COMMENTS

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

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In interpreting X-ray or neutron scattering results on self-aggregating objects constituted by molecules of known volume, like micelles and vesicles, it is important to realize that electronic densities (or scattering length densities) and radii are not at all independent variables and that improper account of molecular packing can lead to unphysical results.

Micelles consist of an apolar core containing the hydrophobic chains surrounded by a polar shell including hydrophilic headgroups and some solvent molecules. If V_a, V_p, ρ_a, and ρ_p are the micellar apolar and polar volumes and electronic densities, v_{tail} and v_{head} and the tail and headgroup volumes of a single amphiphile molecule, and v_{solv} is the water molecular volume, then, for a micellar aggregation number N, molecular packing, and volume conservation[1,2] set the constraints $V_a = N v_{tail}$ and $V_p = N(v_{head} + h v_{solv})$, with h the number of water molecules per amphiphile included in the outer shell. Besides, v_{tail} and v_{head} cannot be arbitrarily chosen since they have to be consistent with the specific volume of the whole amphiphile, which can be assessed precisely by density measurements.[3]

The above constraints should be carefully taken care of, whatever the chosen procedure in the micellar data interpretation. Mainly two approaches are used. On one hand, a model of the micelle is made, and the theoretical scattering spectrum is calculated to be compared with the measured one. It is then straightforward to account for molecular constraints, as the monomer can be explicitly taken as the building unit of a micelle. On the other hand, the smoothed experimental scattering spectrum is mathematically inverted[4] to give a distance distribution function and a scattering length density profile inside the micelle and hence its geometrical representation. This last procedure does not account for molecular constraints, and the results have to be checked a posteriori for molecular consistency.

The object of this comment is to show that absurd micellar structures are proposed in the paper by Hirai et al.[5] just because molecular constraints are disregarded. Disialoganglioside (GD1) micelles are represented by double-shell prolate ellipsoids of revolution with parameters changing with temperatures. Let us focus on the set of parameters reported for 6 °C. (Similar inconsistencies are common to all other data.) Authors state that (a) hydrophobic core axial ratio $AR_c = 1.38$, and whole micelle axial ratio $AR_t = 1.43$; (b) hydrophobic core minor semiaxis $a_c = 24.5$ Å, and whole micelle minor semiaxis $a_t = 48.5$ Å; (c) average scattering density relative to the solvent of the hydrophobic core = 0.58; (d) average scattering density relative to the solvent of the outer shell = 1.42; (e) average scattering density of the hydrophilic sugar head of the GD1 molecule = 12.6×10^{10} cm^{-2}, equivalent to $\rho_{head} = 0.446$ electrons/Å^3; (f) average scattering density of the hydrophobic double tail, the ceramide, of the GD1 molecule = 8.7×10^{10} cm^{-2}, equivalent to $\rho_{tail} = 0.308$ electrons/Å^3; and (g) average scattering density of the water solvent = 9.4×10^{10} cm^{-2}, equivalent to $\rho_{solv} = 0.333$ electrons/Å^3.

From the number of electrons in the GD1 molecule, $n_{etail} = 317$ of the hydrophobic part (the ceramide) and $n_{ehead} = 681$ of the hydrophilic headgroup, and from the given electron densities, (f) and (e), one calculates the volume of the hydrophobic tail $v_{tail} = 317/0.308 = 1029$ Å^3 and the volume of the hydrophilic head $v_{head} = 661/0.446 = 1480$ Å^3.

Just starting from the author assumptions, without discussing their choices, we want to show the strong internal inconsistency of the results they propose at points (a), (b), (c) and (d). First of all, the average scattering densities data reported in (c) and (d), once converted to electron densities as $\rho_a = 0.58 \times 0.333 = 0.193$ electrons/Å^3 for the hydrophobic core of the micelle and $\rho_p = 1.42 \times 0.333 = 0.473$ electrons/Å^3 for the hydrophilic shell, give an immediate warning about consistency, since the core has a much lower electron density than the one of the single ceramides (0.308 electrons/Å^3, see (f)) of which it is made up, and the sugar headgroups should be highly compressed in the hydrophilic shell of the micelle, to give an electron density higher than the one of the headgroup of the individual GD1 molecule (0.446 electrons/Å^3, see (e)). The number h of water molecules in the hydrophilic shell comes out to be negative (inconceivable!) by solving the usual volume-conservation equation[1] $\rho_p = (n_{ehead} + h n_{esolv})/(v_{head} + h v_{solv})$, which expresses that the polar shell volume is made up of water and headgroups and which reconstructs the average electronic density of the polar shell ρ_p starting from the numbers of electrons and volumes of the polar headgroup and solvent molecules.

