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Hierarchical Ordering of Sugar Based Amphiphiles

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Colloidal aggregates of amphiphilic molecules provide an example of self-confined liquid crystal, being finite both in extension, nanometric scale, and in dimension, nearly-two dimensional. In fact, although disperse on the macroscopic scale, amphiphiles demix in dilute aqueous solution on the microscopic scale due to their bifunctional nature and form condensed assemblies with a complex internal structure, dictated by the compromise between hydrophobic and hydrophilic requirements. On raising the concentration, these amphiphile-aggregate containing solutions start to develop organised structures on the mesoscale, evidencing a long-range crystalline order, but still preserving the ability to rearrange on the nanoscale giving rise to rich phase diagrams.

We present here some peculiar and unexpected effects observed for aggregates of gangliosides, glycosidic amphiphiles with both an extended hydrophobic portion, a double-tailed ceramide, and a bulky saccharidic headgroup, displaying large conformational adaptability and showing relevant preferential interactions. While giving rise to a highly cooperative structural unit, with a strong conformational coupling between surface and core of the colloid, ganglioside aggregates respond to crowding in a counterintuitive fashion, exploiting their structural metamorphism.

Keywords: gangliosides; self-confined liquid crystals; sugar based amphiphiles

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Gangliosides are anionic glycosphingolipids, see Figure 1, naturally occurring in the outer leaflet of plasma membranes, where they participate in lipid-driven microdomains [1], involved in diverse functional aspects like cell-cell recognition, cell adhesion, transfer of information, cell growth, proliferation and apoptosis. Besides, many structural aspects of these microdomains deserve attention. For example, they are found only on the outer side of the membrane and they are lipid-driven, suggesting that the physico-chemical properties of the lipid matrix identify the microdomain with respect to the environment, both for the structure and the dynamics.

When dissolved in water, gangliosides form complex aggregated structures going from micelles to vesicles, bilayers or locally-bilayers, bicontinuous or lace structures, depending on the specific molecule and on concentration, for a pictorial sketch of the different aggregated structure see Figure 2. As a common feature, they display a pronounced ability to organize themselves on surfaces according to steric or curvature requirements [2–5].

GM1, sketched in Figure 1, has been widely studied and can be used as a paradigm for the general behaviour of gangliosides. In dilute aqueous solution it forms big micelles with a hydrodynamic radius $R_H = 58.4\,\text{Å}$, made up of 300 monomers, disklike in shape with an axial ratio of about 2. Each molecule requires a huge area on the aggregate surface, of the order of $100\,\text{Å}^2$, in order to pack its bulky headgroup,

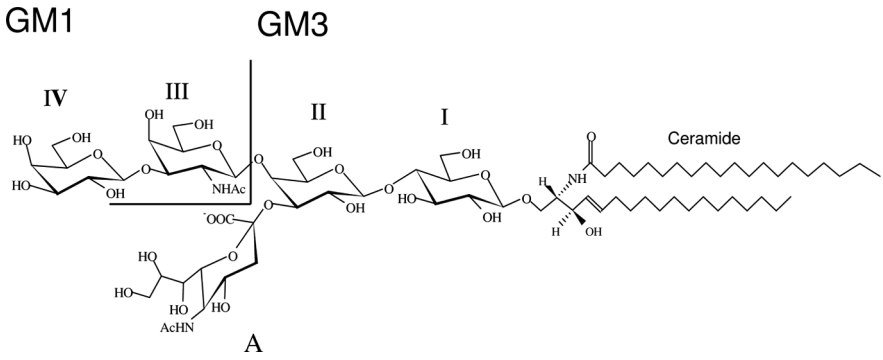

FIGURE 1 Chemical structure of ganglioside GM1 and GM3. The lipid moiety is ceramide (Cer), constituted by a long-chain amino alcohol, sphingosine (Sph), and a fatty acid, connected to each other by an amide linkage. GM1 headgroup has a four-sugar backbone, namely Glc (glucose, I) – Gal (galactose, II) – NeuAc (N-acetylgalactosamine, III) – Gal (galactose, IV), and a sialic acid (N-acetylneuraminic acid, A) branched to the sugar II. GM3 headgroup lacks of III and IV sugar groups.

