Reply to Comment on "Thermotropic Structural Change of Disialoganglioside Micelles Studied by Using Synchrotron Radiation Small-Angle X-ray Scattering"

Mitsuhiro Hirai* and Toshiharu Takizawa

Department of Physics, Gunma University, Maebashi 371, Japan

To answer all comments in the preceding paper,[1] it is very important to explain briefly the scheme of our shell-modeling method. In previous papers[2] we fitted an experimental scattering curve with a model scattering function $I(q)$ of a particle composed of n shells with an ellipsoidal shape of rotation as follows.

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

where q is the magnitude of the scattering vector, j_1 is the spherical Bessel function of the first rank, $\bar{\rho}_i$ is the average excess scattering density (so-called contrast) of the ith shell, V_i is the volume of the ith shell; $R_i = r_i(1 + x^2(v_i^2 - 1))^{1/2}$, and r_i and v_i are the semi-axis and semi-axial ratio of ith shell, respectively. $\bar{\rho}_i$, r_i, and v_i were used as fitting parameters. Based on the following consideration given in our previous paper,[2] the modeling analyses were done by using eq 1. By using the chemical structure of the G_{D1} ganglioside molecule and the empirical expression of a hydrocarbon chain volume[3] and the apparent atomic volumes of the basic chemical elements,[4] we tentatively estimated the volumes and the average scattering densities of the head and tail portions of the G_{D1} ganglioside molecule, which were 1525 Å^3 and 1.26×10^{11} cm^{-2} (equivalent to 0.446 electrons/Å^3) for the head and 1029 Å^3 and 8.7×10^{10} cm^{-2} (equivalent to 0.308 electrons/Å^3) for ceramide. Therefore, the contrasts of the head and tail portions of the G_{D1} molecule were expected to be tentatively 3.2×10^{10} cm^{-2} (equivalent to 0.113 electrons/Å^3) and -0.7×10^{10} cm^{-2} (equivalent to -0.025 electrons/Å^3) since the average scattering density of water is 9.4×10^{10} cm^{-2} (equivalent to 0.333 electrons/Å^3). Then we simplified the ganglioside micellar structure as a double-shelled ellipsoid of rotation composed of a shell (hydrophilic region) and a core (hydrophobic region). Experimentally, we measured the relative scattering intensities from the ganglioside micellar solution, and we used eq 1 for profile fittings of the experimental scattering curves calibrated on the relative intensity scale. As is well-known in the small-angle scattering theory in solution,[5] a difference between the average scattering densities of the solute and the solvent contributes to effective scattering intensities. This is also concretely shown in eq 1; that is, eq 1 contains average excess scattering densities (contrasts) of model components (the shell

and the core), not average scattering densities of them. Alternatively, in eq 1 only relative values of contrasts have physical meanings and can change a profile of a model scattering function. Owing to the above reason, that we placed the average scattering density of the solvent to be 1 at the start of the running of our profile-fitting program, means the normalization of contrasts not by the scattering density of the solvent but by an arbitrary scattering density unit. Therefore, we have to revise both the vertical axes of Figure 5b and the description concerning this figure in our previous paper,[2] and we have to alter the expression from "the average scattering densities" to the relative values of contrasts of the shell and core regions by subtracting 1 from the previous values of the scattering densities. Equation 1 given in our profile-fitting program clearly ensures that the above corrections do not affect the other presented structural parameters presented, so we do not have to change all other parts of our previous papers. This alternation of the expression from the average scattering densities to the contrasts has already been done in our most recent paper where we compare characteristics of thermotropic phase behavior in different types of ganglioside micelles by showing the relative values of contrasts of the shell and core regions.[6] Of course, any modeling method applied to scattering data analysis cannot be used or started without considering molecular constraints of scattering objects. Our analyses definitely account for those constraints, and their comments completely misunderstand or disregard the explanations of the shell-modeling method in our previous paper.

In the preceding paper by Cantú et al.,[1] they executed some calculations to strongly assert inconsistencies of our modeling results by using the structural parameters in our previous paper.[2] We showed very well agreement of the scattering curves, the gyration radii, and the distance distributions obtained from our models with those obtained from the experiments, which successfully indicated that the structural parameters obtained from our modeling analyses were very reasonable and consistent. However, to rebute the criticism by Cantú et al.,[1] from their point of view, we will confirm again the reasonableness of our previous results.

