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Calorimetric and small angle X-ray and neutron scattering studies of mixing gangliosides and methylated albumin[1]

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Abstract

We measured the mixing enthalpies of gangliosides with methylated albumin in aqueous solutions at various concentration ratios, and carried out X-ray, neutron scattering experiments for the mixed solutions at nearly the same concentration ratios. The mixing heats were positive when the mixing ratios of lipid to protein inclined to either side, and negative at the intermediate range of the mixing ratio corresponding to the formation of interfacial fluff in a binary solution reported by Hayashi. The X-ray and neutron scattering analyses of the mixed solutions showed that addition of methylated albumin induces disintegration of the micelle structure of gangliosides and patchlike binding of ganglioside molecules to the albumin molecule appears. The analyses also showed that origomeric particles of the lipoproteins are formed at the intermediate range of the mixing ratios.

Keywords: Mixing heat; Ganglioside; Methylated albumin; X-Ray; Neutron scattering

1. Introduction

Gangliosides are sialic acid containing glycosphingolipid found in vertebrate cell membranes, especially rich in nerves [1–2]. Gangliosides appear to play important roles in a variety of events such as cell–cell recognition, cell contact inhibition of growth and transmission of biochemical signals into cells. Gangliosides are strong amphiphilic substance. A hydrophobic ceramide moiety is inserted into the interior of cell membranes.

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On the other hand, hydrophilic oligosaccharide residues protrude from the outer cell membranes, playing the role of a molecular sensor in the transfer of various chemical signals [3–8].

Ganglioside molecules do not form lipid bilayers, although they have two long hydrophobic chains, and disperse in water as micelles owing to the large hydrophilic head groups. Recently, Sonnino et al. reviewed the critical micelle concentrations (CMCs) of gangliosides, and they concluded the CMCs of GMB, GM2, GM1, GD1a, GT1b are in the range of 3.4×10^{-9} to 3.9×10^{-8}, and the CMCs of natural gangliosides are in the same order of magnitude [9–10].

Gangliosides interact with soluble proteins such as serum albumin. Previously, Hayashi and Katagiri [11] found an interesting phenomenon that the solubility of ganglioside to a biphasic solvent changes drastically with the addition of methylated albumin, which was used as model chemicals of cationic proteins in synaptic membranes. Gangliosides precipitate at an interfacial region between the upper hydrophilic phase and the lower hydrophobic phase by addition of a critical amount of methylated albumin.

In this study we measured the heat of complex formation of ganglioside with methylated albumin, and analyzed the structures of the complexes in solutions by using neutron and synchrotron radiation X-ray scattering methods.

2. Experimental

Crude gangliosides were extracted with hot chloroform/methanol from an acetone/ethanol/light petroleum extracted residue of cephaline [12]. The crude ganglioside in chloroform/methanol was applied to a silicic acid column, and washed by chloroform/methanol/2.5 M NH_4OH. Di-sialogangliosides (G_D) were obtained by stepwise elusion by a modification of Svennerholm's method [13]. The fraction used in the experiment showed one spot of G_D on thin-layer chromatography. The fractionated sample was dialyzed against distilled water several times and lyophilized. The lyophilized ganglioside powder was dissolved in distilled water or in Hepes buffer (pH 7) and served for the experiments. We used crude gangliosides in the scattering measurements, because a large amount of sample was needed in the measurements. The crude sample showed two strong spots on TLCP indicating that the major constituents were G_M and G_D. Methylated albumin (methyl ester of bovine albumin) [14] and HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) were supplied from Sigma Chemical Co.

The turbidity of the solutions was measured by a sensitive turbidity meter which detects scattered light intersecting at right angles to the incident light path.

The heat of mixing in aqueous solution of ganglioside with methylated albumin in aqueous solution was measured using Tokyo-Riko's twin type heat conduction microcalorimeter [15]. The two solutions were kept separately in the two compartments of a cylindrical glass cell, and the same volumes of distilled water were held in the two compartments of the reference glass cell. The solutions in the sample cell and reference cell were mixed by turning the cell upside down. The sensitivity of the calorimeter was higher than $0.16\,\mu V \times \mu W^{-1}$, which enables us to detect the mixing enthalpies arising from a sample less than 1 mg in dry weight. Measurements of the mixing heats were car-

ried out at 20.0°C using 1 ml solutions in each compartment. The concentration of the solution of gangliosides and methylated albumin was changed from 0.2 to 2.0 mg × ml^{-1}.

