Intensive Extrusion and Occlusion of Water in Ganglioside Micelles with Thermal Reversibility

Mitsuhiro Hirai and Toshiharu Takizawa
Department of Physics, Gunma University, Maebashi 371, Japan

ABSTRACT By using a shell-modeling analysis for small-angle scattering data of ganglioside micellar dispersion, we recently reported that the elevation of temperature induces a significant shrinkage of the hydrophilic region of the ganglioside micelle, suggesting that the oligosaccharide chains with sialic acid residues of ganglioside molecules change the conformation, sensitively responding to a change in temperature (Hirai et al., 1996. *Biophys. J.* 70:1761–1768; *J. Phys. Chem.* 100:11675–11680). We have carried out further analyses of the temperature dependence of the structural parameters reported previously, and we have found clear evidence of reversible extrusion and occlusion of a large amount of water in the hydrophilic region of the ganglioside micelle in the physiological temperature range of 6–60°C. The present results suggest a remarkable function of ganglioside molecules: they change the hydrophilicity of the cell surface locally as a response to variations in temperature. This phenomenon might be involved in various surface events, such as cell-cell interaction and cell surface-protein interaction.

INTRODUCTION

Gangliosides have been shown to participate in various important physiological functions in cell surfaces, such as self-organization of tissues, immune response and cell differentiation, through a great variety of ganglioside structures (Hannun and Bell, 1989; Hakomori and Igarashi, 1993; Svennerholm et al., 1994). Ganglioside is composed of a ceramide as a hydrophobic tail and of an oligosaccharide chain containing one or more *N*-acetylneuraminic acid (sialic acid) residues as a hydrophilic head. Such huge negatively charged sugar heads give gangliosides very unique physicochemical characteristics in comparison with those of phospholipids. Many intensive studies have been carried out to clarify the aggregative characteristics of gangliosides (Tettamanti et al., 1985; Maggio et al., 1985; Corti and Cantù, 1990; Corti et al., 1996; Sonnion et al., 1994). Using neutron and x-ray-scattering techniques, we have been studying the structural phase behavior of ganglioside aggregates with regard to temperature, pH, and concentration (Hirai et al., 1995b, 1996a–c), and the complexation of gangliosides with proteins (Hirai et al., 1995a, 1998; Takizawa et al., 1995).

Recently, using shell-modeling data analysis of synchrotron radiation x-ray small-angle scattering measurements, we have reported that the elevation of temperature induces a significant shrinkage of the hydrophilic region (oligosaccharide chain region) of the ganglioside micelle, accompanied by a slight expansion of the hydrophobic region (ceramide region) in buffer solvents, suggesting that oligosaccharide chains with sialic acid residues of ganglioside molecules change conformation, sensitively responding to a change in temperature (Hirai et al., 1996b,c). The thermotropic structural change of the hydrophilic region of ganglioside micelles would be similar to that of liposomes containing phospholipids with covalently attached poly(ethylene glycol) (PEG-lipids) (Kenworthy et al., 1995). In the present report, we will show that the above shrinkage accompanies an intensive extrusion of water occluded in the hydrophilic region of ganglioside micelle, by executing further analyses of the thermotropic structural transition of the ganglioside micelles reported previously (Hirai et al., 1996b,c).

MATERIALS AND METHODS

Sample preparation and SAXS measurements

The gangliosides used were the monosialoganglioside (abbreviated as G_{M1}) and disialoganglioside (abbreviated as G_{D1}) extracted from bovine brain. These gangliosides (0.5% w/v) were dissolved in buffer solvents adjusted to pH 6.8 and used for the scattering experiments. Small-angle x-ray scattering (SAXS) experiments were carried out by using the synchrotron radiation small-angle x-ray scattering spectrometer installed at the synchrotron source (PF) at the National Laboratory for High-Energy Physics (KEK), Tsukuba, Japan (Ueki et al., 1985). The wavelength and the covered q range were 1.49 Å and 0.01–0.25 $Å^{-1}$, respectively, where q is the magnitude of scattering vector, defined by $q = (4\pi/\lambda)\sin(\theta/2)$ (θ is the scattering angle; λ is the wavelength). The temperature of the sample was controlled from 6.0°C to 60°C by a thermostat to within 0.5 degree. Other experimental conditions were as described previously (Hirai et al., 1996b,c).

