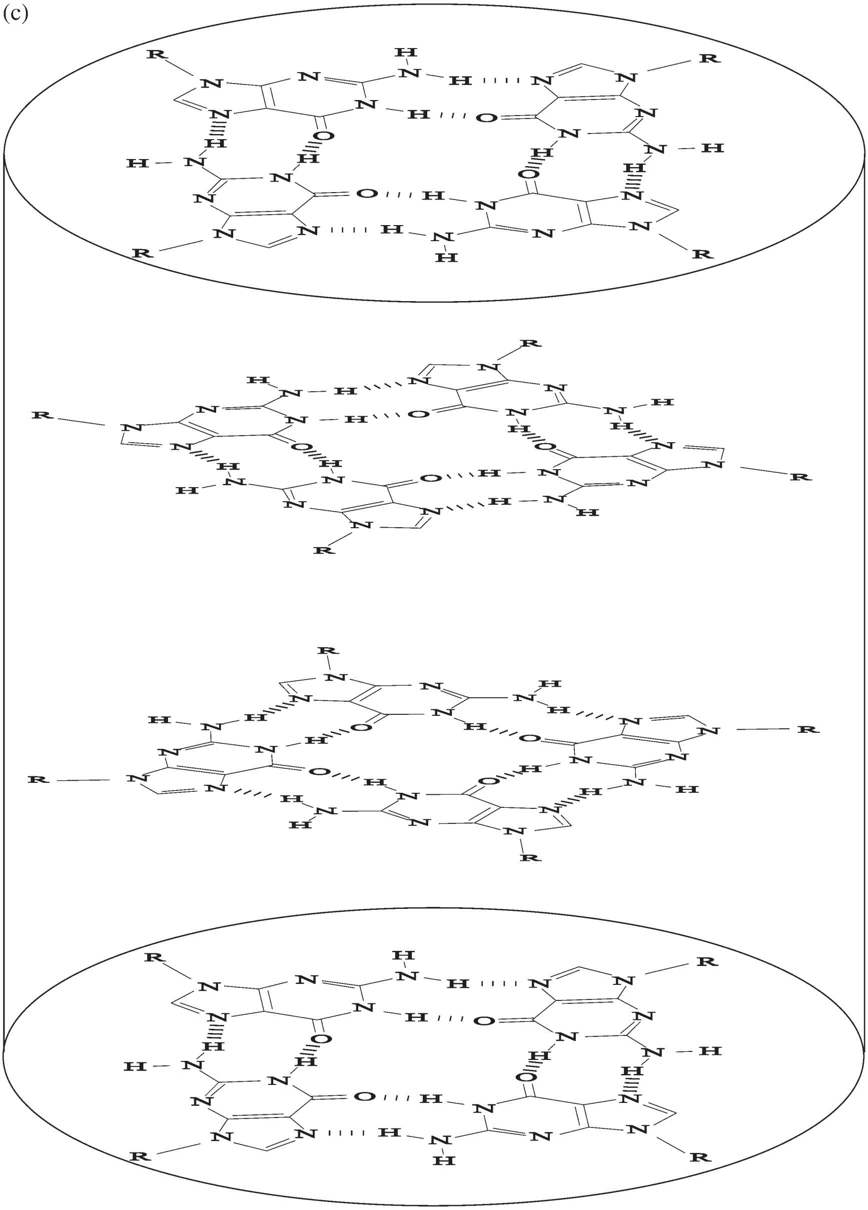

FIGURE 1 Continued.

