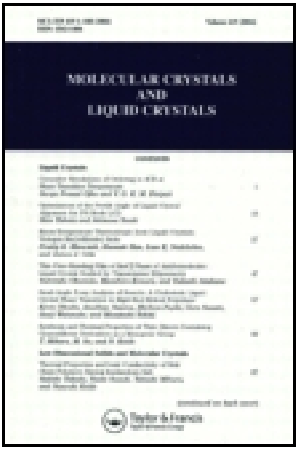

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Dynamics of Guanosine Self-Assembled Aggregates in the Hexagonal Columnar Phase by Quasielastic Neutron Scattering

H. Franz [a], G. Mathe [a], F. Ciuchi [b], P. Mariani [b], W. Petry [a] & W. Schmidt [c]

[a] Technical University of Munich, Physik Department, James-Franck-Str., 85748, Garching, Germany

[b] Istituto di Scienze Fisiche, Facoltà di Medicina, Università degli Studi di Ancona and Istituto Nazionale per la Fisica della Materia, INFM, research unit of Ancona, via Ranieri 65, 60131, Ancona, Italy

[c] ILL, Grenoble Cedex, France

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DYNAMICS OF GUANOSINE SELF-ASSEMBLED AGGREGATES IN THE HEXAGONAL COLUMNAR PHASE BY QUASIELASTIC NEUTRON SCATTERING

H. FRANZ[1], G. MATHE[1], F. CIUCHI[2], P. MARIANI[2], W. PETRY[1], W. SCHMIDT[3]
[1]Technical University of Munich, Physik Department, James-Franck-Str., 85748 Garching, Germany
[2]Istituto di Scienze Fisiche, Facoltà di Medicina, Università degli Studi di Ancona and Istituto Nazionale per la Fisica della Materia, INFM, research unit of Ancona, via Ranieri 65, 60131 Ancona, Italy
[3]ILL, Grenoble, Cedex, France

Abstract Guanosine, one component of the nucleic acids, presents a simple model system to study the self-assembling process and the properties of columnar liquid-crystalline phases. In a recent x-ray study we investigated the structure of the aggregates at medium concentrations and explored the phase-diagram as a function of salt concentration in the solution. A microscopic model has been presented describing the size and distribution of the rod-shaped aggregates consisting of stacked guanosine tetramers.

Here we present a first study of the dynamics of guanosine in the two-dimensional hexagonal phase. Inelastic neutron scattering gives further evidence for the "persistent-flexible-hard-rod" model; displacements in the hexagonal plane are found to be extremely high. To a detection limit of 0.04 meV we find no evidence for propagating phonon modes. The quasielastic signal measured is attributed to relaxational motions that is extremely overdamped motions of the guanosine rods.

INTRODUCTION

From a series of experiments[1-4] the ability of guanosine to self-associate into large aggregates has been well known for about 30 years now. In particular the guanosine-water system shows lyotropic phase behaviour, with a phase sequence isotropic - cholesteric (nematic) - two-dimensional hexagonal. The steric arrangement of the NH_2 groups and the oxygen of the purin-ring allows the formation of a planar aggregate composed of four guanosine monomers bound in the so called Hoogsteen mode[5] (see figure 1).

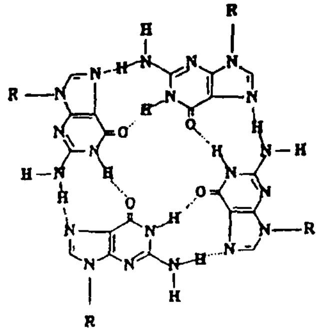

FIGURE 1: Schematic representation of the microscopic structure of a d(pG) tetramer.

The existence of those tetramers is thought to be of great biological importance. It has been proposed that the stability of the chromosomes depends on the guanin-rich sequence found in the so called telomeric ends[6]. Indeed evidence for the formation of guanosine tetramers in backfolded DNA of telomeric ends has been found in several examples[7].

Moreover the self-association seems to play a role in the processes during meiosis, that is the formation of the germ cells. The tetramers supply a mode to induce the parallel pairing of the four homologous chromatides. This is of importance for the possibility to exchange parts of the genetic code and thus provides a mechanism to promote evolution. Finally a prebiotic significance is discussed, as the guanosine stacks present a structure which is rather similar to DNA and forms spontaneously in aqueous solution once a certain concentration of guanosine is reached.