RESULTS AND DISCUSSION

The temperature dependence of the structural parameters of G_{M1} and G_{D1} ganglioside micelles reported previously (Hirai et al., 1996b,c) are summarized in Fig. 1, where Fig. 1 *a* shows the radii and semiaxial ratios of the shell and core regions, and Fig. 1 *b* shows the relative values of these

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Address reprint requests to Dr. Mitsuhiro Hirai, Department of Physics, Gunma University, 4-2 Aramaki, Maebashi 371, Japan. Tel.: 81-272-20-7554; Fax: 81-272-20-7551; E-mail: hirai@sun.aramaki.gunma-u.ac.jp.

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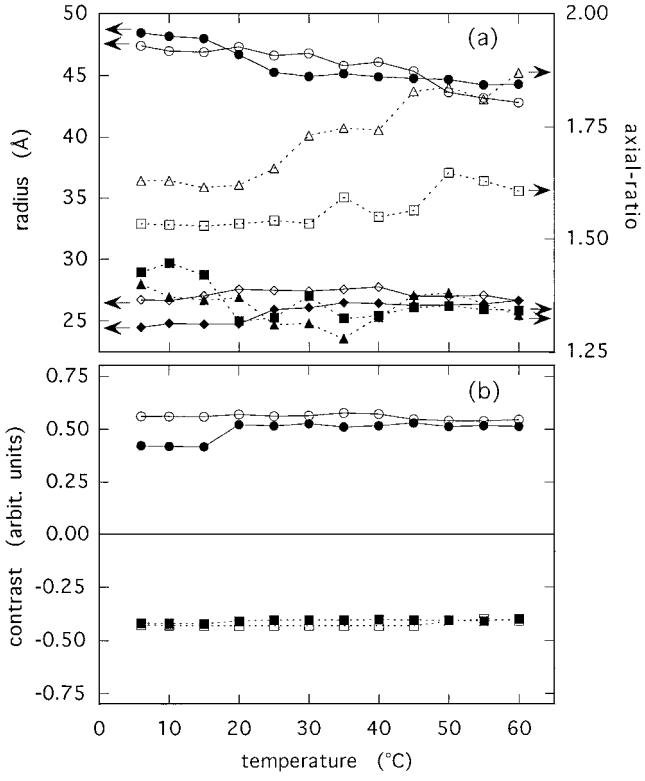

FIGURE 1 Temperature dependence of the structural parameters of ganglioside micelles in aqueous solutions determined by the shell-modeling method, where open and full marks correspond to G_{M1} and G_{D1} micelles, respectively. *(a)* ○, ●, Shell radii; ◇, ◆, core radii; □, ■, shell semiaxial ratios; △, ▲, core semiaxial ratios of the micellar structure model obtained. *(b)* ○, ●, shell contrasts; □, ■, core contrasts. The definitions of the above structural parameters of the model structure are shown in Fig. 2. The parameters in *a* are reproduced, and the parameters in *b* are revised from the previous reports (Hirai et al., 1996b,c).