X-Ray and neutron scattering experiments on the mixed solutions were carried out by using the small angle X-ray scattering spectrometer (SAXES) at the synchrotron source (PF) [16] and the small and medium angle neutron scattering spectrometer (WINKS) at the pulsed neutron source of KENS in the National Laboratory for High Energy Physics, Tsukuba, Japan [17]. A mixture of light and heavy water with 41% v/v D_2O was used as the solvent for the neutron scattering experiment to match the scattered intensity from albumin.

3. Results

Each of the aqueous solutions of di-sialogangliosides (G_D) and methylated albumin (MA) is transparent. The mixed solution was turbid immediately after mixing, thereafter, transparency of the solution recovered gradually. Fig. 1 shows the recovering process of the transparency of the mixed solutions. The recovery is more rapid in the mixed solution with a higher ratio of MA/G_D as shown in the figure.

Heat production of the mixed solutions of G_D and MA in distilled water is shown for samples with the various mixing ratios of MA/G_D in Fig. 2. At the mixing ratio of MA/G_D = 1/0.1 and 1/0.2, sharp exothermic peaks are observed. The sign of the mixing heat reverses at a mixing ratio between MA/G_D = 1/0.2 and 1/0.25, then broad endothermic peaks appear at the mixing ratios of MA/G_D = 1/0.3, 1/0.5 and 1/1. The sign of

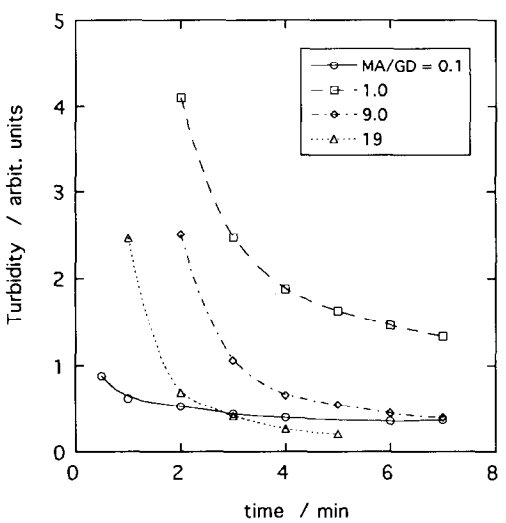

Fig. 1. Recovery of the transparency of the mixed solutions of ganglioside (G_D) and methylated albumin (MA) in distilled water. Weight ratios of MA/G_D are indicated in the figure.

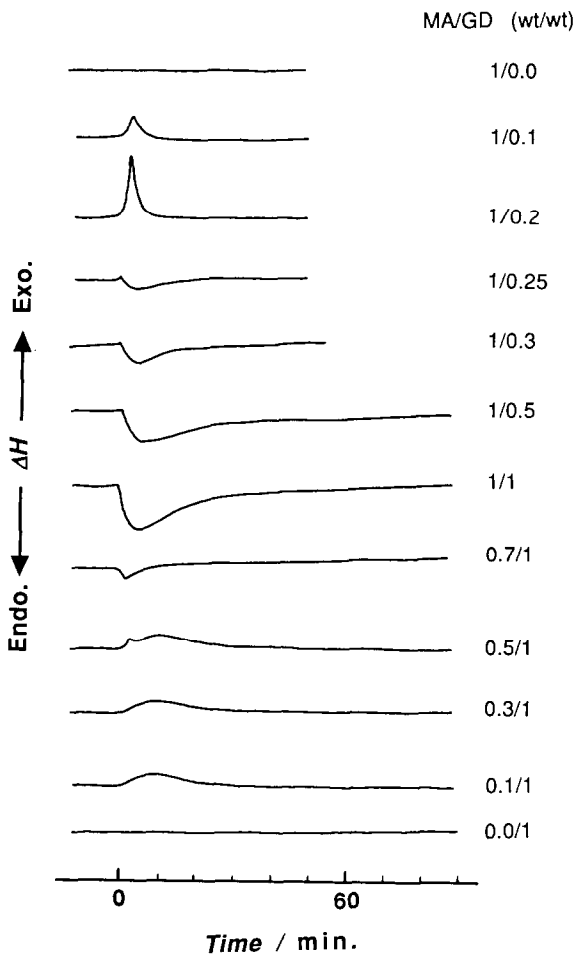

Fig. 2. Heat production of mixing ganglioside (G_D) with methylated albumin in distilled water. Weight ratios of MA/G_D are indicated in the figure. The experimental data of MA/G_D = 1/0.0 and 0.0/1 show the heat of dilution of the aqueous solutions of MA and G_D, respectively.

the mixing heat reverses again nearly at MA/G_D = 0.7/1 and broad exothermic peaks appear at mixing ratios of MA/G_D = 0.5/1–0.1/1.

