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Effect of Alcohols on Glycosphingolipid Aggregates

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Gangliosides, belonging to species of glycosphingolipids (GSLs), are known to be involved in the formation lipid microdomains, so-called lipid rafts, on plasma membrane surfaces. The physicochemical properties of gangliosides deeply relate to the physiological functions of lipid rafts such as cell signaling. Previously we reported various unique characteristics of ganglioside aggregates and their complexes with other lipids. Due to the presence of oligosaccharide chain in the head portion, ganglioside molecules are very hydrophilic and form micellar structures in aqueous solvents. In this report we show the results of the hydration property of ganglioside micelles depending on alcohol concentration and species. The present results indicate that the increase both in the concentration and the number of hydrocarbon of alcohol induces the significant changes in the hydration of the ganglioside head portion and the packing parameter, which becomes to be more evidently seen with increasing the hydrocarbon chain length. It suggests that the competitive hydrophilic and hydrophobic interactions between polar and non-polar groups both in ganglioside and alcohol molecules sensitively affect aggregative properties of gangliosides.
Key words: glycosphingolipid, raft, small-angle scattering, X-ray

1. INTRODUCTION

A common feature of lipid microdomains, so-called rafts, is their peculiar lipid composition, being rich in glycosphingolipids (GSLs), sphingomyelin and cholesterol. Gangliosides, major components of GSLs, are composed of a ceramide linked to an oligosaccharide chain with sialic acids. Gangliosides are localized on the plasma membrane surface and to be rich in central nervous system. Functions of rafts, such as a platform of signal transduction, cell adhesion and lipid/protein sorting [1-6], are assumed to relate to the peculiar features of GSL molecules both in ceramide and oligosaccharide portions. Due to the characteristics of the molecular structure of gangliosides in the head and tail portions, ganglioside molecules are assumed to form complex hydrogen bond network between those lipids and water molecules [7, 8].

Now the concept of lipid raft is well recognized as a crossroad between cell biology and condensed matter physics [9]. The functions of rafts would relate closely to peculiar features of gangliosides. By using neutron and X-ray scattering techniques we have clarified functional characteristics of gangliosides and ganglioside-containing lipid mixtures as follows. 1) Ellipsoidal micellar formation of gangliosides in aqueous solvents; temperature-dependent hydration and dehydration of hydrophilic head region accompanying the changes in the conformation and the charge of oligosaccharide chains [10-17]. 2) Predominantly location of gangliosides at outer-leaflet of ganglioside-phospholipid vesicles [18, 19]. 3) Presence of maximum miscibility of cholesterols to gangliosides in ganglioside-cholesterol binary mixtures; cholesterol-dependent micelle-to-vesicle and Ca-induced vesicle-to-lamellar transitions with the formation of interdigitated structures between oligosaccharide chains [20, 21]. 4) Dynamics of ganglioside micelles and ganglioside-cholesterol-phospholipid ternary mixtures dominated by hydration and bending of oligosaccharide chains; smallest values at the lipid composition as similar as in intact membrane including rafts [21, 22]. 5) Water permeability controlled by potassium ion through ganglioside-domains [23]. Thus, we show the unique potential of the ganglioside microdomains that modulate local charge and hydrophilicity on membrane surfaces and dominate dynamics of membranes. Such properties of gangliosides seem to be essential for accumulating and activating functional proteins in plasma membrane.

To obtain further information of ganglioside molecules on their hydration and aggregative properties, we have carried out small-angle X-ray scattering (SAXS) experiment for ganglioside micelles by varying alcohol concentrations and species since alcohols are expected to induce hydrodynamic properties of aqueous solutions.