columnar stacks (Fig. 1.c). In concentrated solutions the tetrameric stacks exhibit lyotropic liquid crystalline polymorphism and the cholesteric (Ch) and hexagonal phases (H) are formed [2]. These systems have an interesting pretransitional region of a so-called isotropic columnar phase in which the self-assembly has already started but the Ch phase is not yet formed. Our previous dynamic light scattering (DLS) studies of the columnar stacking in deoxyguanosine 5′-monophosphate (dGMP) in the form of diammonium salt showed that solution dynamics of the self-assembled columns is mainly influenced by their polyelectrolyte nature [3,4]. A study of a $c = 5\,\mathrm{wt\%}$ dGMP solution indicated that by increasing the temperature the number of self-assembled columns decreased until the DLS signal from the aggregates abruptly disappeared at some critical concentration [5]. However, such an abrupt transition between the self-assembled and monomeric objects is unusual for linear self-assembling systems [6]. Besides this, a DLS study of a similar system, namely guanosine 5′-monophosphate (GMP) in the form of free acid, showed that self-assembled aggregates dissociate gradually with increasing temperature and that the form of dissociated species in the high temperature region depends on the solution concentration [7]. In order to further resolve the above mentioned discrepancies we decided to investigate the temperature dependence of the columnar stacking of diammonium dGMP in more detail and to make also a comparative study between the diammonium GMP and dGMP derivatives (for structural difference see Fig. 1a).

EXPERIMENTAL

Deoxyguanosine 5′-monophosphate (dGMP) in the form of sodium salt was purchased from Sigma (99% purity). The diammonium salt dGMP was obtained by exchanging the cations using an Amberlite 200 ion exchange resin (Fluka). Guanosine 5′-monophosphate (GMP) in the form of free acid (Sigma, 99% purity) was titrated with NH_4OH to obtain diammonium GMP. Both procedures were followed by subsequent lyophilization. Solutions of the required concentration were then prepared by mixing the lyophilized material with deionized water and analyzed after an equilibration time of 24 hours. To verify the reproducibility of the results obtained from samples that were prepared by different procedures, an additional set of solutions from the commercially available dGMP in the form of ammonium salt (Sigma, 99% purity) was prepared. The self-assembly process of both sets of dGMP solutions was reproducible independently of the sample preparation procedures. A more detailed study on the influence of sample preparation on the self-assembly of dGMP and GMP is currently under way and will be published in a separate paper.

Dynamic light scattering (DLS) experiments were performed using a He-Ne laser as a light source ($\lambda = 632.8\,\mathrm{nm}$), a photomultiplier as a detector and a digital correlator (ALV5000). The scattered light was detected at scattering angles ϑ ranging from 30° to 90°. The polarization of the scattered light was selected to be parallel to the incident polarization. A capillary with the sample was placed into a specially constructed index-matching bath, the temperature of which was regulated with a temperature controller (Oxford ITC4) to within $\pm 0.1^\circ\mathrm{C}$.

The homodyne intensity correlation function of the scattered light probes the dynamical response of the system, which is given as [8,9]

$$g_2(t) = 1 + \left| \sum_i A_i \exp\left[-(t/\tau_i)^{\beta_i} \right] \right|^2, \tag{1}$$

where A_i is the amplitude and τ_i is the relaxation time of the ith relaxation mode. For a solution of nearly monodisperse scatters the Kohlrausch-Williams-Watts (KWW) parameter β_i is close to 1, while in solutions with polydisperse scattering objects smaller β_i values are obtained. The apparent translational diffusion coefficients of the modes were calculated as

$$D_{app,i} = 1/\tau_i q^2 \tag{2}$$

where $q = (4\pi n/\lambda)\sin(\vartheta/2)$ is the scattering vector and n the refractive index of the solution. The corresponding apparent hydrodynamic radii were calculated as

$$R_h = k_B T / 6\pi\eta D_{app} \tag{3}$$

with k_B being the Boltzmann constant and η the solvent viscosity. Usually two diffusive modes are observed in guanosine solutions by DLS. This report focused on the faster of the two modes, which arises from the translational diffusion of the self-assembled guanosine molecules. The second, slower, DLS mode is related to the diffusion of large globular aggregates of unknown inner structure (see ref. [3] for more details).

