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Comparison of Response of Biological Supermacromolecules to Magnetic Field Depending on Surface Structure

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By using time-resolved synchrotron radiation X-ray scattering technique we have observed behaviors of biological supermacromolecules in solutions under magnetic field. Here we treated two different supermacromolecules, namely, tobacco mosaic virus and cucumber green mottle mosaic virus. Both viruses take isomorphous cylindrical structures regarded as a typical rod-shaped colloidal particle. Although these viruses are closely related genetically and take isomorphous structures, we found a quite different behavior between those suspensions under magnetic field. The secondary and tertiary structures of the coat proteins of these viruses are mostly identical with each other. There exists a minor difference in the protein surface structures. The present results suggest that the response of the supermacromolecular assembly to magnetic field depends not only on the diamagnetic anisotropy but also on the surface structure.

Keywords: liquid crystal; phase behavior; magnetic field; biological supermacromolecule; small-angle X-ray scattering

INTRODUCTION

Behavior of biological supermacromolecular suspensions under

electromagnetic field is a very attractive scientific concern and has been studied theoretically and experimentally from the viewpoint relating to cooperative phenomena in liquid crystal phase transitions. On the basis of on some repulsive or attractive interaction, many theoretical works have been done to clarify self-ordering mechanisms of rod-shaped colloidal particles [1-5]. In the present report we treat rod-shaped viruses, namely tobacco mosaic virus (TMV) and cucumber green mottle mosaic virus (CGMMV), which consist of ~2100 coat-protein subunits and of a single strand RNA with ~6400 nucleotides. These viruses are known to take a well-defined isomorphous cylindrical structure of 180 Å in diameter and 3000 Å in height, therefore their suspensions are regarded as a typical system of rod-shaped colloidal particles. These two viruses are closely related genetically, and CGMMV is a strain of TMV group. Previously, we studied the phase behaviors of TMV suspensions and determined the phase diagram of TMV liquid crystal depending on both magnetic-field strength and molecular concentration [6, 7]. In the present study, we have compared the orientational process of CGMMV with that of TMV by using synchrotron radiation small-angle X-ray scattering (SR-SAXS) measurements. In spite of the isomorphous structures of these viruses, we have found a quite different phase behavior of CGMMV suspension under magnetic field in comparison with that of TMV. We show that a minor difference between the surface structures of these rod-shaped colloidal particles plays an essentially important role for a phase behavior under magnetic field.

MATERIALS AND METHODS

These samples used for the present experiment were tobacco mosaic virus (TMV) from Japanese common strain OM [8] and cucumber green mottle mosaic virus (CGMMV) from watermelon strain. These viruses were suspended in 10 mM sodium phosphate buffer adjusted at pH 7.2. The

concentrations of the suspensions were 106 - 266 mg/ml for TMV and 20 - 240 mg/ml for and CGMMV, respectively. These values were determined spectrophotometrically by using an absorption coefficient of $e^{1g/l}_{260nm} = 3.0$ for the virus.

The SR-SAXS experiments were performed by using a SAXS spectrometer installed at the BL10C line of the 2.5 GeV storage ring at the Photon Factory, High Energy Accelerator Research Organization, Tsukuba, Japan. The X-ray wavelength and the sample-to-detector distance were 1.49 Å and 198 cm, respectively. The scattering intensities were recorded with a one-dimensional position sensitive proportional counter (PSPC). The TMV and CGMMV suspensions were contained in a quartz cell with 1-mm path length. The time-resolved measurements under magnetic field were carried out at 25°C on a programmed time-interval setting with the 55-second exposure time for each time-frame. The total exposure time for one sample was below 660 seconds, which was short enough to avoid some radiation damage on the sample. The characteristics of the permanent magnetic circuit used was reported elsewhere [9]. The applied magnetic field strength was selected to be 1.37 tesla. The direction of the applied magnetic field was perpendicular to the directions of the PSPC anode wire and the incident X-ray beam.