Our interest in this system is the lyotropic phase behaviour. In recent papers[8-10] we studied the microscopic structure and the phase diagram of the deoxyguanosine-5'-monophosphate / water system both in presence and absence of salt in the solution. Combining SAS[11] and x-ray diffraction[12] the size and the spatial arrangement of the aggregates have been determined in detail. The number of stacked tetramers was found to be rather low: increasing from about 30 to 55 in the cholesteric phase the number remains constant at this value in the hexagonal phase. The ability of the cations to stabilise the aggregates has been observed via a shift in the concentration of the phase transitions.

For the interpretation of the phase behaviour we compared our data with two theoretical models; the "rigid self-assembled rodlike particle model"[13] and the "persistent flexible hard rod model"[14]. The first starts from spherical monomers which reversibly self-associate to form rigid spherocylinders, displaying liquid-crystalline phase behaviour. The second model starts with polymer like chain molecules with flexibility described by a persistence length. By the formation of liquid-crystalline phases those molecules are confined into tubes with diameters slightly exceeding the molecule extension. The phase behaviour now depends on the fixed length of the molecules and their relative persistence length.

Comparing the theoretical phase diagrams to the one determined experimentally we find quantitative agreement only with the flexible case. Moreover the rigid model predicts a broad distribution of the aggregate length and a strong dependence of the distribution average on temperature. Both facts are not observed experimentally.

Thus the question arises if we can find direct evidence for the importance of flexibility studying the dynamic response of the system. However this is a rather involved problem. The sample consists of flexible large aggregates, displaying a hierarchy of internal degrees of freedom like localised motions of individual atoms or residual groups, different modes of movement of the tetramers, excitations of the stack of tetramers and finally phonon-like excitations of the two-dimensional hexagonal lattice. Moreover, the solvent - water - should play an active role in the dynamics of the system, as it mediates the interaction forces and probably dominates the entropic contribution to the free energy.

For a study of the dynamics of a solid or liquid the most suitable method is inelastic neutron scattering. Using different instruments excitations in a range from some 100 neV up to some 100 meV in a range of wavelengths form subatomic scales to several 100 Å may be detected. In order to test the guanosine / water system for excitations in the two dimensional hexagonal phase, we have to use good resolution both in energy and reciprocal space. Thus a triple-axis instrument using cold neutrons was our first choice.

EXPERIMENTAL

Samples were prepared using commercially available ammonium salt of deoxyguanosine-5'-monophosphate, d(pG), (Sigma 99% purity). A mixture of 50% in weight with heavy water (Sigma 99.9 enriched in deuterium) was left for three days to equilibrate in a separate container, then transferred into a vacuum tight aluminium sample-cell. The size of the final sample was 3.5 cm in diameter with a thickness of 1.7 mm in direction of the neutron beam thus giving approximately 10% of total scattering. After mounting the cell, the sample was heated several times to 68°C in order to guarantee homogeneity.

Measurements were performed at the triple-axis-spectrometer IN12, ILL, Grenoble[15] using two different wavelengths corresponding to incident wavevectors of $k_i = 1.1$ and 1.35 $Å^{-1}$, respectively. The instrument resolution function was measured using a vanadium standard. Values of the full-width at half maximum (FWHM) of 41μeV and 94μeV were found for the two set-ups, respectively. Elastic and inelastic scans in the constant-q mode ($\vec{q} = \vec{k}_f - \vec{k}_i$, with k_f wavevector after the scattering process) were performed for three different sample temperatures: 20°C, 45°C and 68°C. In order to search for periodic excitations we scanned at different values of the scattering vector q in the first three Brillouin zones. For q ≈ G (G = reciprocal lattice vector) the scattering geometry allows a maximum energy transfer of 1.7 meV. For q ≈ 3G 5.5 meV of energy transfer are possible. Elastic scans were frequently repeated to control the stability of the system.

Calculating the scattering cross sections (SCS) for the guanosine tetramers and the water we used the scattering lengths as tabulated in[16]. The amount of exchanged hydrogen/deuterium atoms from the guanosine to the solvent is assumed to be the maximum number of labile hydrogens[17], i.e. five per residual. The number of water molecules per residual is 17 at the concentration chosen. For an isolated tetramer we calculate a *coherent* SCS of 55.65 kbarn (all values for forward scattering), while the SCS for the equivalent amount of water (i.e. 48 D_2O + 20 HDO) is 6.038 kbarn. The total incoherent SCS is 4.1 kbarn. For a stack of 50 tetramers the values are 139 Mbarn versus 0.302 Mbarn for the *coherent* SCS and 0.205 Mbarn for the incoherent one. Those values are correct for scattering from inter-molecular correlations. Thus at low q the scattered intensity will be dominated by the coherent scattering of the guanosine aggregates even for low aggregation.