Small-angle X-ray scattering (SAXS) experiments were performed with an evacuated Kratky compact camera system (Anton Paar, Graz, Austria) with a block collimating unit, attached to a conventional X-ray generator (Bruker AXS, Karlsruhe, Germany) equipped with a sealed X-ray tube (Cu-anode target type) producing Ni-filtered CuK_α X-rays with a wavelength $\lambda = 0.154\,\mathrm{nm}$. The operating power was $35\,\mathrm{kV} \times 10\,\mathrm{mA}$. The temperature of the samples was regulated with a temperature controller (K-PR, Anton Paar) to within $\pm 0.2^\circ\mathrm{C}$. The scattering intensities vs. scattering vector q were measured with a linear position sensitive detector (PSD 50 m, M. Braun, Garsching, Germany). Before evaluation the scattering data were corrected for the absorption [10], empty capillary, and solvent scattering.

Experimental SAXS data were evaluated utilizing the so-called 'Guinier plot' method [11–13]. It emanates from the fact that for the central part of the small-angle scattering curve a universal approximation exists irrespective of the scattering particles' shape. This approximation is described by the following equation [11]:

$$I(q) = I(0)\exp\left(-q^2R_g^2/3\right), \tag{4}$$

where $I(0)$ corresponds to the scattering intensity at zero angle and R_g is the radius of gyration of scattering particles. R_g is defined in analogy to the radius of inertia in mechanics [13]:

$$R_g^2 = \int_V \rho(r_i)r_i^2 dV_i \Big/ \int_V \rho(r_i)dV_i, \tag{5}$$

where $\rho(r_i)$ is the local electron density inside the scattering particle. R_g can thus be obtained from the innermost part of the scattering curve, namely from the slope of the linear descent in a plot of ln $I(q)$ vs. q^2. This method, however, is relevant only for dilute solutions. At higher concentrations of the scattering particles the interparticle correlations usually considerably affect the innermost part of the scattering curve. Consequently, at such concentrations the obtained radii R_g should be taken with some precaution.

From SAXS measurements in dilute solutions one can primarily obtain information on the dimensions of the scattering objects. DLS measurements, on the other hand, probe the dynamic properties of the aggregates and consequently provide also information on the dimensions of the hydration layer and effects of friction. As a result, the R_h values are usually larger than the corresponding R_g data. Combining both methods thus provides a possibility to obtain a more complete picture of aggregation phenomena of guanosine molecules.

RESULTS

The concentration dependence of the apparent diffusion coefficient [3] and the corresponding apparent hydrodynamic radius (Fig. 2) of the fast DLS relaxation mode of the dGMP show a typical polyelectrolyte behavior [14,15]. With increasing solution concentration the apparent values of R_h decrease. This is because with increasing number of

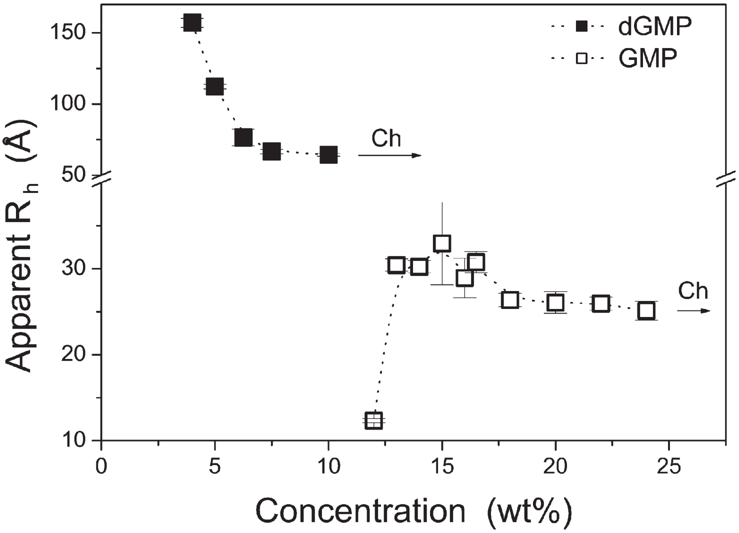

FIGURE 2 Apparent hydrodynamic radius of the fast DLS mode calculated from Eq. (3) as a function of dGMP and GMP concentration at room temperature. The dotted lines are guides to the eye.

