Letter to the Editor

Consistency of Microstructural Modeling of Micelles: Letter Concerning "Thermotropic Behavior and Stability of Monosialoganglioside Micelles in Aqueous Solution"

Electronic densities and radii of self-aggregating objects, like micelles and vesicles, constituted by molecules of known volume, are not at all independent variables. In interpreting scattering results on binary or pseudobinary surfactant systems, this property should always be used. Improper account of the packing of molecules can lead to unphysical results, and wild use of the inverse Fourier transform method, independent of physical constraints, may produce meaningless results, as we show on the specific example of a published paper regarding gangliosides: "Thermotropic behavior and stability of monosialoganglioside micelles in aqueous solution," which appeared in *Biophysical Journal* (1996) 70:1761–1768.

Micelles are made of self-assembled amphiphilic molecules. The most accepted model for their microstructure (Tanford, 1980) consists of an apolar core containing the hydrophobic chains surrounded by a polar shell including hydrophilic headgroups and some solvent molecules. No solvent penetration of the apolar core is allowed, except for core surface roughness. The composition of the solvent outside the polar shell is the bulk composition.

Let us call V_a and V_p the apolar and polar volumes of a micelle of any shape, and ρ_a and ρ_p the apolar and polar scattering length densities, equivalent to electronic densities in the case of X-rays (Stuhrmann, 1978). In addition, let us call v_{tail} and v_{head} the tail and headgroup volumes of a single amphiphile molecule and v_{solv} the volume of a water molecule. For any possible aggregation number N of the micelle, the molecular packing for given molecular volumes requires

$$V_a = N*v_{tail} \quad \text{and} \quad V_p = N*(v_{head} + h*v_{solv})$$

where h is the number of water molecules per amphiphile molecule included in the outer shell, not necessarily bound energetically.

These statements concerning the conservation of volumes, although rather obvious, have nevertheless been explicitly pointed out in the literature by Hayter and Penfold (1983). Furthermore, even if v_{tail} and v_{head} cannot be measured separately, they cannot be arbitrarily chosen, because they have to be consistent with the specific volume of the whole amphiphilic molecule, which can be assessed precisely by density measurements on the micellar solution. In addition, some reasonable guess about the molecular apolar volume can often be made, for example after Tanford (1980) or Small (1986).

The above statements should be taken carefully into consideration, whatever the chosen procedure in the interpretation of data regarding micellar solutions. Two approaches are mainly used in the literature. On one hand, a model of the micelle is made and the theoretical scattering spectrum is calculated to be compared with the measured one. In this case it is rather 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 to give a distance distribution function, a scattering length density profile inside the micelle, and then a geometrical representation of it. This last procedure does not account for molecular constraints, and the results have then to be checked a posteriori for molecular consistency. A detailed comparison between the results obtained according to the two guidelines for the interpretation of scattering data, taking into account the molecular constraints, has recently been made on small micelles of a double-chain surfactant (Arleth, 1997).

The above considerations can be put in other words by saying that, in any modeling of micellar shape and mass, the choice of a set of independently adjustable parameters has to be made in such a way that they are truly independent from each other, and not connected via molecular constraints. For example, aggregation number N and included water molecules h are suitable to be chosen, whereas radii and densities are not, as they cannot be assumed as independent parameters, once the reasonable and widely agreed-upon guidelines for the autoaggregation of amphiphiles, described at the beginning, have been accepted.

We show by the following example that absurd structures can be proposed if an unsuitable choice of adjustable parameters is made, without considering molecular constraints.

In the paper by Hirai et al. (1996), a GM1 micelle is proposed to be reproduced by a double-shell prolate (rodlike) ellipsoid of revolution. Different sets of physicochemical parameters are given at different temperatures and conditions. As an example, let us consider the set at 6°C:

1. hydrophobic core minor semiaxis a_c = 26.7 Å; whole micelle minor semiaxis a_t = 47.5 Å;
2. hydrophobic core axial ratio AR_c = 1.63; whole micelle axial ratio AR_t = 1.53;
3. average scattering density relative to the solvent of the hydrophobic core = 0.573;

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4. average scattering density relative to the solvent of the outer shell = 1.56.

In addition, the following values are used:

5. average scattering density of the hydrophilic head of the GM1 molecule = 12.3×10^{10} cm^{-2}, equivalent to ρ_{head} = 0.435 electrons/Å^3;
6. average scattering density of the hydrophobic tail, the ceramide, of the GM1 molecule = 8.7×10^{10} cm^{-2}, equivalent to ρ_{tail} = 0.308 electrons/Å^3;
7. average scattering density of the water solvent = 9.4×10^{10} cm^{-2}, equivalent to ρ_{solv} = 0.333 electrons/Å^3.