Nearly the same tendency is observed in mixing heats of G_D and MA in HEPES buffer at pH 7, although sharp exothermic peaks are scarcely observed even at the mixing ratio of MA/G_D = 1/0.1 as shown in Fig. 3. Mixing enthalpies of G_D with MA in distilled water are plotted in Fig. 4 as a function of the mixing ratio of MA/G_D. In Fig. 4, enthalpies of mixing are shown in normalized values against the weight of G_D and MA.

Figs. 5 and 6 show the X-ray and neutron scattering curves of mixed solutions of crude gangliosides and MA in distilled water at various ratios of mixing. The scattered intensities are plotted as a function of momentum transfer, $q = (4\pi \sin \theta)/\lambda$. Here, λ is the wavelength and 2θ is the scattering angle. In neutron scattering experiments, 41% v/v

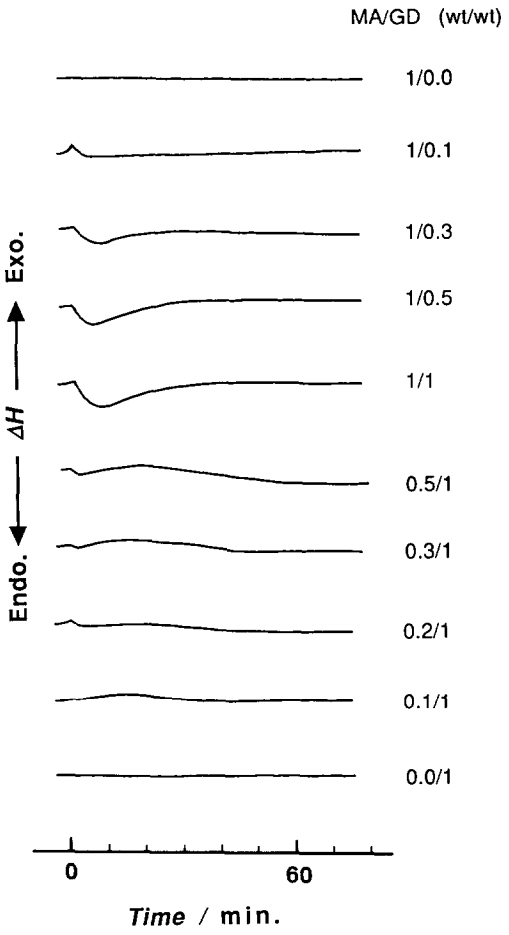

Fig. 3. Heat productions of mixing ganglioside (G_D) with methylated albumin in Hepes buffer (pH = 7.0). Weight ratios of MA/G_D are indicated in the figure. The experimental data of MA/G_D = 1/0.0 and 0.0/1 show the heat of dilution of the aqueous solutions of MA and G_D, respectively.

D_2O was used as a solvent of contrast matching albumin. Therefore, the scattering curve reflects directly the structure of aggregates of gangliosides in solution.

As shown in Fig. 6, the scattering curve changes from a simple bell-shape to the profile with a subsidiary hump (indicated by the arrows in the figure) around $q = 1$ nm^{-1} as the concentration of MA increases. Finally, at the highest concentration of MA, the scattering curve becomes almost flat.