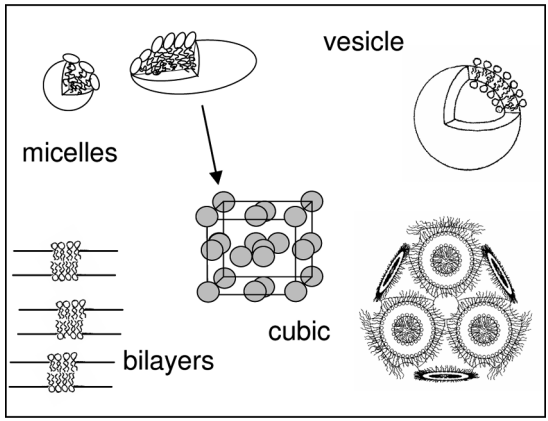

FIGURE 2 Pictorial sketch of the different aggregated structure formed by gangliosides (micelles, vesicles, bilayers). The complex internal structure is characterized by large conformational adaptability and preferential interactions among the bulky saccaridic head groups. On raising the concentration, long range interactions lead to different organised structures on the mesoscale (cubic of micelles, bicontinuous cubic, lamellar phase), but still preserving the ability to rearrange on the nanoscale giving rise to rich phase diagrams.

with a volume of roughly 1000 $Å^3$, having a lateral hindrance larger than that of the hydrophobic chains. It is worth mentioning that all the common lipids of biological interest bear headgroups whose size is much smaller than that of the hydrophobic tails. This is the reason why GM1, although double tailed, forms micelles rather than membrane-type aggregates in dilute solution, headgroups paying a strong contribution to the hydrophobic-hydrophilic balance. According to the well known scheme of Israelachvili [6], gangliosides have a packing parameter close to one half. As a result, GM1 aggregates present a strong steric surface packing, as evidenced by their unusual behaviour as a function of the ionic strength of the solution. In fact, as ganglioside head groups contain a sialic acid residue (Fig. 1), micelles are partially dissociated, bearing a micellar charge of 48 e.u., corresponding to a 16% dissociation [7] Thus, ganglioside micelles undergo electrostatic repulsive inter-particle interactions. In the very dilute region, interactions can be suppressed by increasing the ionic strength of the solution. In response to the addition of salt, micelles of usual ionic amphiphiles, like SDS, increase in size, due to a decrease in the fractional charge of the micelles following counterion adsorption at the micellar surface [8]. Contrary to the general case, addition of salt doesn't change the size and the shape of GM1 micelles.

This property can be appreciated by looking at Figure 3, reporting Small Angle X-ray Scattering (SAXS) spectra obtained on GM1 micelles ($\phi = 0.0008$) in pure water or in 100 mM NaCl. In the non-interacting case, the shape of the spectrum is due to the micelle form factor, corresponding to an interfacial area of 95.4 Å^2. In the absence of added salt, interactions induce a non-random distribution of micelles, showing up with the presence of a structure factor peak at some q-value, corresponding to a positive correlation in the pair distribution function at some distance. It can be seen that the two spectra are superimposable in nearly all the q-range, indicating that micelles keep the same geometry. They only differ in a shallow q region, where the presence of a structure factor peak is appreciable. Moreover, head-group organization stands the increase of interactions due to micellar crowding. In fact, on increasing concentration all over the disordered micellar phase, L1 phase, the GM1 micelle dimension does not change, as well as the aggregation number, from the very dilute region up to 0.25 volume fraction [9], where interparticle distances are smaller than twice the micelle dimension. This is again a peculiar behaviour as compared to SDS, that promptly responds to increasing interactions due to micellar crowding by elongating in bigger micelles, thus reducing the number of micelles and increasing their interparticle distance.

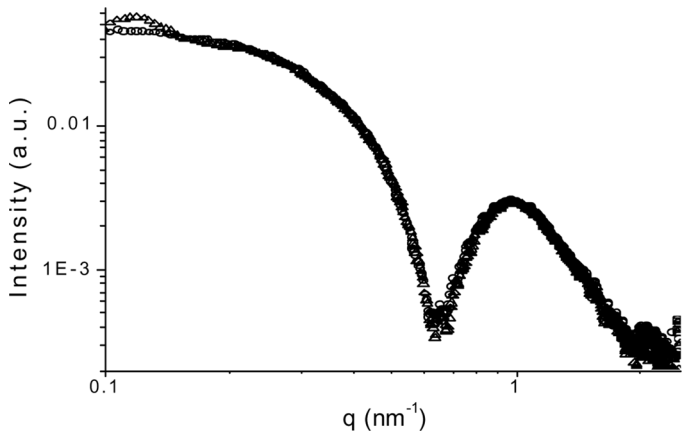

FIGURE 3 Small Angle X-ray Scattering spectra of two GM1 micelle solutions ($\phi = 0.0008$) at different ionic strengths: no added salt (triangles) and 100 mM NaCl (circles). SAXS experiments were performed on the ID02 instrument at the ESRF high-brilliance synchrotron facility (Grenoble, FR) in the range of momentum transfer $q = 0.015$–$0.3\,\text{Å}^{-1}$ (wavelength of the incident beam $\lambda = 1\text{Å}$).