regions. The shell and core regions mostly correspond to the hydrophilic head and hydrophobic tail regions of ganglioside molecules. For the following reason, in Fig. 1 *b* we have made a slight modification in the structural parameters reported previously (Hirai et al., 1996b,c). We used the following equation for profile fittings of the experimental scattering curves to determine the structural parameters in Fig. 1:

$$I(q) \propto \int_0^1 \left[3\left\{ \bar{\rho}_1 V_1 j_1(qR_1)/(qR_1) + \sum_{i=2}^{n} (\bar{\rho}_i - \bar{\rho}_{i-1}) V_i j_1(qR_i)/(qR_i) \right\} \right]^2 dx \quad (1)$$

where $I(q)$ is the spherically averaged scattering function of a particle with an ellipsoidal shape of rotation composed of n shells with different average excess scattering density $\bar{\rho}_i$ (so-called contrast); i is the number of the shell; j_1 is the spherical Bessel function of the first rank. R_i is defined as $R_i = r_i\,(1 + x^2(v_i^2 - 1))^{1/2}$, where r_i and v_i are the radii and semiaxial ratio of the ith ellipsoidal shell, respectively. $\bar{\rho}_i$, r_i and v_i can be used as fitting parameters. We simplified the ganglioside micellar structure to be a double-shelled ellipsoid of rotation composed of a core surrounded by a shell, namely, we gave $n = 2$ in Eq. 1. Fig. 2 shows schematically the definitions of structural parameters used in Fig. 1. According to the solution by small-angle scattering theory (Stuhrmann and Miller, 1978), a difference between the average scattering densities of the solute and the solvent produces effective scattering intensities. This situation is essentially as reflected by Eq. 1, namely, relative values of contrasts can change $I(q)$ profiles of the model scattering functions expressed by Eq. 1. In addition, we measured the relative scattering intensities experimentally. Then the previous description of the average scattering densities (Hirai et al., 1996a–c) is inappropriate only on this point; therefore we have revised our report to show the relative values of contrast in Fig. 1 *b* instead of the average scattering densities. As ensured by Eq. 1, other structural parameters in Fig. 1 *a* reported previously are not affected by the above modification.

Based on the empirical expressions for a hydrocarbon chain volume (Israelachvili et al., 1976) and on the apparent atomic volumes of the basic chemical elements (Zamyatin, 1972), we can estimate the volumes of the hydrophilic head and hydrophobic tail of the ganglioside molecule; that is, the volumes of the heads are 1215 Å^3 for G_{M1} and 1525 Å^3 for G_{D1}, and the volume of the ceramide is 1029 Å^3. Because we know the chemical components of ganglioside molecules, the average scattering densities of the head and tail portions turn out to be 1.23×10^{11} cm^{-2} for the G_{M1} head, 1.26×10^{11} cm^{-2} for the G_{D1} head, and 8.69×10^{10} cm^{-2} for ceramide. The average scattering density of water is 9.4×10^{10} cm^{-2}. Then the values of contrast of the head and tail portions of the ganglioside molecule are 2.9×10^{10}

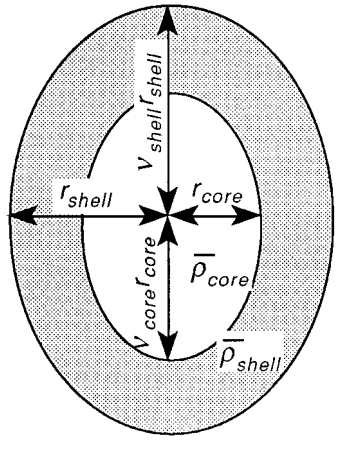

FIGURE 2 Definitions of the structural parameters of the model structure used in the present shell-modeling analysis. We simplified a ganglioside micellar structure as a double-shell ellipsoid of rotation composed of a core surrounded by a shell. $\bar{\rho}_{shell}$, $\bar{\rho}_{core}$, r_{shell}, r_{core}, ν_{shell}, and ν_{core} are the relative values of contrasts, the radii, and the semiaxial ratios of the shell and the core, respectively.

cm^{-2} for the G_{M1} head, 3.2×10^{10} cm^{-2} for the G_{D1} head, and -0.7×10^{10} cm^{-2} for ceramide, respectively. On the other hand, as in the cases of other hydrated phospholipid membranes (Worcester and Franks, 1976; Büldt et al., 1978), the micellar hydrophobic core can be assumed to be virtually devoid of internal water. In addition, the volume of ceramide in the micelle can be assumed to be mostly constant in the temperature range measured, because hydrocarbon chains in micelles contain many gauche configurations (Gruen, 1981; Gruen and de Lacey, 1984; Dill et al., 1984). Then the ratio between the relative values of contrast of the shell and core regions in Fig. 1 *b* ($\sim 0.5/-0.5$) is very different from the above empirical value of $\sim 3/-0.7$, suggesting directly the presence of a large amount of water in the hydrophilic shell region of the ganglioside micelle.

