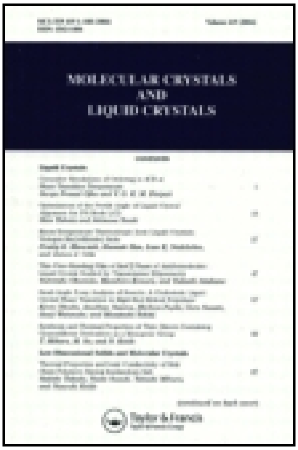

Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl19

Elongation of Ganglioside (GD_{1a}) Micellar Structure Depending on Cholesterol Content

Tomohiro Hayakawa [a] & Mitsuhiro Hirai [a]

[a] Department of Physics, Gunma University, Maebashi, 371-8510, Japan

Version of record first published: 24 Sep 2006

To cite this article: Tomohiro Hayakawa & Mitsuhiro Hirai (2001): Elongation of Ganglioside (GD_{1a}) Micellar Structure Depending on Cholesterol Content, Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals, 367:1, 631-640

To link to this article: http://dx.doi.org/10.1080/10587250108028684

Mol. Cryst. and Liq. Cryst., 2001, Vol. 367, pp. 631-640
Reprints available directly from the publisher
Photocopying permitted by license only

Published by license under the
Gordon and Breach Science Publishers imprint,
a member of the Taylor & Francis Group,
Printed in the USA

Elongation of Ganglioside (GD_{1a}) Micellar Structure Depending on Cholesterol Content

TOMOHIRO HAYAKAWA and MITSUHIRO HIRAI*

Department of Physics, Gunma University, Maebashi 371–8510, Japan

The influence of cholesterol with various molar fractions on the structure of disialoganglioside (GD_{1a})/cholesterol mixed micelle was investigated by using synchrotron radiation small-angle X-ray scattering (SR-SAXS). The radius of gyration (*Rg*) of the micelles, estimated from the obtained data, was increased with increasing cholesterol content. A multi-shell model analysis for micelles, that was successfully applied to the structure estimation of GD_1 micelles by Hirai et al. (1996), revealed that the cholesterol-dependent increase of *Rg* of the micelles is due to an elongation of its prolate spherocylindrical structure. In the present analysis, we assumed the micelle of the ganglioside/cholesterol mixture as a triple-shell structure taking into account the high electron density of the steroid ring of cholesterol that is inserted in the hydrophobic region of the micelle. The examined molar ratios of [ganglioside]/[cholesterol] were 1:0, 1:0.05, 1:0.1, 1:0.2, 1:0.4, 1:0.7 and 1:1. The ganglioside concentration for all samples was constant at 0.5 % w/v.

Keywords: ganglioside; GD_{1a}; cholesterol; micelle; synchrotron radiation small-angle X-ray scattering

INTRODUCTION

Polysialogangliosides, glycosphingolipids containing more than one sialic

---

* Corresponding author.

acid residue, are abundant in neuronal synapse membranes and are speculated to be involved in various membrane-mediated physiological phenomena [1-4]. Due to their strong amphiphilic properties and bulky headgroups, polysialogangliosides such as disialoganglioside and trisialoganglioside form small micelles [5,6] rather than vesicles or planar bilayers in an aqueous solution. Even when they form vesicles with other lipids, they tend to give a high curvature to the membrane structure [7,8]. Such properties of gangliosides would modulate not only membrane fluidity and fusion but also activities of membrane-bound enzymes. However, compared with that of phospholipid mixtures, structural information on the association of polysialogangliosides with other membrane lipid components is still limited and ambiguous. In this present study, the characteristics of the structures of ganglioside (GD_{1a})/cholesterol mixed micelles with various molar ratios were investigated by using SR-SAXS. According to the shell-modeling method [9-12], to estimate the structural parameters (such as radii, axial ratios and contrasts) of the mixed micelles, we assumed the structures of the micelles as a triple-shell structure composed of the three regions, *i.e.*, a core, an inner-shell and an outer-shell, because incorporated cholesterol within the hydrophobic region of the micelles is predicted to be anchored with its hydroxyl group at the hydrophilic-hydrophobic interface. Due to the high electron density of the steroid ring of cholesterol, the scattering density in the hydrophobic interfacial region would be much different from that in the hydrophobic core region, where low electron density methyl groups are located.