RESULTS AND DISCUSSION

Elastic scans

In order to check the crystallinity of the sample we performed elastic scans ($\Delta E = 0$) covering the first three Debye-Scherrer rings for each sample temperature. As an example figure 2 shows the (1,0), (1,1) and (2,0) peaks of the two-dimensional hexagonal lattice at 20°C. The background consists of contributions from incoherent scattering and scattering from water. Also lattice imperfections may contribute to this part of the intensity. As already observed by x-ray scattering[12] the thermal expansion coefficient is negative and has a value of $\alpha = -1.6 \cdot 10^{-3}\ K^{-1}$. Analysing the scattering vector and temperature dependence of the Bragg intensity we calculated the lateral mean square displacement of the tubes in the hexagonal lattice. At 20°C we found a value for the root mean square displacement of 4.9 Å. The comparison of the displacement with the lattice parameter (a = 32.6 Å at 20°C) evidently gives a further indication of the importance of flexibility. A rigid lattice would not withstand displacements as high as some 15% of the lattice parameter.

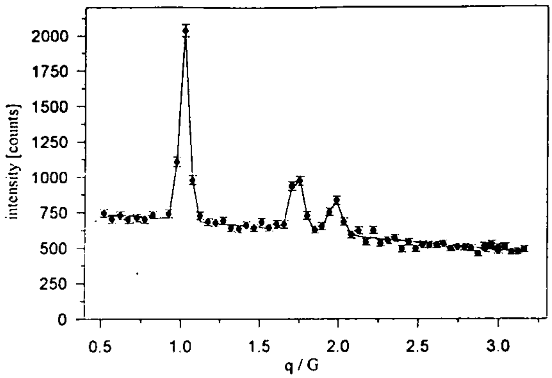

FIGURE 2: Elastic scan from 0.11 to 0.71 Å^{-1} measured at ambient temperature. The three peaks are the (1,0), (1,1) and (2,0) Debye-Scherrer rings of the two-dimensional hexagonal lattice, with lattice spacing 32.6 Å. The q-axis is scaled in units if the reciprocal lattice vector G.

Inelastic scans

Performing inelastic scans we were searching for excitations in the meV and sub-meV range with wavelength $2\pi/q$ corresponding to the distance between the cylinders in the hexagonal lattice that is inter-molecular excitations. Spectra have been measured at reciprocal lattice points and in-between and at different temperatures. They all show a similar shape, though being different in detail. Figure 3 gives an example for a scan across the (2,0) Debye-Scherrer ring.

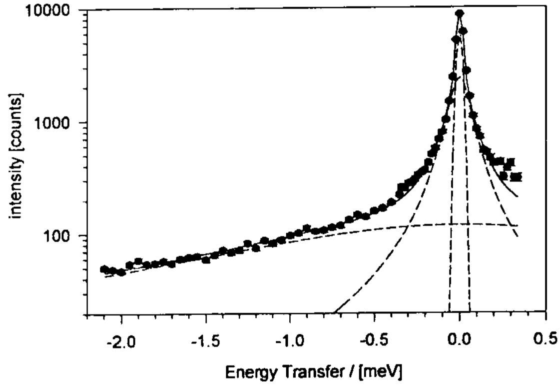

FIGURE 3: Inelastic scan (symbols) across the (2,0) Bragg peak of the two-dimensional hexagonal lattice. Sample temperature 68°C. The solid line is a fit with two Lorentzians and one Gaussian line as explained in the text. The dashed lines show the three contributions separately.

Besides the elastic intensity which is modelled by a Gaussian with the width of the instrument resolution function, we find a rather unstructured broad distribution of inelastic scattering centered at $\Delta E = 0$. To model this distribution a fit using two Lorentzian lines was found to be sufficient for all spectra measured. However this is only thought to be a parametrisation of an unknown inelastic lineshape. Attempts to describe the inelastic excitation by a damped oscillator failed completely. The first Lorentzian shows a full-width at half maximum (FWHM) of roughly 1 meV, the second one a FWHM of approximately 100 μeV. The amplitude of the narrow line is one order of magnitude higher than the broad one. As a function of temperature the quasielastic intensity increases while the elastic (gaussian) intensity decreases. Figure 4 summarises the results of the fitting procedure displayed as temperature and q dependence of the Lorentzian line widths. While the dependence on temperature is very weak - the width stays essentially constant - the q dependence is found to be more complex. While the general trend also in this case seems to be a constant value, some points however do deviate significantly from the average value. On the basis of the data obtained up to now it is not possible to correlate those deviations with the two-dimensional hexagonal lattice.