self-assembled columns present in the solution their translational motion becomes faster due to electrostatic interactions [9]. At concentrations above 10 wt% the solutions become liquid crystalline and the cholesteric phase is formed (Fig. 2). From the value of $R_h = 157 \pm 3$ Å measured for the lowest concentration at which self-assembly is observed ($c = 4$ wt%), the length of the dGMP stacks was calculated to be approximately 400 Å [3]. This corresponds to 110 stacked tetramers.

At room temperature ($T = 24$°C) the phase diagram of GMP solutions is shifted to higher concentrations as compared to dGMP (Fig. 2). The fast diffusive mode is observed only for $c \geq 12$ wt%, while the cholesteric phase appears at $c > 25$ wt%. In the 12 wt% GMP solution R_h has a value of 12.3 ± 0.2 Å. Using the bead model of Jurga-Nowak *et al.* [7], this value corresponds to 4 stacked tetramers (Fig 1c). However, already at $c = 13$ wt% the value of R_h increases to 30.4 ± 0.7 Å indicating an abrupt transition to larger aggregates with the length of 120 Å. By further increasing the GMP concentration the aggregate motion again becomes faster and correspondingly R_h decreases, although to a much smaller extent than observed for the dGMP derivative.

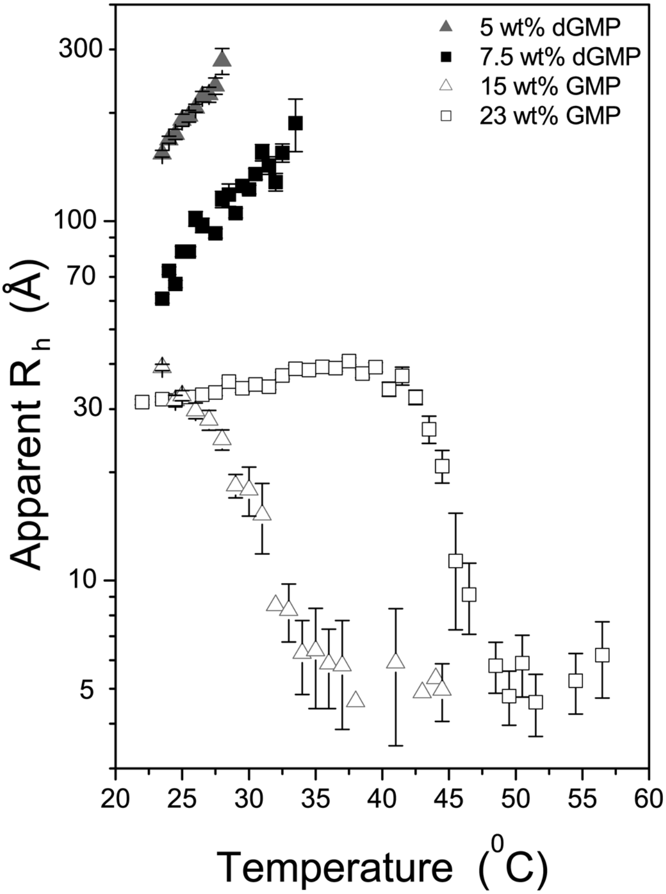

FIGURE 3 Aggregate melting curves for dGMP and GMP solutions resolved by DLS measurements. In dGMP the DLS signal associated with translational motion of the aggregates can not be measured above the critical temperature.