MATERIALS AND METHODS

Materials

Disialoganglioside ($IV^3NeuAc\alpha$-,$II^3NeuAc\alpha$-$GgOse_4Cer$ abbreviated as GD_{1a}) and cholesterol were purchased from SIGMA Chemical and used without further purification. Required quantities of ganglioside and cholesterol for each sample were dissolved in a 2:1 (v/v) mixture of chloroform and methanol. The solvent was removed in a stream of nitrogen gas, and the samples were dried at 45 °C in vacuo for overnight. The dried samples were dissolved in 50 mM Hepes (*N*-(2-hydroxymethyl)piperazine-*N'*-(2-ethane-sulfonic acid)) buffer adjusted to pH 7.0, and the mixture was vortexed for several minutes. The final concentration of ganglioside for all samples was 0.5 % w/v. The molar ratios of [ganglioside] : [cholesterol] (g:c) for each sample was 1: 0, 1:0.05, 1:0.1, 1:0.2, 1:0.4, 1:0.7 and 1: 1. The all samples were prepared more than 24 hours before the measurements.

Scattering Method and Analysis

Small-angle X-ray scattering measurements using synchrotron radiation were carried out by a SAXS spectrometer installed at the BL-10C beam line of the 2.5 GeV storage ring at the Photon Factory at the High Energy Accelerator Research Organization, Tsukuba, Japan. X-ray scattering patterns were recorded with a position-sensitive photon counter (PSPC) and associated electronics. The X-ray wave length, the sample-to-detector distance, and the exposure time were 1.49 Å, 198 cm, and 480 seconds respectively. The obtained scattering data were analyzed based on the same procedures presented previously [9-12]. The radius of gyration (R_g) of the micelles was obtained by the Guinier equation. The structural parameters (radius, axial ratio, and contrast) of the micelle were derived from the model scattering function for a particle with an ellipsoidal shape of revolution composed of *n* shells with different average excess scattering density (so-called contrast) [9-12]. In the present analysis, we applied a triple-shell model (composed of a core, an inner-shell and an outer-shell) for the estimation of the structural parameters of the micelles taking into account the high electron density of steroid ring of cholesterol.

RESULTS AND DISCUSSION

Cholesterol Dependence of Scattering Curve and Evaluation of Gyration Radius

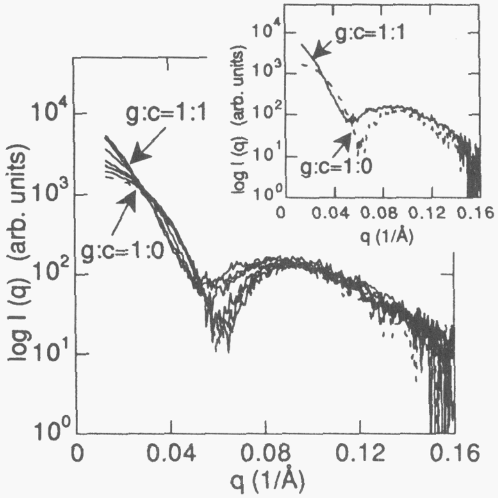

Figure 1. Cholesterol dependence of the scattering curve *I(q)* of the micelle of ganglioside (GD_{1a})/cholesterol mixture in 50 mM Hepes buffer at pH 7.0. Inset in the figure shows the difference between the profiles for g:c = 1:0 and g:c = 1:1.

Figure 1 shows the scattering curves $I(q)$ from the GD_{1a}/cholesterol mixed micelles at 25 °C with increasing the cholesterol molar fraction up to 50 mol % (g:c = 1:1). All scattering curves are reflecting the micellar structures. The scattering curve from the pure GD_{1a} micelles (g:c = 1:0) has a minimum at $q = \sim 0.06$ Å^{-1} and a rounded peak at $q = \sim 0.09$ Å^{-1}. With increasing the cholesterol content, the minimum position of the scattering curve shifts to a small-q region, and the slope of the scattering curve below $q = \sim 0.06$ Å^{-1} which represents the overall size of the solute particle becomes to increase. The above changes of the scattering curves show that the incorporation of cholesterol into the GD_{1a} micelles significantly modifies the size and shape of the micelles.