2. EXPERIMENTAL

2.1 Sample Preparation

Type-III ganglioside from bovine brain purchased from SIGMA Chemical Co. (USA) was used without further purification. The major components of type-III ganglioside were checked to be mostly monosialoganglioside (G_{M1}) and disialoganglioside (G_{D1a}) by using a thin-layer chromatography which was described in detail [24]. We used five different types of alcohols. The alcohols used were methanol, ethanol, 1-propanol, 1-butanol, and 1-pentanol, which were purchased from WAKO Pure Chemical Co. The type-III powder was suspended in 100 mM HEPES (*N*-(2-hydroxymethyl) piperazine-*N'*-(2-ethane-sulfonic acid)) H_2O buffer adjusted at pH 7. This 1.0 % w/v ganglioside solution was mixed with the solvents containing appropriate amounts of alcohol by 1:1 (v/v) ratio. The final concentration of Type-III ganglioside was 0.50 % w/v (2.9×10^{-3} M). The molar concentrations of alcohols in the alcohol-water binary solvents were set to be 0, 0.005, 0.01, 0.025, 0.05, 0.075, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, and 3 M, where the concentration of alcohol in each binary solvent was varied in the range from 0 to 3 M for methanol, ethanol, and propanol, from 0 to 1 M for butanol, and from 0 to 0.25 M for pentanol, respectively.

2.2 X-ray Scattering Measurements and Analyses

SAXS measurements were carried out by using the BL10C SAXS spectrometer at the synchrotron source, Photon Factory (PF) at the High Energy Accelerator Research Organization (KEK), Tsukuba, Japan. The X-ray wavelength, the sample-to-detector distance and the exposure time were 1.49 Å, 85 cm, and 300 seconds, respectively. The temperature of the samples contained in the sample cells with a pair of ~20 μm mica windows were held at 25 °C by using a water-bath circulator.

To obtain the scattering curve $I(q)$ from the ganglioside aggregates, the changes in the mass absorption coefficient and the transmission of the solvents depending on the alcohol concentration and species were considered when the background subtraction were done. The corrected scattering curves $I(q)$ were subjected to the following standard analyses. The beginning of the scattering curve $I(q)$ depends on the Guinier equation in the form of

$$I(q) = I(0)\exp(-q^2R_g^2/3). \quad (1)$$

where $I(0)$ and R_g designate the zero-angle scattering intensity and the radius of gyration, respectively. q is the magnitude of scattering vector defined by $q = (4\pi/\lambda)\sin(\theta/2)$ (θ, the scattering angle; λ, the wavelength). The distance distribution function $p(r)$ was calculated by the Fourier inversion of the scattering intensity $I(q)$ as

$$p(r) = \frac{2}{\pi}\int_0^\infty rqI(q)\sin(rq)dq. \quad (2)$$

The $p(r)$ function is known to reflect the particle shape, the intra-particle scattering density distribution and the inter-particle correlation [25]. The maximum dimension D_{max} of the particle is estimated from the $p(r)$ function satisfying the condition $p(r) = 0$ for $r > D_{max}$. To avoid inherent systematic artifacts for estimating R_g by the use of the Guinier approximation, the Glatter's method was also applied [25], where R_g is given as follows.

$$R_g^2 = \frac{\int_0^{D_{max}} p(r)r^2dr}{2\int_0^{D_{max}} p(r)dr}. \quad (3)$$

3. RESULTS AND DISCUSSION

3.1 SAXS Curves of Ganglioside Micelles in Alcohol-Water Binary Solvent: Concentration Dependence

Figure 1 shows the SAXS curves of the type-III ganglioside micelle depending on the alcohol concentrations and species, where A, B, C, D, and E correspond to the alcohol-water binary solvents containing methanol, ethanol, 1-propanol, 1-butanol, and 1-pentanol solvents, respectively. As shown in Fig. 1, with increasing the hydrocarbon chain length of alcohol, the changes of SAXS curves depending on alcohol concentrations become to be significant. In the case of the methanol- or ethanol-water binary solvent the changes of the SAXS profiles are relatively smaller up to the maximum concentration of 3 M than in those of other binary solvents. The effect of the presence of alcohols is evidently seen above the chain length of butanol even at low alcohol concentrations. However, the significant changes of the SAXS curves start from the different concentrations depending on the alcohol species, namely, from ~1.5 M for propanol, ~0.5 M for butanol, and ~0.75 M for pentanol, respectively.