GM1 monomers keep their packing until, at $\phi \sim 0.25$, a mesoscale-liquid-crystal phase is reached. Such a phase (Pm3n) is characterized by a regular array of spheroidal interacting micelles ordered in a cubic lattice (see Fig. 2) [10].

The strong steric packing of gangliosides and their curvature requirements are so demanding that they drive to spatial segregation of different gangliosides in nanoscale mixed aggregates [11], indicating that they can preferentially dislocate even on the scale of a microdomain on the membrane surface, contributing to its structural and dynamic properties and promoting other features like for instance preferential solubility of biomolecules. Nonetheless, their role could be much more complex, as the packing of the ganglioside cluster can be modified.

On heating in the 30°–55°C temperature range, ganglioside micelles undergo an irreversible collective conformational rearrangement resulting in smaller micelles. This peculiar behaviour displays not only at low concentrations [12–14], but extends to the whole L1 phase, where changes on a local scale have a deep effect on the structure and

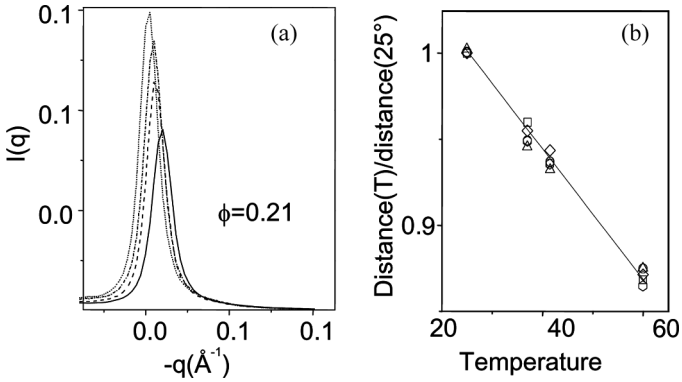

FIGURE 4 Small Angle Neutron Scattering results on GM1 micellar solutions in the range $0.025 < \phi < 0.21$ as a function of equilibration temperature. Panel a: spectra are relative to temperatures of 25 (dots), 35 (dot-dash), 45 (dash) and 60°C (full line), left to right, for a $\phi = 0.21$ GM1 solution. Panel b: relative variation of the characteristic inter-micellar distance, as obtained by SANS spectra for different GM1 solutions, namely $\phi = 0.025$ (squares), 0.116 (triangles), 0.155 (diamonds), 0.21 (exagons). The line represents the linear fit to the data. SANS experiments were performed on the PAXE instrument at LLB Orphèe (Saclay, FR) in the range of momentum transfer = 0.0014–0.15 $Å^{-1}$ ($\lambda = 8$ Å). Ganglioside solutions were prepared in D_2O and put in quartz cells.

ordering over a larger spatial scale. Figure 4 reports results on GM1 micelle solutions at different concentrations as obtained from Small Angle Neutron Scattering (SANS). At different equilibration temperatures, micellar solutions assume different mesoscale arrangement, resulting in a shift in the structure factor peak position, as it is clearly visible in Figure 4a for $\phi = 0.21$ solution. Figure 4b reports the corresponding variation of the characteristic inter micellar distance for different micelles concentration ($\phi = 0.025$, 0.077, 0.116, 0.155, 0.21): it is seen that, as temperature raises, micelles get closer at constant volume fraction, thus a higher number of smaller micelles populates the solution. The fractional variation in the intermicellar characteristic distance, and then in the packing arrangement, is linear in the investigated temperature interval and the same slope is observed at all concentrations. Moreover, very careful SANS experiments as a function of the equilibration time at a given temperature, enable us to follow the kinetics of the micellar structure arrangement after a T-jump (5°C). In Figure 5a the SANS spectra in the region of the structure factor peak are reported for different equilibration times after a T-jump from 25° to 35°C. The peak slightly shifts towards higher q values, as expected for closer and smaller micelles. The micellar rearrangement is slow, as can be seen in Figure 5b, where the time evolution of the inter micellar