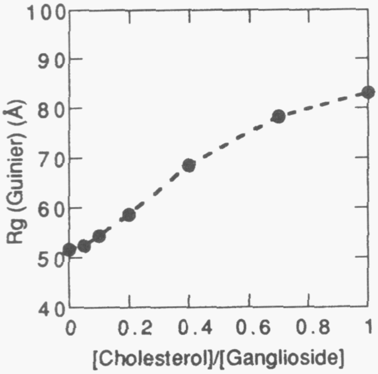

Figure 2.
Change of the gyration radius R_g of the mixture micelle depending on cholesterol molar fraction determined by the Guinier method.

Figure 2 shows the dependence of the gyration radius R_g of the GD_{1a}/cholesterol mixted micelle on the cholesterol molar fraction. The R_g value increases with increasing the cholesterol fraction. Although both changes in the shape and volume of the micelle affect the R_g value, a separation of these effects from the R_g is generally difficult especially for a particle with a heterogeneous scattering density distribution. Hence, we employed a modeling analysis for a quantitative discussion as shown below.

Triple-shell model analysis

To obtain a quantitative information on the structures of the ganglioside/cholesterol mixture micelles, a shell-modeling analysis was performed by the procedures described previously [9-12]. In the cases of the cholesterol containing bilayers of phosphatidylcholine [13] or sphingomyelin [14], cholesterol is assumed to be anchored with its hydroxyl group at the hydrophilic-hydrophobic interface. The orientation of cholesterol within a micellar system of gangliosides would occure in a similar manner. The estimated hydrocarbon volume of cholesterol is

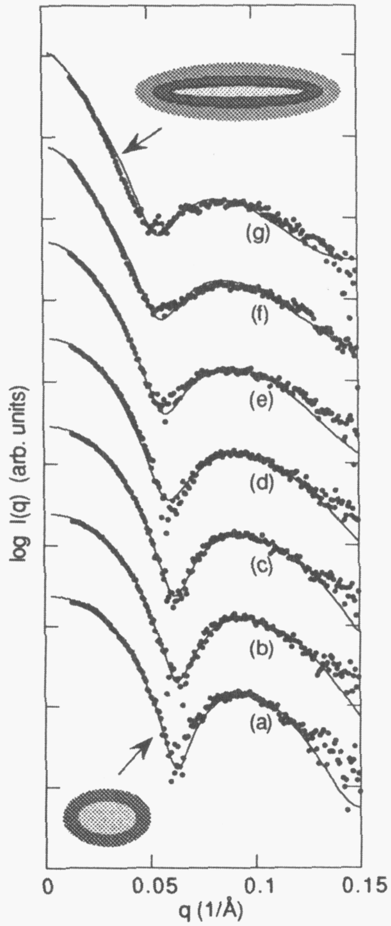

Figure 3. Scattering curves $I(q)$ from the model structures optimized for the experimental data. (a) g:c = 1:0, (b) g:c = 1:0.05, (c) g:c = 1:0.1, (d) g:c = 1:0.2, (e) g:c = 1:0.4, (f) g:c = 1:0.7, (g) g:c = 1:1. Full lines represent theoretical scattering curves fitted to the respective experimental data.

~400 Å^3, the length and the surface area are 17.5 Å and 19 Å^2 respectively [15]. Therefore, the scattering density of the hydrocarbon region of cholesterol, calculated using the above values, should be ~14.6×10^{10} cm^{-2}. This value is higher than that of GD$_1$ sugar head (12.6×10^{10} cm^{-2}) [10]. Such a high value of cholesterol results from the steroid ring because the scattering density of CH$_2$ chains is generally lower than that of water solvents (~9.4×10^{10} cm^{-2}). In the present analysis, therefore, we applied a triple-shell model for the structure estimation of the mixted micelles, taking into account of the high electron density of the steroid ring of cholesterol. The pure GD$_{1a}$ micelle (g:c = 1:0) was analyzed as a double-shell structure as we performed previously. Figure 3 shows the optimized theoretical curves can well simulate the respective scattering curevs from the measurements. The schematics in Figure 3 show the micellar structures that give the