According to the above revision of the vertical axes of Figure 5b, the ratio of relative contrasts between the shell and core regions of the model structure of the G_{D1} ganglioside micelle at 6 °C is given to be $(1.42 - 1)/(0.58 - 1) = 0.42/-0.42$. This ratio is greatly different from the tentatively estimated value of 3.2×10^{10} cm$^{-2}/-0.7 \times 10^{10}$ cm$^{-2} = 3.2/-0.7$. As mentioned in our previous paper,[2] we can assume that the micellar hydrophobic core is virtually devoid of internal water as same as other hydrated phospholipid membranes. Then the average scattering density of the hydrophilic shell turns out to be $(-0.7 \times 10^{10}) \times (0.42/-0.42) + 9.4 \times 10^{10} = 10.1 \times 10^{10}$ cm^{-2} (equivalent to 0.358 electrons/Å^3). This value is very reasonable and clearly suggests that a large amount of water is occluded in the hydrophilic shell region of the micelle. In our early paper[7] we also mentioned this fact by using both neutron and X-ray scattering methods with the shell-modeling analysis, where we gave the contrast ratio of $1.1/-1.0$. We can also show high reasonability and consistency of the obtained structural parameters by the estimation of the number n_w of water molecules occluded in the shell region as follows. According to a standard description,[8] the values of contrasts of the shell and core are given as

* Corresponding author. Telefax INT+81 272-20-7552 or 7551; Phone INT+81 272-20-7554; e-mail hirai@sun.aramaki.gunma-u.ac.jp.

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

$$\bar{\rho}_{\text{core}} = \frac{\sum_{\text{cer}}b}{V_{\text{cer}}} - \frac{\sum_{\text{water}}b}{V_{\text{water}}} \qquad (2)$$

where $\sum b$ is the total scattering amplitudes of water molecule, the sugar head, and ceramide portions of the ganglioside molecule, V_{water}, V_{head}, and V_{cer} are the excluded volumes of the above, and n_a is the aggregation number of the micelle. Then the ratio $\Delta_{\text{shell}}/\Delta_{\text{core}}$ of the relative values of contrasts, which is obtained by the shell-modeling analysis, is given by

$$\frac{\Delta_{\text{shell}}}{\Delta_{\text{core}}} = \frac{\bar{\rho}_{\text{shell}}}{\bar{\rho}_{\text{core}}} = \left(\frac{n_w\sum_{\text{water}}b + n_a\sum_{\text{head}}b}{V_{\text{shell}}} - \alpha\right)\bigg/\left(\frac{\sum_{\text{cer}}b}{V_{\text{cer}}} - \alpha\right) \qquad (3)$$

where $\alpha = \sum_{\text{water}}b/V_{\text{water}} = 9.4 \times 10^{10}$ cm^{-2}; $V_{\text{shell}} = n_w V_{\text{water}} + n_a V_{\text{head}}$, $n_a = V_{\text{core}}/V_{\text{cer}}$, and V_{shell} and V_{core} are the volumes of the shell and the core. Therefore the number n_w is given by

$$n_w = \left[\left\{\frac{\Delta_{\text{shell}}}{\Delta_{\text{core}}}\left(\frac{\sum_{\text{cer}}b}{V_{\text{cer}}} - \alpha\right) + \alpha\right\}V_{\text{shell}} - n_a\sum_{\text{head}}b\right]\bigg/\sum_{\text{water}}b \qquad (4)$$

As we know $\sum_{\text{water}}b = 2.81 \times 10^{-12}$ cm, $\sum_{\text{head}}b = 1.92 \times 10^{-10}$ cm for the G_{D1} head, and $\sum_{\text{cer}}b/V_{\text{cer}} = 8.69 \times 10^{10}$ cm^{-2}, we can estimate the number n_w by using eq 4 based on the obtained structural parameters of the G_{D1} ganglioside micelle at 6 °C which are 24.5 and 48.5 Å for the core and shell radii, 1.38 and 1.43 for the semiaxial ratios of the core and shell, and $\Delta_{\text{shell}}/\Delta_{\text{core}} = |0.42/{-}0.42|$. When the aggregation number n_a is given by $V_{\text{core}}/V_{\text{cer}}$, the number n_a of the G_{D1} ganglioside micelle at 6 °C comes out to be $1.38 \times (4\pi \times 24.5^3/3)/1029 = 83$. The value n_w turns out to be 1.56×10^4. Without using the ratio $\Delta_{\text{shell}}/\Delta_{\text{core}}$, we can simply evaluate the value n_w by using another equation.

$$n_w = (V_{\text{shell}} - n_a V_{\text{head}})/V_{\text{water}} \qquad (5)$$

where $V_{\text{water}} = 30$ Å^3. The n_w value at 6 °C estimated by using eq 5 is 1.55×10^4. Both values of n_w obtained by using eqs 4 and 5 are in well agreement, indicating high reasonableness and consistency of the structural parameters reported. As clearly shown in the above, our previous results[2] must not show any presence of highly compressed sugar headgroups or any negative number of water molecules in the hydrophilic shell which are discussed by Cantú et al. as the major critical inconsistencies of our modeling results. Even if their comments are derived from the unsuitable expressions in Figure 5b, they seem to present an awfully meaningless and unphysical discussion. The comment on the distance distribution analysis is not the case for our previous papers since we calculated all distance distributions directly from the experimental and modeling scattering functions independently. They extremely distort our modeling method and results in our previous paper.