Let us now calculate the core and shell volume, V_a and V_p, of the prolate micelles with the dimensions given in (a) and (b): $V_a = (4/3)\pi a_c^3 AR_c = 84\,993$ Å^3 and $V_p = (4/3)\pi(a_t^3 AR_t - a_c^3 AR_c) = 598\,238$ Å^3 . Then, the total number of electrons in the core comes out to be $n_{ecore} = V_a \rho_a = 16\,404$, corresponding to 52 ceramides of 317 electrons each, while the total number of electrons in the shell is $n_{eshell} = V_p \rho_p = 282\,996$, corresponding to 416 headgroups of 681 electrons each. (No water is included in the shell since h is even negative.) If the calculation is carried out on the whole micelle, disregarding the attribution of volumes and electrons to the different parts, one finds that the total volume of the micelle $V = V_p + V_a = 683\,231$ Å^3 contains 300 whole molecules with 681 + 317 = 998 electrons each.

It is hard to imagine how 52 ceramides can associate with 416 headgroups to make a micelle of 300 whole GD1 molecules (strong inconsistency!). The situation becomes even worse if

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one argues that not all of the 300 ceramides are wholly embedded in the core. Then a volume corresponding to (300−52) = 248 ceramides, that is (84993/52) × 248 = 405351 Å^3, has to be included in the outer shell, leaving an extremely small volume for the 300 sugar headgroups in the outer shell, resulting into an absolutely unfeasible density of the sugar moiety (more than twice the one of dry sugar!!)

The authors handle their data with the mathematical inversion method[4] determining an explicit distance distribution function, which, afterward, they fit with a core−shell model with no molecular constraints. Then, it is not surprising that they get inconsistent and even unrealistic values for the micellar physical parameters, as discussed above. In general, one cannot predict the precise values of the physical and geometrical parameters since they are interconnected, but one can establish some reasonable ranges. For instance, the core average electron density should be close to the one of pure hydrocarbons and the shell average electron density should be lower than the one of the sugar headgroups.

In any case, the inconsistency of the micellar parameters reported by the authors still remains even considering only the geometrical parameters, without considering the electronic densities data. With a simple geometrical calculation, one realizes that the proposed micellar core hosts 83 ceramides of 1029 Å^3 each. According to the commonly accepted micellar picture, this means that the aggregation number of the whole micelle is of the order of 80, which is too low, about one-third of the GD1 aggregation number reported throughout in the literature, including papers by the authors themselves.[6] This is a direct consequence of the wrong assumption of a prolate micellar shape instead of the oblate one, a mistake the authors could have avoided if they had looked at the absolute scattered intensities. Furthermore, in a double-shell ellipsoidal model for a micelle, either oblate or prolate, one should satisfy the geometrical condition that the external axial ratio is smaller than the internal one (contrary to what claimed for GD1, see (a)).

The evident and nonnegligible problems of internal consistency clearly prevent from attributing reliability to the results and to the picture the authors give about the thermotropic behavior of ganglioside micelles (which, by the way, are known to be oblate and not prolate in shape[7]!).

Indeed, ganglioside micelles do exhibit a peculiar and very interesting thermotropic behavior, including thermal hysteresis and bistability which have been first accidentally encountered[4] and then widely and deeply investigated with both light and X-ray scattering techniques. A rather detailed landscape of the results, including the comparison among different gangliosides together with a quite complete theoretical interpretation of the observed behavior in terms of a cooperative conformational transition of the ganglioside headgroups, has already appeared.[8−11]

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