According to the following scheme, we can evaluate the number of water molecules occluded in the hydrophilic region from the values of the structural parameters shown in Fig. 1. Based on a standard expression (Stuhrmann and Miller, 1978), the values of contrasts of the shell and core regions are given as

$$\bar{\rho}_{\text{shell}} = \frac{n_w \Sigma_{\text{water}} b + n_a \Sigma_{\text{head}} b}{n_w V_{\text{water}} + n_a V_{\text{head}}} - \frac{\Sigma_{\text{water}} b}{V_{\text{water}}} \tag{2}$$

$$\bar{\rho}_{\text{core}} = \frac{\Sigma_{\text{cer}} b}{V_{\text{cer}}} - \frac{\Sigma_{\text{water}} b}{V_{\text{water}}} \tag{3}$$

where $\Sigma_{\text{water}} b$, $\Sigma_{\text{head}} b$, $\Sigma_{\text{cer}} b$, V_{water}, V_{head}, and V_{cer} mean the total scattering amplitudes and the excluded volumes of water molecule, and the head and tail portions of the ganglioside molecule, respectively; n_w is the number of water molecules occluded in the shell region; n_a is the aggregation number of ganglioside micelles. The following equation is derived from Eqs. 2 and 3:

$$\frac{\Delta_{\text{shell}}}{\Delta_{\text{core}}} = \frac{\bar{\rho}_{\text{shell}}}{\bar{\rho}_{\text{core}}} = \left(\frac{n_w \Sigma_{\text{water}} b + n_a \Sigma_{\text{head}} b}{V_{\text{shell}}} - \alpha \right) \Big/ \left(\frac{\Sigma_{\text{cer}} b}{V_{\text{cer}}} - \alpha \right) \tag{4}$$

where $\alpha = \Sigma_{\text{water}} b / V_{\text{water}} = 9.4 \times 10^{10}$ cm^{-2}; $V_{\text{shell}} = n_w V_{\text{water}} + n_a V_{\text{head}}$; $n_a = V_{\text{core}}/V_{\text{cer}}$; V_{shell} and V_{core} are the volumes of the shell and core regions estimated from Fig. 1 *a*; $\Delta_{\text{shell}}/\Delta_{\text{core}}$ is the ratio between the shell and core contrasts obtained by the shell-modeling analysis in Fig. 1 *b*. Then the number n_w of water molecules in the shell region is given by

$$n_w = \left[\left\{ \frac{\Delta_{\text{shell}}}{\Delta_{\text{core}}} \left(\frac{\Sigma_{\text{cer}} b}{V_{\text{cer}}} - \alpha \right) + \alpha \right\} V_{\text{shell}} - n_a \Sigma_{\text{head}} b \right] \Big/ \Sigma_{\text{water}} b \tag{5}$$

As we know the chemical components water molecule, G_{M1} head, G_{D1} head, and ceramide, we can also calculate $\Sigma_{\text{water}} b = 2.81 \times 10^{-12}$ cm, $\Sigma_{\text{head}} b = 1.50 \times 10^{-10}$ cm for the G_{M1} head and 1.92×10^{-10} cm for the G_{D1} head, $\Sigma_{\text{cer}} b/V_{\text{cer}} = 8.69 \times 10^{10}$ cm^{-2}.