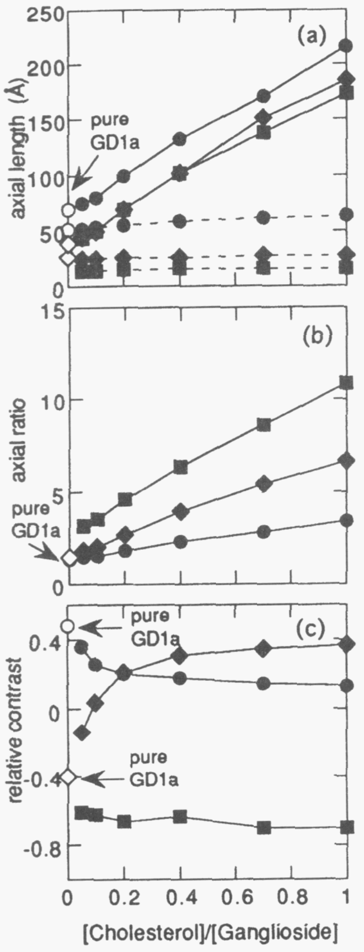

Figure 4. Structural parameters from the model fitting. (a) axis length; (b) axial ratio; (c) contrast. Circles (●) for outer-shell; diamonds (◆) for inner-shell; square (■) for core. In (a), dashed lines correspond to the short-axis (radius), and full lines for the long-axis.

theoretical curves of g:c = 1:0 and g:c = 1:1 respectively. The cholesterol dependence of the structural parameters of the micelles estimated from the shell-modeling analyses are plotted in Figure 4. Figure 4(a) shows that the length of the long axis of each shell region (core, inner-shell and outer-shell) increases linearly with increasing the cholesterol molar fraction, whereas the short axis (radius) for each region does not show such a significant change. Namely, the long-axis length of the outer-shell is 69 Å at g:c = 1:0 and increases to 216 Å at g:c = 1:1, while the short-axis length of the outer-shell shows relatively a minor increase from 51.7 Å at g:c = 1:0 to 63.5 Å at g:c = 1:1. The axis lengths of the inner-shells and the cores shown in Figure 4(a) vary in a similar manner as the outer-shell. These results indicate that the cholesterol-dependent increase of *R*g of the micelle results directly from an elongation of the prolate ellipsoidal structure of the micelle. The cholesterol dependence of the axial ratio is shown in Figure 4(b). The relative values of the average excess scattering densities (contrasts) of the respective regions in the micelle are also shown in Figure 4(c). The contrast of the outer-shell region decreases rather significantly from 0.48 at g:c = 1:0 to 0.2 at g:c = 1:0.2, and then decreases slightly but continuously until g:c = 1:1. The contrast of the inner-shell, on the contrary, increases with increasing the cholesterol content. Above g:c = 1:0.2, the inner-shell contrast becomes higher than that of the outer-shell. The core contrast is mostly stable against the variation of the cholesterol content, though it shows a slight decrease from -0.6 at g:c = 1:0 to -0.7 at g:c = 1:1.

These results indicate that most of the steroid rings of cholesterol molecules are located in the inner-shell region of the micelle. Therefore, the decrease of the outer-shell contrast would be explained by the increase of the amount of water molecules within the outer-shell region, that is, the incorporation of cholesterol molecules within the inner-shell region tends to give a more distance between the ganglioside sugar heads in the outer-shell region. The low electron density of the terminal methyl groups of the lipid hydrocarbon chains would correspond to the low contrast of the micellar core region. The stable values of both the contrast and radius of this region indicate that the cholesterol molecules would not be inserted deeply into this region even at the high cholesterol contents for the elongated micellar structures, *i.e.*, g:c = 1:0.7 and g:c = 1:1. Alternatively, cholesterol molecules have a role as a kind of spacer molecules at the hydrophilic-hydrophobic interface of the mixed micelles. In addition, the significant change of the micellar axial ratio with a little increase of the radius suggests that the cholesterol molecules lower the micellar curvature along the long-axis direction due to their complementary packing structures with those of the gangliosides. Although the radius increase in each region is not negligible, this would be explained as an increase of some inhomogeneity of the molecular packing, that is, the lipid molecules are not associated exactly in a same manner at a same level at the micellar surface.