RESULT AND DISCUSSION

Comparison of Lyotropic Phase Transition of CGMMV with That of TMV

Figures 1(A) and 1(B) show the scattering curves depending on concentration of TMV and CGMMV, respectively. The abrupt decrease of the scattering intensity at a very small angle below $q = 0.006$ Å^{-1} (the absolute value of the scattering vector $|q| = q = 4\pi\sin\theta/\lambda$, scattering angle 2θ, wavelength λ) is attributed to the beam stopper. In Figure 1(A), with increasing the TMV concentration from 106 to 201 mg/ml the broad peak

at ~0.018 $Å^{-1}$ becomes sharper, suggesting the appearance of the repulsive nearest-neighbor correlation between the TMV particles. The hump at q =

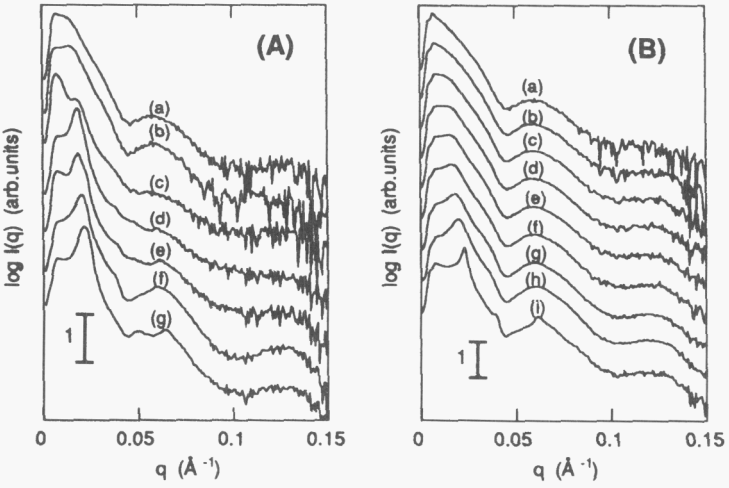

Figure 1. Changes of scattering curves of TMV (A) and CGMMV (B) suspensions caused by lyotropic phase transitions. In (A), (a), 106 mg/ml; (b), 137 mg/ml; (c), 171 mg/ml; (d), 201 mg/ml; (e), 211 mg/ml; (f) 248 mg/ml; (g), 266 mg/ml. In (B), (a), 20 mg/ml; (b), 40 mg/ml; (c), 60 mg/ml; (d), 80 mg/ml; (e), 92 mg/ml; (f), 109 mg/ml; (g), 133 mg/ml; (h), 171 mg/ml; (i), 240 mg/ml of CGMMV suspension. The TMV data have been shown elsewhere [7].

~0.06 $Å^{-1}$ changes significantly at 171 mg/ml. In the TMV concentration from 201 to 266 mg/ml, the position of the peak at ~0.018 $Å^{-1}$ shifts to 0.022 $Å^{-1}$, and other subsidiary humps above q = ~0.03 $Å^{-1}$ become to show evident peak shapes from a higher-ordering. On the other hand, in Figure 1(B) the concentration dependence of the scattering curve of CGMMV is different from that of TMV. Namely, in the concentration from 20 to 171 mg/ml the scattering curves at q = 0.03 - 0.1 $Å^{-1}$ are mostly hold except for the appearance of the repulsive correlation effect between the CGMMV particles. At 240 mg/ml the subsidiary humps and peaks become to show an evident higher-ordering, which results from the

transverse ordering within the layers in a smectic phase as described below.

To analyze a higher-ordered arrangement of solute particles, we have separated the intraparticle and interparticle structure factors from the scattering curve. As described in detail [7], based on a multi-convolution theory the scattering function $I(q)$ from a solution composed of identical particles is given as

$$I(q) \propto \left\langle \left| \Im\{\rho(\mathbf{r}) * w(\mathbf{r})\} \right|^2 \right\rangle = \left\langle I_s(\mathbf{q}) \right\rangle \left\langle W(\mathbf{q}) \right\rangle = I_s(q) W(q) \tag{1}$$