By applying Eq. 5 to the ratio of the contrasts in Fig. 1 *b*, we can estimate the number n_w. In Fig. 3 the value n_w per ganglioside molecule at each temperature is presented. With elevating temperature, the number of water molecules in the shell region significantly decreases in cases of G_{M1} and G_{D1}. At low temperature the n_w value per ganglioside molecule in the G_{D1} micelle is much lager than that in the G_{M1} micelle. The decreasing tendencies in the two cases are greatly different; that is, in the G_{D1} micelle the extrusion of water occurs drastically at $\sim$20°C, whereas in the G_{M1} micelle the extrusion proceeds continuously. This difference is attributable to the structural characteristics of ganglioside molecules, namely, the conformational flexibility and the number of sialic acid residues of the oligosaccharide chain portions. As can be seen in Fig. 3, the extrusion of water from the hydrophilic shell region occurs reversibly despite the evident thermal hysteresis of the micellar shape (Hirai et al., 1996a). The reliability factors defined by $R = \Sigma |I_{\text{obs}}(q) - I_{\text{model}}(q)| / \Sigma I_{\text{obs}}(q)$ in the shell-model fittings were shown previously to be $\sim$0.02–0.04, and a thermal expansion of the core region through, e.g., a chain-melting transition, if it actually occurs, would be expected to be less than 6% (Marsh, 1990), because the volume ratio of a CH_2 or CH_3 group between gel and fluid states is 25.5 $Å^3$/27.0 $Å^3$ or 51.0 $Å^3$/54.0 $Å^3$. Then the relative error of the n_w values estimated in the above is below 20%. By using another equation (Eq. 6), we can also evaluate the n_w value:

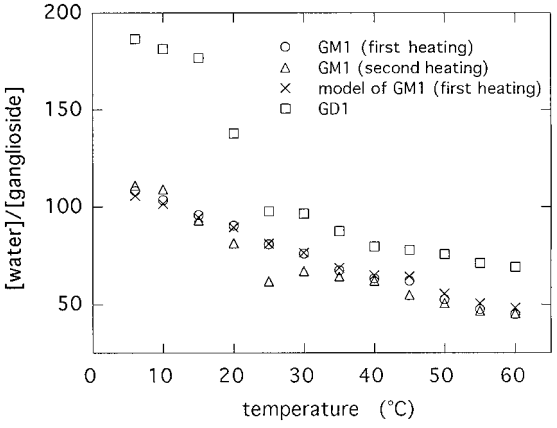

FIGURE 3 Temperature dependence of the number of water molecules per ganglioside molecule occluded in the micelle. ○, △, G_{M1} micelle at first and second heating processes, respectively; □, G_{D1} micelle. These values were estimated by using Eq. 5. ×, Values estimated by using Eq. 6 for the G_{M1} micelle at first heating.

$$n_w = (V_{\text{shell}} - n_a V_{\text{head}}) / V_{\text{water}} \tag{6}$$

where $V_{\text{water}} \approx 30$ $Å^3$, and we simply consider the geometrical packing criterion of micellar formation (Israelachvili et al., 1976). As shown in Fig. 3, the n_w values estimated by using Eq. 5 are in good agreement with those estimated by using Eq. 6, clearly indicating that the structural parameters obtained by using the shell-modeling analysis are very reasonable and consistent. Such reasonability and consistency of the above estimation can be also confirmed by the comparison of the zero-angle scattering intensity $I(0)$ ob-

tained from the model structure with that from the experimental data. Fig. 4 shows the temperature dependence of $I(0)$ obtained by using three different methods, namely the Guinier plot, the distance distribution function, and the shell-modeling analysis. The former two methods afford the experimental values of $I(0)$. In Fig. 4 the $I(0)$ obtained by using the shell-modeling analysis was calculated by the following equation:

$$I(0) = (\bar{\rho}_{\text{shell}}V_{\text{shell}} + \bar{\rho}_{\text{core}}V_{\text{core}})^2 \cdot (C - C_{\text{cmc}})/n_a \propto (\Delta_{\text{shell}}V_{\text{shell}} + \Delta_{\text{core}}V_{\text{core}})^2 \cdot (C - C_{\text{cmc}})/n_a \quad (7)$$