CONCLUSION

The structures of the micelles of the ganglioside/cholesterol mixtures have been investigated using SAXS technique and a shell-modeling analysis. In this study, the elongation of the prolate spherocylindrical structure of the micelle was indicated in a manner depending on the cholesterol molar fraction. These micellar structures of the mixtures derived from our present analysis would explain the experimental results that the addition of cholesterol to a ganglioside aggregate containing LAURDAN fluorescence increase the LAURDAN GPex values [16]. Namely, the cholesterol molecules incorporated in the micellar hydrophobic region lower the curvature along the long-axis direction of the micelles and increase the packing density in the hydrophobic region due to a preferential packing between cholesterol and ganglioside acyl chain. Such an effect results in a low water content in the hydrophobic-hydrophilic interface of the mixed micelles. Recently, sphingolipids, especially glycosphingolipids, has been found to be localized in lipid microdomains in a variety of cells, including polysialoganglioside-rich neuronal cell [17]. To organize such lipid domains in the planar membranes, incorporation with cholesterol would be favorable or even essential for polysialogangliosides having bulky head groups. In the present study, it is suggested that the intermolecular distance of ganglioside head groups within the mixed micelles is increased by the cholesterol addition. The physicochemical properties of ganglioside head groups in this situation are expected to be different from that in the case of the pure micelles [9], which will be reported elsewhere.

Acknowledgments

The synchrotron radiation X-ray scattering experiments were done under the approval of the Photon Factory Program Advisory Committee (Proposal No. 98G185 & 2000G148) of High Energy Accelerator Research Organization, Tsukuba, Japan.

References

[1] H.A. Hansson, J. Holmgren and L. Svennerholm, *Proc. Natl. Acad. Sci. U.S.A,* **74**, 3782 (1977).

[2] H. Rahmann, H. Rosner, K.H. Kortje, H. Beitinger and V. Seybold, *Progress in Brain Research,* Vol. 101, *Biological Function of Ganglioside,* Elsevier, Amsterdam, 127 (1994).

[3] H. Rahmann, *Trends Glycosci. Glycotech.,* **2**, 450 (1990).

[4] H. Rahmann, H. Rosner and H. Breer, *J. Theor. Biol.* **57**, 231 (1976).

[5] M. Hirai, T. Takizawa, S. Yabuki and K. Hayashi, *J. Chem. Soc., Faraday Trans.* **92**, 4533 (1996).

[6] B. Ulrich-Bott and H. Wiegandt, *J. Lipid Res.,* **25**, 1233 (1984).

[7] B. Maggio, J. Albert and R.K. Yu, *Biochim. Biophys. Acta,* **945**, 145 (1988).

[8] P.D. Thomas and M.J. Poznansky, *Biochim. Biophys. Acta,* **978**, 85 (1989).

[9] M. Hirai, T. Takizawa, S. Yabuki, Y. Nakata and K. Hayashi, *Biophys. J.,* **70**, 1761 (1996).

[10] M. Hirai, T. Takizawa, S. Yabuki, T. Hirai and K. Hayashi, *J. Phys. Chem.,* **100**, 11675 (1996).

[11] M. Hirai and T. Takizawa, *Biophys. J.,* **74**, 3010 (1998).

[12] M. Hirai, H. Iwase, T. Hayakawa, *J. Phys. Chem. B,* **103**, 10136 (1999).

[13] N.P. Franks and W.R. Lieb, *J. Mol. Biol.,* **133**, 469 (1979).

[14] P.R. Maulik and G.G. Shipley, *Biochemistry*, **35**, 8025 (1996).

[15] S. Carnie, J.N. Israelachvili and B.A. Pailthorpe, *Biochim. Biophys. Acta,* **554**, 340 (1979).

[16] L.A. Bagatolli, E. Gratton and G.D. Fidelio, *Biophys. J,* **75**, 331 (1998).

[17] A. Prinetti, V. Chigorno, G. Tettamanti and S. Sonnino, *J. Biol. Chem.,* **275**, 11658 (2000).