Figure 3 shows the temperature dependence of R_h measured in dGMP solutions with concentrations $c = 5\,\text{wt}\%$ and $7.5\,\text{wt}\%$. In both cases R_h increases until the fast DLS mode disappears. This behavior is analogous to an increase of the R_h value when the solution concentration is lowered (Fig. 2), thus indicating that the number of self-assembled dGMP stacks decreases with increasing temperature. The $R_h(T)$ dependence of GMP solutions was investigated for the $c = 15\,\text{wt}\%$ and $23\,\text{wt}\%$ solutions. In this material the whole melting process is clearly evident (Fig. 3) and once the aggregates break apart

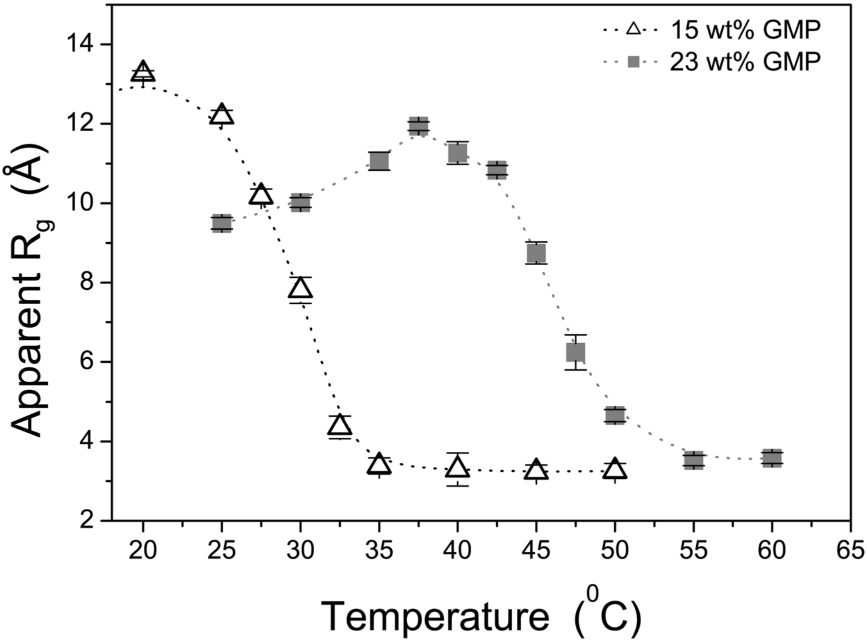

FIGURE 4 Radius of gyration of the GMP aggregates as a function of the temperature. The dotted lines are guides to the eye.

only the dissociated species remains in the solution. The average R_h value of the melted GMP species is 5.5 ± 0.6 Å.

Based on the SAXS data, the radius of gyration R_g of the self-assembled GMP stacks was obtained from the Guinier plot procedure explained in the previous chapter. The results are shown in Figure 4. The aggregate melting curves resulting from the SAXS measurements follow the trend resolved by the DLS experiments. After an initial increase of the apparent R_g values the aggregates start to break apart until only the melted species are present in the solution. The average value of R_g for melted species is 3.4 ± 0.14 Å and is (within the experimental error) the same for both investigated GMP concentrations (15 wt% and 23 wt%). This indicates that in the region where only the melted species is present the interparticle interactions do not have considerable effect on the obtained R_g values and the Guinier approximation is relevant.

DISCUSSION

The small structural difference between the dGMP and GMP molecules (−H or −OH group at the C2′ position, see Fig. 1a) has a dramatic influence on the self-assembly process of the two derivatives. In dGMP the tetramers start to stack into columnar aggregates at much lower concentrations ($c \geq 4\,\mathrm{wt\%}$) than in GMP ($c \geq 12\,\mathrm{wt\%}$). The dGMP columns form the cholesteric phase already at $c > 10\,\mathrm{wt\%}$ and have much bigger length (Fig. 2). The assembly of GMP tetramers is less favorable and consequently the complete phase diagram is shifted to higher concentrations, while at the same time the aggregate length is smaller. The apparent hydrodynamic radius R_h of the two molecules decreases with increasing concentration, indicating that in both cases the translational dynamics of the self-assembled stacks is dominated by electrostatic interactions. Eventually, close to the I-Ch phase transition, the R_h reaches an asymptotic value, which is a sign that the system has entered into the semidilute polyelectrolyte regime [14,16].