where $\langle\ \rangle$, $\Im\{\ \}$, and * mean the spherical average, Fourier transform, and convolution integral, respectively. $I_s(q)$ and $W(q)$ are the spherical-averaged scattering functions of $I_s(\mathbf{q})$ and $W(\mathbf{q})$; $I_s(\mathbf{q})$ and $W(\mathbf{q})$ obtained by the square of the Fourier transform of the intraparticle scattering density distribution $\rho(\mathbf{r})$ and of the interparticule translational distribution function $w(\mathbf{r})$, respectively. The $I(q)$ from a solution in isotropic phase is regarded as $I_s(q)$ in Eq. (1). As we can observe the $I(q)$ of the TMV and CGMMV suspensions both in isotropic phase (ex. 20 mg/ml for CGMMV), the interparticle correlation scattering function $W(q)$ at high concentration can be deduced from $I(q)/I_s(q)$. Figure 2 shows the $W(q)$ functions of the CGMMV suspensions at various concentrations. Concerning the $W(q)$ of the 266 mg/ml TMV suspension in the insert of Figure 2 was previously discussed in detail [7]. In Figure 2 the $W(q)$ of the 240 mg/ml CGMMV suspension has several sharp peaks. These peak positions are well described by the Miller indices of two-dimensional hexagonal lattice with a paracrystalline disorder, namely by a disordered smectic B phase. The hexagonal lattice ordering of the 240 mg/ml CGMMV suspension is higher than that of the 266 mg/ml TMV suspension since the lattice peaks are much clearly seen for the CGMMV suspension.

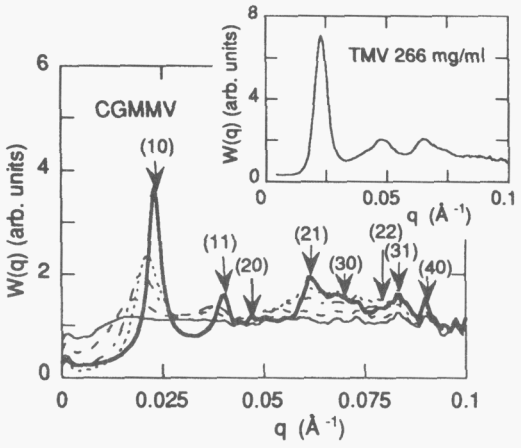

Figure 2. Interparticle correlation scattering functions $W(q)$ of the CGMMV suspensions. Thin full line, 40 mg/ml; dash line, 80 mg/ml; dash line with dots, 133 mg/ml; dotted line, 171 mg/ml; thick full line, 240 mg/ml. The insert shows the $W(q)$ of the TMV suspension of 266 mg/ml, which was shown previously [7].

Comparison of Orientational Processes of CGMMV and TMV Under Magnetic Field

Figures 3(A) and 3(B) show the time courses of the changes of the scattering curves of the TMV (88 mg/ml) and CGMMV (92 mg/ml) suspensions under the magnetic field strength of 1.37 tesla, respectively. As discussed previously [6], the magnetic orientational energy of TMV particle from the diamagnetic anisotropy at 1.37 tesla is much smaller in about three orders of magnitude than the Brownian motional energy. In spite of such a small orientational energy, in Figure 3(A) the scattering curve of the TMV suspension changes with a defined relaxation time (243 ± 22 s for 88 mg/ml TMV at 1.37 tesla). This is explained as the magnetically induced orientation of the nematic liquid crystal microdomains of the TMV particles along the magnetic field direction [6]. On the other hand, in spite of the isomorphous structure, the CGMMV

suspension does not show any magnetically induced orientation, as seen in Figure 3(B). This indicates that in the case of the CGMMV suspension a growth from nematic polycrystalline domains to a single domain does not occur. In other words, the CGMMV particles prefer to take an isotropic phase and resist to take a nematic phase even by applying magnetic field. The reason is attributable to a differences between the surface structures of the TMV and CGMMV particle, as discussed in the following section.

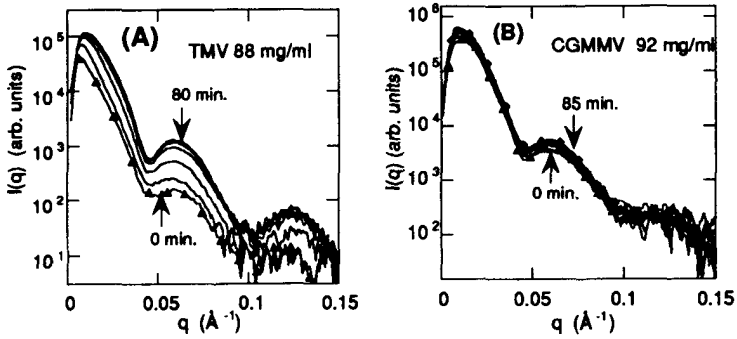

Figure 3. Time courses of the changes of the scattering curves of the TMV (88 mg/ml) and CGMMV (92 mg/ml) suspensions under the magnetic field strength of 1.37 tesla. TMV data has been shown previously [6].