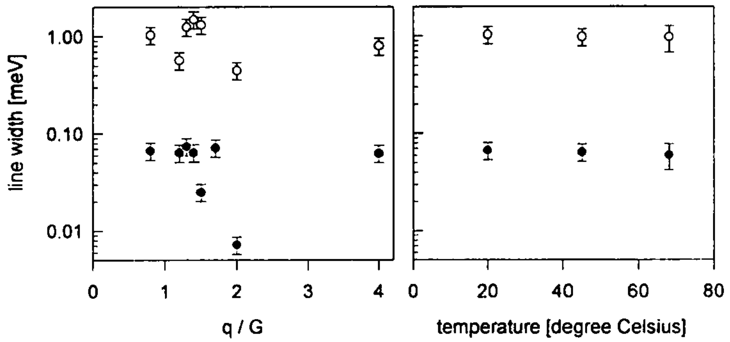

FIGURE 4: Temperature (right) and wavevector (left) dependence of the quasielastic line widths. Open symbols: broad line, full symbols: narrow line.

At low q values there are mainly two relaxational processes which have to be considered for the interpretation of the data measured.

Firstly diffusional processes, i.e. long range transport of matter, give rise to a quasielastic signal with a linewidth Γ proportional to the diffusion constant D. Free diffusion shows a characteristic dependence of the line width on both q and temperature[18]:

$$\Gamma(T,q) = 2\hbar D(T)q^2.$$

The increase with temperature usually obeys an Arrhenius behaviour, while q enters in a quadratic way. For low q ($qr_G < 1$) this result is also valid for long flexible polymer molecules[19]. Our data clearly show that this is not the case for this system. We thus exclude diffusion as a cause for the quasielastic intensity observed though the inelastic *lineshape* would be compatible with such an explanation.

The second processes are phonon-like excitations of the two-dimensional lattice. If the damping is low they will result in a maximum in the inelastic intensity at an energy transfer different from zero. For an only partially ordered sample - like the one at hand - we expect to observe some projection of the phonon density of states (DOS) which will give a broad distribution in energy, starting however at zero for zero energy transfer[20]. The corresponding incoherent inelastic lineshape is approximately constant at low energies[21]. If we elvaluate the q-averaged data - to a good approximation equal to the incoherent scattering - we observe an excess intensity at low excitation energies. This excess intensity is ascribed to damping of the phonon modes due to the anharmonic soft interaction potential. The detailed lineshape depends on the potential, which for our system is not known. However, we expect only small variations of the lineshape with temperature.

Finally we have to consider localised motions of water or of the stacked guanosine tetramers. These excitations have their main contribution to the scattering intensity at q values larger than the inverse of r_G (see for example[19]). Regarding the contribution of water we have to take two contributions into account: freely moving molecules and those bound to the guanosine. Calculating the available volume between the aggregates we conclude that one monolayer of water consumes 50% of the molecules. So at maximum two layers of water may form around the columnar aggregates. These layers are known to be bound rather tightly to the guanosine aggregates[22] and consequently differ from bulk water concerning their dynamics. Inelastic scattering of bound water has been studied in several systems, such as DNA[23] or proteins[24,25]. Typical excitation energies found in those studies are in the range of 3-50 meV and thus outside our interval of energy-transfer. Besides those excitations quasielastic scattering in the 100 μeV range has been found, which might contribute to the measured signal. As all data were taken at low q values however, the maximum contribution of water is estimated to some percent (see above).

Thus damped phonon like excitations of the hexagonal lattice are the most probable source for the inelastic intensity. The case of flexible polymer chains in *isotropic* solution has been treated in several neutron and light scattering experiments and in theoretical papers (for a recent review see[19]). Our results clearly show that this description is not sufficient our high concentrated system. The liquid crystalline order has a strong influence on the dynamics as it suppresses diffusion (no q^2 behaviour) and allows for phonon-like excitations which however are overdamped. Obviously thermal excitations are more similar to localized motions known from amorphous systems[26].

We want to state again, that the spectral shape used to fit the measured data in this work is entirely empirical and serves only to quantify the dependence of the spectra on the experimental parameters. To confirm the influence of the liquid crystalline order, which is reflected in the deviations from a constant q behaviour of the line width more data have to be collected. In particular it would be useful to vary the water concentration in the sample in the limits of the hexagonal phase region. In this way the relative contribution of water could be treated in a more quantitative manner.

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