4. Discussion

The purpose of this study was to clarify the structures and processes of formation of

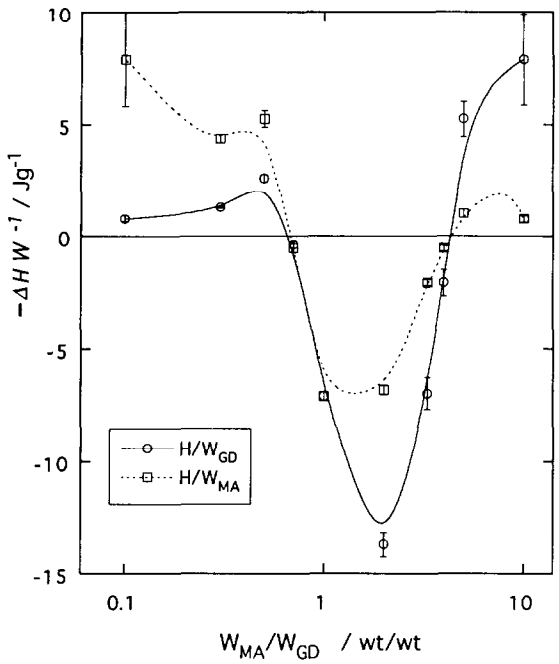

Fig. 4. Mixing enthalpies of ganglioside (G_D) with methylated albumin in distilled water versus mixing ratios Enthalpies are normalized according to the weight of ganglioside (WG_D) or methylated albumin (WMA).

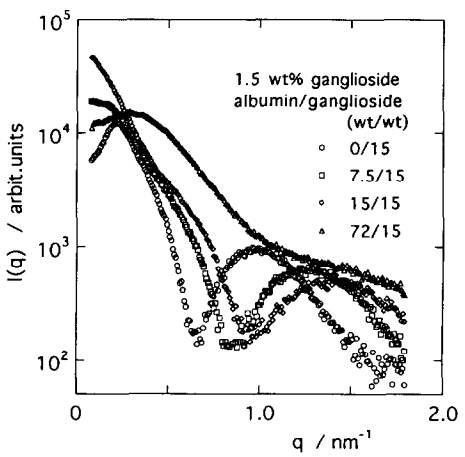

Fig. 5. X-Ray scattering curves of the complexes of gangliosides (G) and methylated albumin (MA) in distilled water. Weight ratios of MA/G are indicated in the figure.

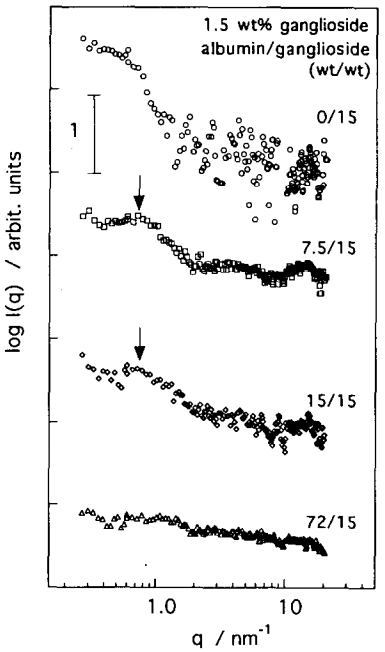

Fig. 6. Neutron scattering intensities of complexes of crude gangliosides (G) and methylated albumin (MA) in distilled water. The humps in the curves are indicated by arrows. Weight ratios of MA/G are indicated in the figure.

the complexes of gangliosides and methylated albumin with special reference to the reported change in the solubility of ganglioside in a biphasic solvent of chloroform/methanol/water with addition of methylated albumin [11]. Previously Tomasi et al. studied the interaction of G_{M1} ganglioside with bovine serum albumin by using absorption and fluorescence properties of the protein chromophores. They showed that the interaction between G_{M1} and albumin was mostly hydrophobic and two complexes were formed; type I had one ganglioside micelle and one polypeptide chain, and type II was a dimer of type I.

Recently Sonnino et. al. reviewed the aggregative properties of gangliosides in solution. They cited their group's result mentioned above and concluded that the complex of G_{M1} and bovine serum albumin involves one micelle of ganglioside and one molecule of albumin. By using serum albumin purified from other sources, on the other hand, they showed that the interaction process of ganglioside albumin was dependent on the structure of the protein [10]. We used methylated albumin whose surface structure seems quite different from native serum albumin.

The mixed solution with methylated albumin is turbid at the initial stage of mixing, although the mixed solution with pure albumin remains transparent. Methylated albumin is a highly basic protein, and we adopt the following assumption and discuss our experimental results.

When the micellar dispersions of gangliosides are mixed with methylated albumin, instantaneous large complexes of ganglioside micelles with methylated albumin may be

formed by the electrostatic attractive forces between negative charges on the micelles of ganglioside and positive charges on the basic protein. At the next stage, however, the ganglioside micelles become unstable by the attachment of positive charges on the molecular surfaces of methylated albumin, and the micelles of gangliosides disintegrate forming new lipoprotein structures.