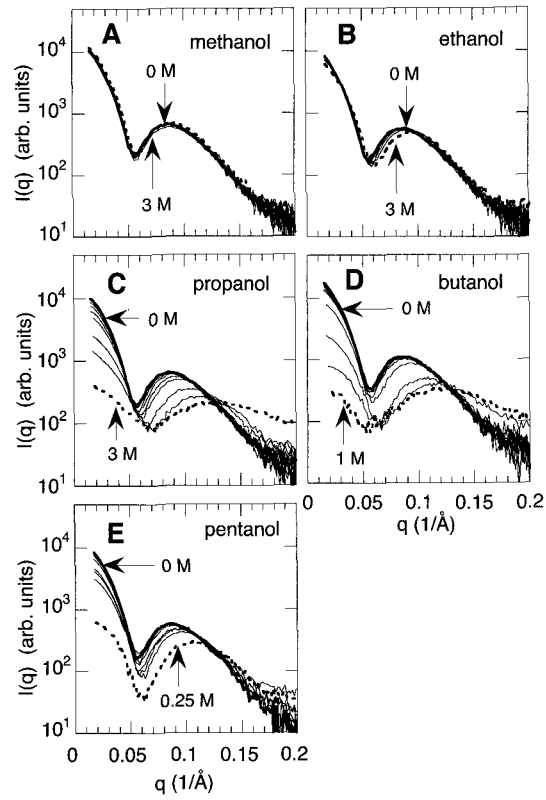

Fig. 1. SAXS curves of ganglioside micelles depending on the alcohol concentrations and species, where A, B, C, D, and E correspond to the solvents containing methanol, ethanol, 1-propanol, 1-butanol, and 1-pentanol, respectively.

3.2 Variation of Distance Distribution Function Depending on Alcohol Concentration

The distance distribution functions $p(r)$ calculated by Equation (3) are given in Fig. 2, where A, B, C, D, and E correspond to the different alcohol-water binary solvents as in Fig. 1. All of the $p(r)$ functions in Fig. 1 have common features, that is, the broad main peak around 70-80 Å and the shoulder or the sub-peak around 23-30 Å. As shown previously in detail [11, 12], these characteristics in the $p(r)$ profiles result from the micellar structures of gangliosides. Namely, the hydrophilic-shell region of the ganglioside micelle consists of the large head portions of oligosaccharide chains that occlude amounts of water. Therefore, the contrasts (the average excess scattering densities of solutes to the scattering density of the solvent) between the shell and core (ceramide portion) regions of the micelle are quite different. The shell and core contrasts in water solvents take positive and negative values, respectively, that is, around 0.4 ~ 0.5 for the shell and -0.4 ~ -0.45 for the core in relative scale [11, 12]. Especially, the shoulder or the sub-peak in the short distance region of the $p(r)$ function corresponds to the hydrophilic shell region of the micelle, which sensitively varies depending on the change of the chain conformation and hydration [15, 17]. The changing manners of the $p(r)$ functions in Figs. 2B-2E caused by the increase of the alcohol concentration are quite similar to those reported previously in the

experiments of temperature elevation of ganglioside micelles [11, 12, 17]. In Figs. 2B-2E, by the increase of the alcohol concentration the sub-peak becomes to be gradually evident from the shoulder profile with the shift of its maximum position to a shorter distance, indicating both the contrast elevation and the shrinkage of the micellar shell region. These changes become to be seen more clearly with increasing the hydrocarbon chain length of alcohol. The above changes are understood to result from the bending of the oligosaccharide chains with their dehydration as similar as those observed at the temperature elevation process of ganglioside micelles [15, 17].