Their comment that "the G_{D1} aggregation number 83 given in the above is too low, about one-third of reported throughout in the literature" is not correct, lies so far to their own results, and disregards other previous papers. For example, the aggregation numbers reported by Ulrich-Bott and Wiegandt[8] are 99 for G_{D1a}, 87 for G_{D1b}, and 53 for G_{T1b}. On the other hand, Cantú and Corti et al. reported these values to be 226 for G_{D1a}, 168 for G_{D1b}, and 176 for G_{T1b}.[9] The reported relative molecular

masses of ganglioside micelles are also greatly different. The relative mass of G_{M1} is 280 000−340 000 by Ulrich-Bott and Wiegandt,[8] 532 000 by Corti et al.,[10] and 450 000 by Tomasi et al.[11] Thus, the aggregation numbers reported in previous papers are greatly confused. Even by the same authors, Cantú et al., significantly different values were given for the same species of gangliosides. Namely, the aggregation number of the G_{M2} ganglioside micelle is 529 in one paper,[9] whereas it is 451−261 in the other paper.[12] In our other previous paper,[13] where we treated the pH dependence of micellar structures of different types of gangliosides, we estimated the aggregation number n_a by using the equation of n_a = (total volume of micelle)/(volume of monomer), for example, $1.32 \times (4\pi \times 46.2^3/3)/2554 = 213$ for 0.5% w/v G_{D1a} in 0.07 M phosphate buffer at 25 °C at pH 7.0. This value is on the same order as 226 reported by Cantú et al. In the present case we used the equation n_a = (total volume of core)/(volume of ceramide) and obtained n_a = 83. When we use the standard equation n_a = (total volume of core)/(volume of hydrocarbon chain), $n_a = 1.38 \times (4\pi \times 24.5^3/3)/850 = 100$, which is in good agreement with that of 99 ± 9 reported by Ulrich-Bott and Wiegandt. Thus, estimated values of aggregation numbers depend on solution conditions (pH, temperature, buffer, ionic strength, and so on), on species of gangliosides, and on equations adopted. The third equation is well-known; however, the simple geometrical packing criterion of micelles might be necessary to be modified in a system composed molecules with significantly huge hydrophilic heads such as gangliosides. Anyhow, the aggregation numbers reported[13] does not contradict our previous paper[2] and does not show any inconsistency or unreasonableness of the shell-modeling results.

Their comment of a possibility of an oblate micellar structure is evidently derived from their own previous results; therefore, we cannot but comment on their results briefly. In a recent paper,[12] they showed four X-ray scattering curves and presented some structural parameters of oblate ellipsoidal structures of ganglioside micelles. Although they showed the model scattering functions with the experimental ones in a normal scale plot, readers can recognize a significant deviation between the model and experimental scattering curves in the small q range below 0.05 Å^{-1}. The scattering curve in this range mostly reflects a micellar shape, and as is well-known, scattering functions of prolate and oblate ellipsoidal particles are very different. A consistency of a model structure with experimental data is easily checked by comparing a gyration radius from a model scattering function with that from an experimental one. If Cantú et al. want to insist on an oblate micellar structure, they have to all show agreement in the scattering curve, the distance distribution function, and the radius of gyration of their oblate ellipsoidal model with those obtained from the experimental data. This is very important for readers to confirm the validity of a presented model. Unfortunately, they did not show any agreement. Thus, their comment of an oblate shape is not a common understanding of a ganglioside micellar structure. Under our experimental conditions ganglioside micelles take prolate shapes, not oblate ones. This is ensured by the consistency and reasonableness of our shell-modeling results, which is shown in the above response as well as in our previous paper.

In their last comment they insist a priority of the finding of a peculiar thermotropic behavior of gangliosides including thermal hysteresis, and they cited their paper in the abstracts of Third International Glycobiology Symposium.[14] Our coauthors also reported such a phase behavior of the ganglioside−water

system in detail by using differential scanning calorimetry,[15] which would be earlier than their report.

As explained in the above, there is no doubt that our previous results clearly satisfy the physical constraints of micellar structure quoted by Cantú et al. within the experimental and modeling resolutions. Finally, it is worth noting that the shell-modeling method is a very powerful tool to determine 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 and understanding of the usage of this method.

References and Notes

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