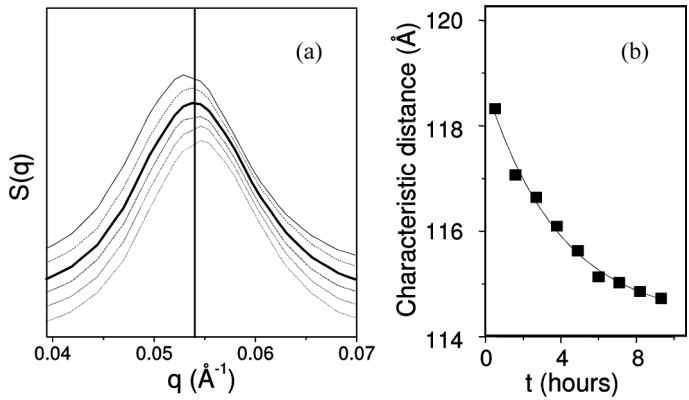

FIGURE 5 GM1 micelle solution ($\phi = 0.21$) after a T-jump from 25°C to 35°C. Panel a: stack presentation of SANS spectra in the q region of the structure factor peak collected at different times, the bold line identifies the spectrum relative to the characteristic-time delay, the vertical line is drawn through the corresponding q-value. Panel b: time evolution of the characteristic inter-micellar distance as obtained from structure factor peak position. The time constant of the exponential decay is $\tau = 3.9$ hours.

distance is reported, together with the exponential fit. The time constant of the exponential decay is 3.9 h, comparable to ganglioside micellar lifetime [11], as expected for amphiphiles with low cmc's, 10^{-8} M for GM1. This result indicates that the new structure is formed through the process of monomer exchange.

The packing of the ganglioside monomers in the aggregate can be switched also by increasing concentration close to the phase boundary between the L1 mesophase (disordered micelles) and Pm3n mesophase (micelles arranged in cubic simmetry), occurring around $\phi = 0.25$. Adopting again a counterintuitive behaviour in response to interparticle interactions, ganglioside micelles suddenly get smaller and easily form the cubic ordered mesophase, in the ϕ-range 0.25–0.4, where small micelles are found [10], rather than the hexagonal mesophase, constituted of elongated micelles, usually adopted by ionic amphiphiles like SDS. At higher concentration, direct bicontinuous cubic mesophases are formed and then lamellar above $\phi = 0.5$, always showing a behaviour related to the spatial arrangement of the heads, giving rise to coexisting mesophases, that are normally encountered in ternary rather than in binary systems [15].

All of the observed behaviours support the idea that the organization of ganglioside amphiphiles within the colloidal aggregate is dictated by their bulky but flexible headgroups. They may assume different conformations, corresponding to different packing densities and geometries on the surface, resulting in different surface coverage ability. Different conformations can be switched collectively from one another, giving rise to a bistable device, that could be activated by an approaching body. It is interesting to notice that this property is maintained till a significative surface dilution with a spacer molecule [16] with the same headgroup of a common membrane amphiphile, with which gangliosides are admixed in microdomains.

Moreover, the surface collective rearrangement causes a response in the hydrophobic core of the aggregate, that is, headgroup and chains conformations of gangliosides is strongly coupled. This hypothesis has been proved by our calorimetry measurements of ganglioside micelles [17]. The data show: a) two different transitions associated to the melting of the headgroups and hydrophobic core, respectively; b) the transition temperature of the tails depends on the molecular arrangement taken by the headgroups. This result is in sharp contrast with the usual thermal behavior of the common lipids in the hydrated lamellar mesophases where only the order-disordered transition associated to the cooperative melting of the tails is observed. This feature can be potentially exploited within the microdomain, in the outside/inside transfer of information process.

The ability of gangliosides to modify the structure of their aggregate by changing the molecular surface area and protrusion, contributing to the energy balance between inter- and intra-aggregate interactions, is particularly intriguing in the case of GM3. GM3 is a tri-saccharide ganglioside, see Figure 1, which plays this metamorphic capability over the single aggregate surface, assuming different packing in different regions of the aggregate. Actually, GM3 has quite an equilibrated hydrophilic-hydrophobic balance, with a preferred molecular packing parameter very close to one half, in the region where discrimination between the world of flat and convex shapes occurs. In the very rich phase diagram of GM3 [18–21] we find structures that are quite non-homogeneous in the surface curvature, such as finite portions of flat lamellas (about 600 nm diameter), spontaneously formed unilamellar vesicles, ribbons with cross-section axial ratio of about two. All of these structures are characterized by the requirement of stabilizing the energy loss on the curved edges of their geometry. Spontaneous vesiculation itself is a rare event in mono-component systems, dealing with the possibility of curving the inner and the outer leaflet of the membrane surface in an asymmetric way without energy cost. This is usually obtained by mixing different components, non homogeneously populating the two leaflets. Figures 6a and 6b consist of Cryo-TEM images relative to GM3 solutions, showing the types of aggregates formed at different GM3 concentration, namely 0.57% and 5.7% volume fraction. Here GM3 carries Na^+ counterion. At low concentration lamellar aggregates are preferentially formed, like the finite portions of flat lamellas (white arrows) and spherical vesicles (black arrow) shown in Figure 6a, coexisting with very few ribbons.