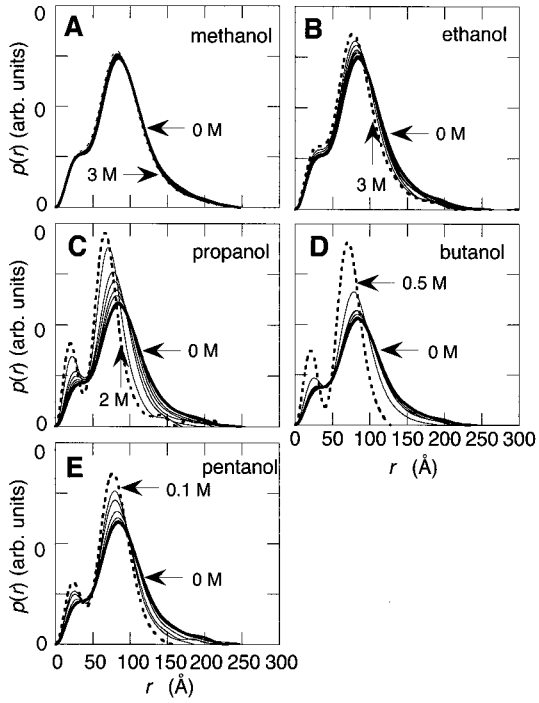

Fig. 2. Distance distribution functions $p(r)$ obtained from Fig.1, where A, B, C, D, and E correspond to the solvents containing methanol, ethanol, 1-propanol, 1-butanol, and 1-pentanol, respectively. In the cases of 1-propanol, 1-butanol, and 1-pentanol solvents, the $p(r)$ functions at higher alcohol concentration show strongly-rippling profiles (at 3 M for 1-propanol, above 0.75 M for 1-butanol, and at 0.25 M 1-pentanol. These $p(r)$ functions are not displayed due to over-scaling in plotting.

As shown in Fig. 3 the evident shift of the main-peak position $p(r)_{max}$ starts from ~0.75 M for methanol, ~0.5 M for ethanol, ~0.25 M for propanol, ~0.025 M butanol, and ~0.01 M for pentanol, respectively. In addition the maximum dimension D_{max} of the micelle, estimated from the intercept of the $p(r)$ function at long distance region ($r > 0$) also become shorter with the increase of the alcohol concentration in Figs. 2B-2E. In the case of the pentanol-water binary solvent (Fig. 2E) the D_{max} value significantly decreases from ~210 Å to ~140 Å. The above changes suggest that the addition of alcohol affects not only the hydrophilic shell region of the micelle, but also the whole structure to induce the contraction of its maximum dimension.

Figure 4 shows the radius of gyration R_g depending on the alcohol concentration. R_g value is known to be a conventional index evaluating a solute particle size for a mono-dispersed solution such as globular particles. As shown in Fig. 4, in the case of methanol- or ethanol-water solvent the increase of alcohol concentration slightly reduces the R_g value, whereas the R_g values decrease considerably in the cases of the water solvents containing propanol, butanol, and pentanol. These changes in the R_g values mostly agree with the results shown in Figs. 2 and 3. The decrements of R_g depending on alcohol concentration are much larger than those of D_{max}. For all cases the R_g values decrease mostly linearly with increasing alcohol concentration, suggesting the decrease of the aggregation number N_a of ganglioside micelles, which is also discussed in below.

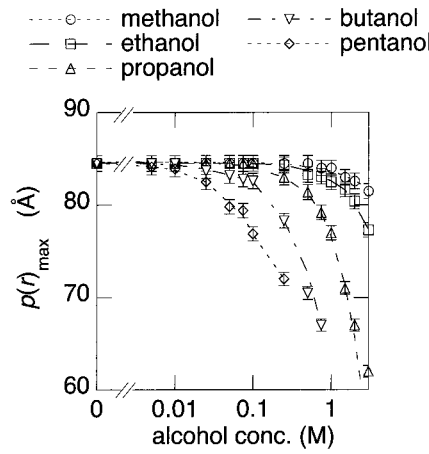

Fig. 3. Main-peak position $p(r)_{max}$ of the distance distribution functions depending on alcohol concentration.

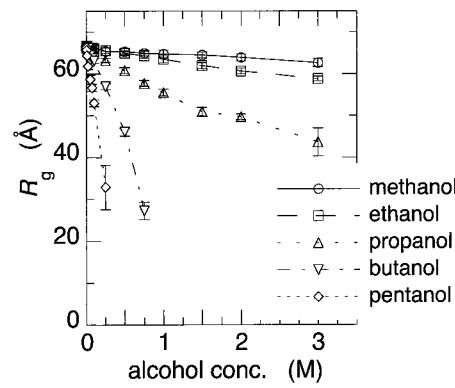

Fig. 4. Radius of gyration R_g depending on alcohol concentration. The lines show the least-square-fitted ones.