Some values are known from the chemistry of the GM1 molecule:

8. number of electrons of the hydrophobic part, the ceramide, n_{etail} = 317;
9. number of electrons of the hydrophilic headgroup, n_{ehead} = 528.

Some values used by the authors, not explicitly mentioned, can then be inferred after the ones they quote:

10. volume of the hydrophobic moiety of GM1, from 6 and 8, v_{tail} = 317/0.308 = 1029 Å^3;
11. volume of the hydrophilic moiety of GM1, from 5 and 9, v_{head} = 528/0.435 = 1213 Å^3.

Without going into the details of the choices of the authors, we wish to show the internal inconsistency of their results, points 1–4, starting from their own assumptions.

First of all, the average scattering densities relative to the solvent recalled in points 3 and 4 can be expressed in terms of electron densities to give $\rho_a = 0.573 \times 0.333 = 0.191$ electrons/Å^3 for the hydrophobic core of the micelle and $\rho_p = 1.56 \times 0.333 = 0.519$ electrons/Å^3 for the hydrophilic shell.

At a glance, these values should give a warning about consistency, as the following observations can readily be made:

- A core of such very very low electron density (and density, of course) should be built up by ceramides, which have the much higher electron density of 0.308 electrons/Å^3 (see 6).
- Nothing but highly compressed sugar headgroups should be allowed to participate to the hydrophilic shell of the micelle, not even a few water molecules, to have an electron density that is already significatively higher than the one quoted in 5 for the headgroup of the individual GM1 molecule (0.435 electrons/Å^3).

In any event, let us assume that the quoted densities are right and try to draw the microstructure of the GM1 micelle. As already noticed, in this case no water is allowed into the hydrophilic shell, which is usually determined by solving the following equation:

$$\rho_p = (n_{ehead} + h*n_{esolv})/(v_{head} + h*v_{solv})$$

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. In the present case h comes out to be negative, as, astonishingly, the determined ρ_p is higher than the one of the headgroup of the individual GM1 molecule.

The quoted geometrical dimensions of the GM1 micelles recalled in 1 and 2 allow us to calculate the volumes of the core V_a and the shell V_p according to the prolate ellipsoidal shape:

$$V_a = (4/3)*\pi*a_c^3*AR_c = 129{,}960 \text{ Å}^3$$

and

$$V_p = (4/3)*\pi*(a_t^3*AR_t - a_c^3*AR_c) = 556{,}888 \text{ Å}^3$$

The total number of electrons in the core is $n_{ecore} = V_a * \rho_a$ = 24,692, corresponding to 78 ceramides of 317 electrons each, whereas the total number of electrons in the shell is $n_{eshell} = V_p * \rho_p$ = 289,025, corresponding to 547 headgroups of 528 electrons each. If the calculation is carried out on the whole micelle, disregarding the attribution of volumes and electrons to the hydrophobic or hydrophilic parts of the GM1 molecules, one finds that the total volume of the micelle, $V = V_p + V_a$ = 686,848 Å^3, contains 371 whole molecules with 528 + 317 = 845 electrons each.

It is hard to imagine how 78 ceramides can associate with 547 headgroups to make a micelle of 371 whole GM1 molecules. One could argue that not all 371 ceramides resulting from the global evaluation of the electrons content of the micelle are wholly embedded in the core; then a volume corresponding to (371 − 78) = 293 ceramides, that is, (129,960/78) × 293 = 488,183 Å^3, has to be included in the outer shell, leaving for the sugar headgroups a volume of only (556,888 − 488,183) = 68,705 Å^3, a very shallow place to host 371 headgroups. In addition, the presence of tails in the outer shell would impose a dramatic reduction of its scattering length density, which is against the initial assumptions of the correctness of its value (0.519 electrons/Å^3, which is already too high).

The same kind of internal inconsistencies are found even if the purely geometrical features of the proposed micellar model are considered. In fact, if one compares the molecular volumes recalled in points 10 and 11 with the already calculated volumes of the core and shell of the proposed micelle, one finds that (129,960/1029) = 126 ceramides included in the core combine with (556,888/1213) = 459 headgroups included in the outer shell to form a micelle of ((129,960 + 556,888)/(1029 + 1213)) = 306 whole GM1 molecules, revealing a problem of chemical and physical balance as before, although with different numbers.