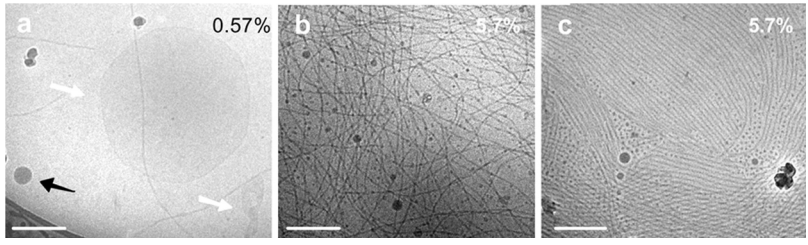

FIGURE 6 Cryo-TEM images of ganglioside GM3 aggregated structures formed at different amphiphile concentrations and with different counterions: (a) $\phi = 0.0057$, Na^+ counterion; (b) $\phi = 0.057$, Na^+ counterion; (c) $\phi = 0.057$, Rb^+ counterion. Cryo-TEM images were obtained on thin ($\sim$5000 Å) aqueous films, vitrified by cooling down the solution to liquid nitrogen temperature [22]. White bars correspond to 200 nm distance.

At higher concentration, as shown in Figure 6b, the flat arrangement is no more preferred, the system evolving toward the strongly curved ribbon structure, that appear to involve a major fraction of the solute and give rise to a disordered network on the mesoscale. This behaviour displayed by GM3 is again opposite to the usual paradigm, telling that aggregates curvature can only decrease as concentration increases. Figure 6c consists of a Cryo-TEM image relative to a GM3 solution at the same concentration of Figure 6b (5.7%) showing the effect of counterion substitution. In fact, here GM3 carries Rb^+ counterion instead of Na^+. Ribbons are still formed but apparently much less flexible and much more interacting, arranging themselves in a highly ordered hexagonal lattice rather than in a disordered network. It is interesting to notice that the degree of order on the mesoscale can be finely tuned by changing a local physical condition of the monomer, like by counterion substitution.

Moreover, the GM3 thermotropic behaviour is rather complex suggesting that, besides the phase behaviour of the lipid chains, spatial rearrangement of the hydrophilic headgroups is involved. In fact, the order parameter of the headgroups region of the ganglioside aggregate couples to that of the hydrophobic chains, passing from a fluid disordered to the solid ordered phase along the melting transition. The GM3 molecule, with a flat headgroup and two long chains, can be geometrically depicted as a disk connected to two cylinders. These markedly different geometries packed into a nanometric aggregate give rise to an intermediate phase between the usual fluid and gel phases [23,24]. This intermediate phase, called solid-disordered, is

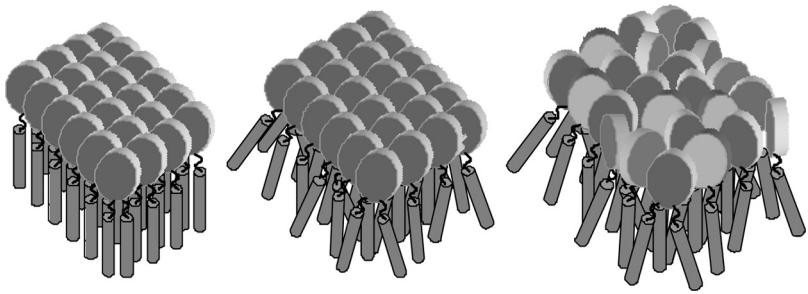

FIGURE 7 Pictorial sketch of the different arrangements assumed by flat headgroups and long chains of GM3 ganglioside molecules inside the aggregate at different temperatures, crossing the corresponding order-disorder transitions. The different geometries are represented by disks and rods. In between the fluid and the gel phases, usually found in lipid lamellae, an intermediate solid-disordered phase is seen.

characterized by an ordered headgroup region overhanging a disordered chain core, as sketched in Figure 7. The ability of this particular ganglioside, GM3, to modulate the geometry and the mechanical properties of the aggregated structure is invoked to explain its central role in tumour progression [1].

The role of gangliosides could then be played on different scales and dimensionalities in real membranes. Surface cooperativity and surface–core coupling could determine the basis of their structural and functional role within microdomains, while their ability to template the mesostructure onto strongly correlated and tunable nanostructures could constitute their contribution to the cell communication with the extracellular world.

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