3.3 Zero-Angle Scattering Intensity and Aggregation Property of Gangliosides in Alcohol-Water Solvents

From the change of the zero-angle scattering intensity $I(0)$ depending on alcohol concentration shown in Fig. 5, other property of ganglioside aggregates can be derived and discussed. As is well known, the $I(0)$ for a mono-disperse solution composed of a unique component is simply given as follows.

$$I(0) \propto n(\overline{\rho}v)^2. \quad (4)$$

n, $\overline{\rho}$, and v are the number concentration of the solute particle, its contrast and volume, respectively. The $I(0)$

values in Fig. 5 were corrected by the expected change of the contrast of ganglioside micelle as shown below. The scattering densities of alcohol-water solvents depending on alcohol concentrations in Fig. 6 were calculated based on the parameters listed in Table 1. Under the present experimental condition we used relatively low alcohol concentrations (molar fractions of alcohols used were below ~0.08). Therefore, in Fig. 6 we ignored the changes of the excess and partial volumes alcohol-water binary solvents [26-28] depending on alcohol concentrations and species, and also ignored the change of the specific volume of ganglioside molecule (G_{M1}:G_{D1}=1:1). As shown in Fig.6, with increasing alcohol concentration the excess scattering density of ganglioside molecule to solvent increases.

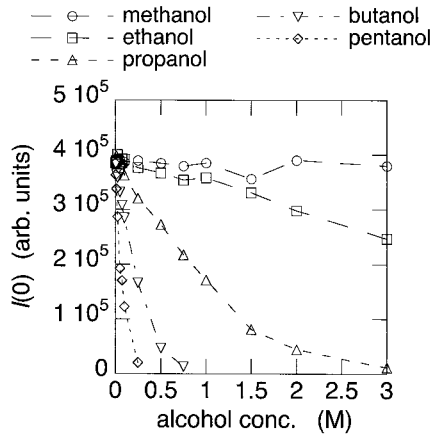

Fig. 5. Zero-angle scattering $I(0)$ depending on alcohol concentration, which was obtained by the extrapolation of the Guinier equation. $I(0)$ values are normalized by the expected changes of the contrasts depending on alcohol concentrations obtained from Fig. 6.

	specific gravity	scattering amplitude per molecule (cm)	scattering density (cm^{-2}) ($x10^{10}$)
Methanol	0.789	5.06×10^{-12}	0.751
Ethanol	0.790	7.31×10^{-12}	0.756
Propanol	0.804	9.56×10^{-12}	0.771
Butanol	0.810	11.81×10^{-12}	0.778
Pentanol	0.814	14.06×10^{-12}	0.783
water	1	2.81×10^{-12}	0.940

Table 1. Parameters used for the estimation of the change of the contrast of ganglioside molecule in alcohol-water binary solvents. The values of specific gravity are from Friedman and Scheraga [26].

For mono-disperse micellar solutions we can rewrite $I(0)$ as

$$I(0) \propto n(\bar{\rho}v_0 N_a)^2 = (\bar{\rho}v_0)^2 N_a (N - N_{cmc}). \quad (5)$$

where N_a, N, N_{cmc} and v_0 are the aggregation number of the micelle, the total number of ganglioside molecules, the critical micellar number concentration and the volume per ganglioside molecule. Here, $v = v_0 N_a$, and $nN_a = N - N_{cmc}$. The contribution of the monomers of ganglioside molecules to the scattering intensity at small q region should be small enough to be ignored since the monomers are much smaller than their micellar aggregates. In addition N = (2.9×10^{-3}) x n_a is constant under the present experimental condition and the reported value of N_{cmc} in water solvents is in the range of 10^{-5}-10^{-5} x n_a [29, 30] (n_a, Avogadro number). Therefore, if N_{cmc} was independent of alcohol concentration and species, $I(0)$ value would be turned to be directly proportional to N_a value. However, the decreases of $I(0)$ in Fig. 5 are much significant compared to those of R_g in Fig. 4 and to the shifts of $p(r)_{max}$ in Fig. 3. When the micelle structure is simplified as a hard sphere, N_a is mostly proportional to R_g^3. Fig. 7 shows the relation between $I(0)/R_g^3$ and alcohol concentration, where the $I(0)/R_g^3$ values are normalized by that at 0 M alcohol. In the case of methanol- or ethanol-water binary solvent the $I(0)/R_g^3$ is mostly constant in the wide range of alcohol concentration, suggesting that the $I(0)$ is proportional to the R_g^3 value. On the other hand, from butanol to pentanol the deviation from such proportionality becomes significant. Around 10 % deviation from 1 in the $I(0)/R_g^3$ value starts from 2 M for ethanol, ~0.75 M for ethanol, ~0.25 M for propanol, ~0.05 M for butanol, and ~0.01 M for pentanol, respectively. Thus, the deviation from 1 in Fig. 7 would be attributable to the increase of N_{cmc}. Namely, the significant decrease of $I(0)$ shown in Fig. 5 results both from the decrease of the aggregation number and from the elevation of the critical micelle concentration of gangliosides.