This assumption explains that the transparency of the mixed solution recovered faster and the sharp exothermic reactions were observed at higher ratio of MA/G_D, because much more positive charge of the methylated albumin molecules interacts with the charges on the micellar surface of a ganglioside molecule at the higher ratio of MA/G_D.

Next, we discuss the structures of dispersions of aqueous solutions of gangliosides and methylated albumin. Hereafter, we use mainly the experimental data without buffer in order to compare the result of the solubility change in a biphasic solvent without buffer reported by Hayashi et al. [11]. In spite of the large difference in size between a micelle of gangliosides and a molecule of methylated albumin, a clear minimum of the scattering intensity is observed for the mixed solution as shown in Fig. 5. The existence of the minimum indicates that the dispersions consist of globular structures with a unique size. The minimum is observed even at the lowest concentration of methylated albumin, showing that the micelles of gangliosides transform to lipoprotein complexes with a characteristic size. The position of the minimum shifts to higher q as the content of methylated albumin increases. At the highest concentration, the scattering curve corresponds nearly to that for methylated albumin.

The humps in the neutron scattering curves at the lipid to protein ratio from 3.75/15 to 15/15 in Fig. 6 indicate the existence of domains of gangliosides which are smaller than the micelles of gangliosides. This means patch-like binding of gangliosides on the molecular surfaces of gangliosides. The peak around $q = 0.2$ nm^{-1} in Fig. 5 is assumed to be due to the interference effect of an electrostatic repulsive interaction between solute particles with anionic surface charges of gangliosides. This peak gradually disappears with increase in methylated albumin.

The tendency of the rapid increase of the scattering intensity below $q = 0.3$ nm^{-1} at a concentration ratio of 15/15 in Fig. 5 indicates the presence of oligomeric particles in the solution.

Finally, we discuss the results of the mixing heat of gangliosides with methylated albumin in aqueous solutions. Mixing heat of gangliosides with methylated albumin in distilled water is exothermic when the concentration ratio inclined to either side of the constituents, and endothermic at the intermediate range of the concentration ratio as shown in Figs. 2 and 3.

Combining the structural information obtained by the analysis of X-ray and neutron scattering from the mixed dispersions of ganglioside and methylated albumin, the exothermic heat comes from the disintegration of micelles and the formation of lipoprotein complexes.

The gradual production of heat at low concentrations of methylated albumin implies that the micelles of gangliosides are relatively stable at the low concentration of methylated albumin, resulting in the slow disintegration of the micelles.

The mixing enthalpies are positive at the intermediate ratio of concentration of the constituents as shown in Fig. 4, and the X-ray scattering pattern suggests the presence of

oligomeric particles at the same ratios of concentration. Therefore, the endothermic reactions are assumed to be caused during the formation of the oligomeric complexes of lipoproteins.

Especially in biological materials, hydrophobic interaction in aqueous solutions is known as one of the important driving forces to construct native structures [18–20]. The hydrophobic agreggative process is endothermic with the increase of entropy of the whole system. The endothermic reactions at these mixing ratios suggest that the oligomeric particles are formed by entropy driven processes by the hydrophobic interactions of tail to tail contacts of patched ganglioside molecules on the surfaces of molecules of methylated albumin.

In Fig. 3, a slow endothermic reaction is observed after the rapid production of heat even at the highest ratio of $MA/G_D = 1/0.1$ in Hepes buffer (pH 7). This suggests that the hydrophobic interaction between the lipoprotein complexes is stronger at neutral pH than at the lower pH of the ganglioside solution in distilled water in Fig. 2.

The concentration ratio at which oligomeric particles are formed in distilled water corresponds to the critical region at which the lipoprotein complex is incorporated into the interface of the biphasic solvent system [11].

In conclusion, micelles of gangliosides are disintegrated by addition of methylated albumin, and monomeric dispersions of lipoproteins with characteristic sizes appear in the solutions. At the intermediate mixing ratios of gangliosides and methylated albumin, the lipoproteins aggregate to form oligomeric particles. The disintegration of the micelles of gangliosides is exothermic, and the formation of oligomeric particles of lipoproteins is endothermic.

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