No water has been included in this last evaluation, which is, of course, unphysical, but the paper we are dealing with gives no explicit value for the water content of the hydrophilic region of the micelle; as pointed out before, one could at most deduce from the already criticized proposed electron

densities that absolutely no water is assumed to be allowed into the outer shell. Inclusion of water would reduce both the 459 headgroups and the 306 whole molecules, leaving their values unmatched.

One could argue that only 126 GM1 molecules form the micelle, according to the 126 ceramides of the core, so that only 126 headgroups participate in the outer shell, occupying a volume of $(126 \times 1213) = 152{,}838$ Å^3, the remaining $(556{,}888 - 152{,}838) = 404{,}050$ Å^3 being occupied by water molecules, each one taking the well-known volume of 30 Å^3. The number of water molecules comes out to be $(404{,}050/30) = 13{,}468$, that is, 107 for a single GM1 headgroup, which is a notably large amount of water. Of course, the inclusion of water in the outer shell reduces its scattering density to a value significantly lower than the one quoted by the authors. The only way to come out of this trouble would be to assume for only those water molecules which are embedded in the hydrophilic shell an electron density ~70% higher than usual, corresponding to a specific gravity of ~1.7 g/cm^3 and to a molecular volume of less than 18 Å^3, and then to fill up the shell with 180 densest H_2O molecules for each ganglioside headgroup. After all, the authors of the paper we are dealing with should know from the literature that the aggregation number of GM1 micelles is not 126 but more than twice as large, as determined by means of experimental techniques, such as laser light scattering, which are more straightforward than X-ray scattering for micellar mass assessment.

As a result, an unphysical micelle is drawn starting from the physical parameters proposed. Exactly the same inconsistencies are present in the rest of the data sets proposed for ganglioside micelles at different temperatures and for different histories.

Overlooking molecular constraints in the modeling of micelles is not at all new in the literature, giving rise to similar problems of inconsistency (for example, in the modeling of the CTAB micelle proposed by Tabony, 1984), which are avoided by taking into proper account the molecular volumes, as shown by Cabane and Zemb (1985) and by Zemb and Charpin (1985).

Unfortunately, as already said, the inverse Fourier transform method (Glatter, 1982), which is additionally used by the authors of the paper we are dealing with, also ignores molecules as building units of micelles, so that it is not a way out of the inconsistency loop. This last method for the mathematical treatment of the scattering results in the absence of interactions carries to the determination of an explicit distance distribution function, and may give directly the value of the maximum chord inside the scattering object. Then, in a second step, this distance distribution function is reproduced, in the case of micelles, with a core-shell model with no molecular constraints. It is not surprising then that, also in this case, users are not prevented from getting inconsistent and even unrealistic values for the micellar physical parameters, such as densities and dimensions, which have at least to be checked a posteriori.

Finally, we should say that extending the significant data to q-values that are as large as possible is always useful, as it enhances resolution. In any event, most small-angle scattering measurements, both x-ray and neutron, ($q < 0.4$ Å^{-1}), do not have better resolution than a CH_2 group size, so that the limit between polar and apolar parts of the micelle is a matter of definition. In addition, it has to be pointed out that geometrically distinct models predict similar shapes for the form factor up to the second and third oscillations. Therefore, the check with molecular packing constraints, when feasible as in the case of micellar systems, is important (Cabane et al., 1985).

Two warnings are then to be given regarding the paper. One is general, and refers to the correct accounting of consistency constraints, which have to stand together with any method for data interpretation. Such a warning is general; that is, it applies to both small-angle x-ray and neutron-scattering data interpretation.

The second warning is specific and refers to the topic of the paper itself. The evident and nonnegligible problems of internal consistency, which have been explicitly and extensively shown to affect the data interpretation, clearly prevent the reader from attributing any reliability to the results and to the picture that has been drawn regarding the thermotropic behavior of ganglioside micelles. In addition, besides some details like the questionable choice of the GM1 hydrophilic volume, it should be underlined that gangliosides have been quite well assessed to form oblate (disklike) rather than prolate (rodlike) micelles, which is obviously effective in the modeling procedure.

Indeed, ganglioside micelles do exhibit a peculiar and very interesting thermotropic behavior, including thermal hysteresis and bistability, which was first accidentally encountered (Cantù et al., 1986; Corti, 1994) and then widely and deeply investigated with both light and x-ray scattering techniques. Quite a detailed landscape of 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, can be gained by looking at the papers by Sonnino et al. (1995), Cantù et al. (1996a, b), and Corti et al. (1996).

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