For both derivatives the observed temperature dependencies of the R_h reveal an aggregate melting process with a sharp transition to the dissociated regime (Fig. 3). For dGMP an increase of the R_h value is observed, which takes place up to some specific temperature at which the corresponding fast mode DLS mode suddenly disappears. This indicates that the aggregates dissociate. An absence of the DLS signal from the melted species at higher temperatures is attributed to the relatively low monomer concentrations (5 and 7.5 wt%) which result in a low light scattering intensity.

Similarly to the dGMP, the temperature dependence of the size of the GMP aggregates also shows a sharp transition to the melted regime (Fig. 3). But, as GMP solutions have higher concentrations, a DLS signal could be measured also above the melting temperature. The average value of the corresponding hydrodynamic radius of the melted species is $\langle R_h \rangle = 5.5 \pm 0.6\,\text{Å}$, which is in excellent agreement with the value $R_h = 5.37 \pm 0.36\,\text{Å}$ obtained by Jurga-Nowak *et al.* [7] for hydrated GMP monomers.

The abrupt dissociation process of the GMP aggregates revealed from the DLS experiments (Fig. 3) is evident also from the SAXS measurements. Plotting the R_g values as a function of the temperature (Fig. 4) again the solution dynamics at lower temperatures, which is driven by inter-aggregate interactions, is followed by the relatively narrow region where the self-assembly completely disappears. The dissociated species has an average R_g of $3.4 \pm 0.14\,\text{Å}$ which corresponds to nonhydrated GMP monomers. Therefore by comparing the DLS and the SAXS data in the high temperature region a very good agreement is obtained. Both methods identify the melted species as single GMP molecules, nevertheless, while SAXS gives pure molecule dimensions, DLS gives also the size of the hydration layer formed around the molecules.

In the lower temperature region the R_h values are notably higher than the R_g values. This discrepancy can not be explained only by the presence of a hydration shell surrounding the aggregates. We attribute it to the modification of the effective solvent viscosity due to the presence of the guanosine molecules. However, a more detailed study is needed to explain the observed effects.

Interestingly, the melting process of diammonium GMP aggregates ends with single monomers regardless of the solution concentration (Figs. 3 and 4), which is in contrast to the melting behavior reported for a GMP system in the form of free acid [7]. Another remarkable difference is that aggregates formed in the free-acid GMP exhibit gradual melting, while a sharp cross-over region is observed in diammonium GMP and dGMP. These features can be explained by taking into account that the assembly of guanosine molecules is actually a two-step process (Fig. 1). The stability of the columnar aggregates depends on the difference in the association energies of the minimum aggregate Φ_0 (tetramer) and the second-step aggregate Φ_1 (stacked tetramers) [6]. For $\Phi_0 \gg \Phi_1$ the system resembles a simple linear aggregation scheme with guanosine tetramer playing the role of the basic aggregation unit. We believe that such behavior was observed for free-acid GMP [7]. The presence of counterions, on the other hand, can drastically increase the stability of the stacks and consequently the value Φ_1 increases [3,17]. For $\Phi_0 \ll \Phi_1$ the assembly becomes cooperative and an abrupt transition from monomers to long aggregates is expected with increasing concentration [6]. Evidently, such a behavior takes place in diammonium dGMP and GMP.

CONCLUSION

The relatively small structural difference between the dGMP and GMP molecules has a great influence on their self-assembly process. Formation of the columnar aggregates is more favorable for dGMP which starts to assemble at notably smaller concentrations and forms longer columns than GMP. Regardless of these differences, the temperature dependence of aggregation properties for both derivatives is very similar and can be divided into three characteristic regions: (i) interaction-governed regime at lower temperatures, (ii) narrow region of intense aggregate dissociation with an abrupt decrease of the R_h and R_g values, (iii) high temperature region where in GMP the melted species is identified as GMP monomers. The abrupt melting process is attributed to the two-step mechanism of the aggregate formation.

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