Physicochemical and Structural Properties of CGMMV and TMV Coat Proteins

To discuss the above difference in the behaviors of the CGMMV and TMV suspensions under magnetic field, at first we compare the physicochemical properties of the coat proteins of these viruses. The sequence homology between the TMV and CGMMV proteins is 36.7 % [10]. According to the structural data of the TMV and CGMMV proteins from the Protein Data Bank (PDB file codes of 2TMV [11] and 1CGM

[12]), the helix and sheet contents are 53.9 % helix and 5.2 % sheet for TMV, and 43.1 % helix and 5.0 % sheet for CGMMV. The ratios between the acidic / basic amino acid contents and between the uncharged polar / nonpolar amino acid contents are 10.6 % / 8.8 %, 33.8 % / 46.9 % for CGMMV, and 9.7 % / 8.4 %, 39.0 % / 42.9 % for TMV. Then, at neutral pH the net negative charge of the CGMMV protein would be higher than that of the TMV protein, which has been already shown experimentally by the potentiometric titration and turbidity measurements [13]. The average net surface charge z (mol / mol) at 0.01 M NaCl is –5.5 for TMV protein, and –6.9 for CGMMV protein. The isoelectric point in distilled water is pH = 3.64 for TMV protein, and pH = 4.27 for CGMMV protein. The average net negative charge of the CGMMV protein has been shown to affect greatly an self-assembling process of cylindrical structure [14].

Based on the atomic coordinates of the TMV and CGMMV proteins from PDB, we can compare directly the three dimensional structures of these proteins by using a structural multiple-alignment data base called FSSP [15]. The result is shown in Figure 4. Although the tertiary structures of the TMV and CGMMV proteins mostly resemble with each other, we can recognize that an evident difference in the regions which locate at the surface area of the viruses. Thus, this region of the CGMMV protein corresponds to the number of amino acid sequence from 153 to 160, namely Ser-Glu-Ala-Thr-Thr-Ser-Lys-Ala. This polypeptide region takes an extended structure from the virus surface, which would act as an steric hindrance to disturb a local alignment of solute particles.

In addition, we can compare the diamagnetic anisotropy between TMV and CGMMV proteins caused by the intramolecular structures. Here, we have developed a program to calculate a diamagnetic anisotropy of a protein based on the atomic coordinates from the PDB file. This program considers the orientations of peptide-bond planes and aromatic amide residues, which are presented normal unit vectors. The anisotropy of diamagnetic susceptibility of a molecule, $\Delta\chi$, is given by the difference

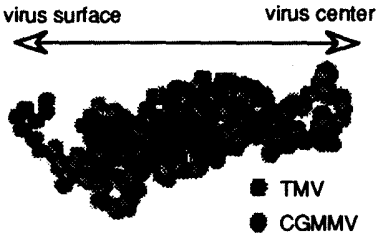

Figure 4. Comparison of 3-D structures between TMV and CGMMV coat proteins. The fitting was done by using Fold classification based on Structure-Structure alignment of Proteins program called FSSP.

between the maximum and minimum values of the diamagnetic susceptibility. According to the above estimation, the values of $\Delta\chi$ (/magnetic susceptibility of peptide unit) are 34.7 for TMV protein and 35.2 for CGMMV protein. Thus, the magnetic orientational energy of CGMMV would be comparable to that of TMV, which does not explain the different orientational behavior between TMV and CGMMV to magnetic field.

In conclusion, the present results evidently show that a response of a supermacromolecular assembly to magnetic field depends not only on a diamagnetic anisotropy, but also on surface characteristics (structure and net charge) of the supermacromolecule. In the present case, the latter factors would affect a short-distance interparticle interaction among the CGMMV particles to restrict a local density fluctuation forming nematic microdomains, which would result in a week response of the CGMMV suspension to magnetic field.

Acknowledgments

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