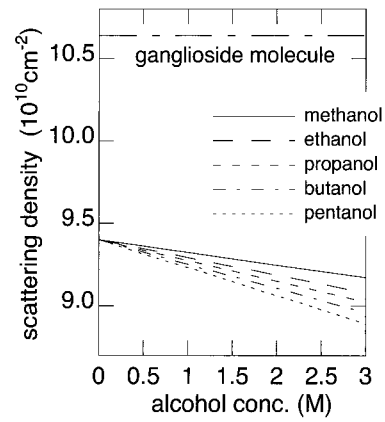

Fig. 6. Variation of the scattering density of alcohol-water binary solvent depending on alcohol concentration. The value of ganglioside molecule without hydration is also shown.

4. CONCLUSION

As shown in the above, in spite of relatively low molar fractions of alcohol in the present experiments (0-0.072 for methanol, 0-0.076 for ethanol, 0-0.080 for propanol, 0-0.024 for butanol, and 0-0.0056 pentanol, respectively), the present results indicate that alcohols not only induce the dehydration and shrinkage of hydrophilic oligosaccharide chain regions of ganglioside aggregates but also affect the solubility of gangliosides in solvents. In addition the increase of hydrocarbon chain length of alcohol drastically reduces the size of ganglioside aggregate, especially for butanol and pentanol. It is clear that the above difference in the effects of alcohols on ganglioside aggregates is attributable to molecular

characteristics of hydrophobic and hydrophilic moieties both in ganglioside and alcohol molecules.

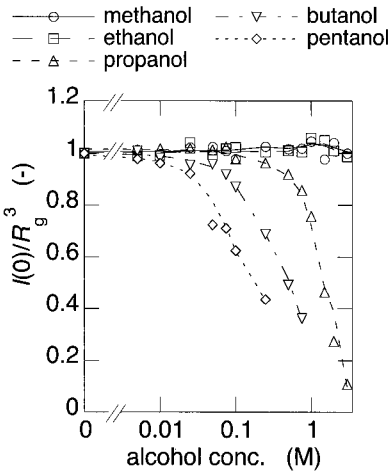

Fig. 7. Alcohol concentration dependence of zero-angle scattering $I(0)$ normalized by R_g^3, which was obtained from depending on , which was obtained by Figs. 4 and 5.

Thermodynamics and physicochemical properties of alcohol-water solvents are themselves of interest in many fields of inquiry and have been studies for a long time. On the other hand, from the point of view of the relation between the physicochemical property of water and its biological function, effects of alcohols on proteins and membranes have been also studies intensively by using various spectroscopic methods such as FTIR and NMR [31, 32]. The mechanism of the effects of alcohols on biological functions was discussed traditionally from the point of view of alcohol penetration into lipid bilayer to induce some changes in membrane fluidity. From 1990s alcohol-induced dehydration has been considered to be one of the key factors of alcohol effect for biological functions due to the bipolar nature of alcohols [31, 33, 34]. In other words alcohols are assumed to be able to affect biological functions through both hydrophobic and hydrophilic properties of themselves.

Thus, as we have shown in the above results so far, the present findings on the effects of alcohols on ganglioside micelles agree well with the previous results [33-35]. In addition the differences in the effects alcohols depending on their hydrocarbon chain lengths indicate that the aggregative property of gangliosides is sensitive to the competitive hydrophobic and hydrophilic interactions between ganglioside and alcohol molecules. Such an intrinsic characteristic of ganglioside molecules against alcohols might correlate with physiological effects of alcohols through lipid rafts.

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