where C and C_{cmc} are the concentrations and the critical micellar concentrations of ganglioside molecules, which in the present case are $C = 3.1 \times 10^{-3}$ M for G_{M1} and 2.7×10^{-3} M for G_{D1}, $C_{\text{cmc}} \approx 2\text{–}3 \times 10^{-8}$ M for G_{M1} and G_{D1}. As we have shown in the above paragraphs and in our previous reports, the consistency and the reasonability of the structural parameters of the obtained models are ensured by the good agreement of the model scattering functions, distance distribution functions, gyration radii, and relative zero-angle scattering intensities with the experimental ones. It would be worth noting that the shell-modeling method is a powerful tool for determining an internal structure of micelle directly from experimental scattering data, especially for a highly monodispersed system such as ganglioside systems, when based on the concrete scheme of the usage of the shell-modeling method. In addition, even under a repulsive interparticle interaction that is known to strongly deform scattering data in a small-angle region, the use of the shell-modeling method, combined with a suitable interparticle correlation potential, enables us to determine directly both an intramicellar structure and an intermicellar interaction potential from experimental scattering data (Hirai et al., 1996a).

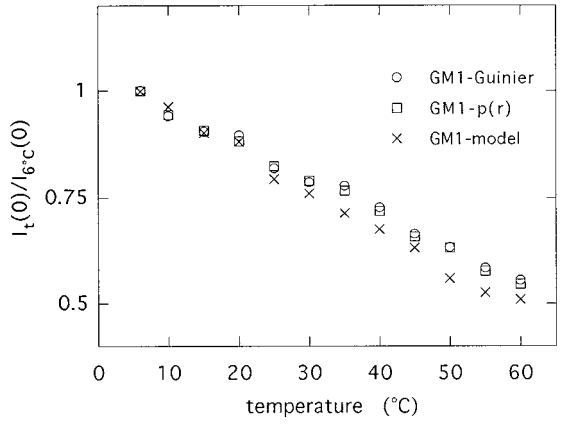

FIGURE 4 Temperature dependence of the zero-angle scattering intensity. The zero-angle scattering intensities for the G_{M1} micelle at first heating obtained from the three different methods are compared. ○, Guinier method; □, distance distribution function; ×, shell-modeling method. The former two methods give us the experimental values of the zero-angle scattering intensity.

The hydration of saccharides has been studied intensively (Uedaira and Uedaira, 1969; Nomura et al., 1982), and it has been shown that the dynamic hydration number n_{DHN} of oligosaccharide can be expressed by a linear relation of the mean value of the number of equatorial OH groups, n(e-OH) (Uedaira et al., 1989, 1990). According to this relation, the number n_{DHN} per G_{M1} or G_{D1} molecule can be expected to be in the range from 50 to 60. Then the number n_w at low temperatures estimated above is about two or three times larger than n_{DHN}, and the elevation of temperature seems to reduce the number n_w to a value approaching the dynamic hydration number n_{DHN}. This indicates that the number n_w estimated here includes not only hydrated water but also simply occluded water, because in the present analyses we cannot distinguish between hydrated water and free water in the hydrophilic region of the ganglioside micelle. Thus the present results suggest that the ganglioside molecules can reversibly occlude or extrude a large amount of water, like a water cavity, through the conformational changes of the oligosaccharide chains as they respond to the temperature variation in the physiological temperature range of 6–60°C. This phenomenon might be a common feature of oligosaccharide gels (Nishinari and Watase, 1991), the mechanism of which might be explained as a kind of critical kinetics of volume phase transition of hydrogel (Tanaka, 1981, 1985). We can assume that the temperature variation affects the local hydrophilicity of the cell surface, because ganglioside molecules are assumed to localize heterogeneously in the outer cell membrane. Such a change in local hydrophilicity might modulate an adhesion between cells or protein binding to the cell surface.

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