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## Phase transition at the surface of mixed micelles of the ganglioside GM1 and dodecylphosphocholine

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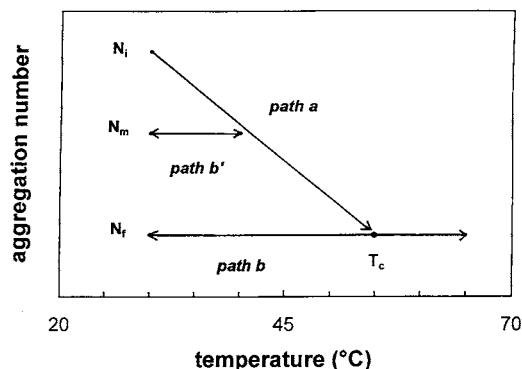
**Abstract.** A temperature-dependent irreversible variation of the average aggregation number of GM1 micelles, with no change in the chemical structure of the molecule, has been observed by light and x-ray scattering. GM1 is an amphiphilic molecule of biological origin, similar to phospholipids but with an extended headgroup, made up of many sugar units. A simple model has been developed to describe the experimental results. It assumes that the polar headgroups of GM1 monomers may exist in two different stable conformations, each of them with a very similar energy, dependent on its own internal structure and displaying preferential interactions with the surrounding heads once inserted in the micelle. The interconversion between the conformational minima is then described as a cooperative transition occurring at the micelle surface, overcoming a naturally emerging barrier due to collective effects. To assess the extent of interactions among the GM1 conformer headgroups different amounts of an amphiphilic spacer have been inserted in GM1 micelles. The thermal hysteresis phenomenon was still observed on mixed micelles until a molar ratio GM1/spacer  $\sim 1/3$  was reached, corresponding to the critical concentration calculated from the mean-field theory of dilute magnetic alloys in a 2D lattice. The observed behaviour, then, appears as a critical phenomenon of topological nature happening in a confined two-dimensional system, that is the micelle surface.

### 1. Introduction

A series of previous papers [1–5] discusses interesting thermal hysteresis effects in ganglioside micelles which have been investigated by light scattering, x-ray and calorimetric techniques. Gangliosides are biological amphiphilic molecules with a double-tail hydrophobic part, like phospholipids, while the headgroup is made up of several sugar units [6]. The typical behaviour for ganglioside micelles is reported in figure 1, where the average aggregation number  $N$  is plotted against temperature. A micellar solution, prepared at room temperature by dissolving the dry ganglioside in aqueous solvent, is heated to temperatures higher than 55 °C: starting from the initial value  $N_i$ , the average aggregation number decreases considerably, following path a. Then, on cooling,  $N$  stays at its lowest value  $N_f$ —path b—and any subsequent heating and cooling procedure follows path b. Irreversible cycles are obtained also for smaller temperature changes, below 55 °C. In fact if the temperature is raised to an intermediate value, say 40 °C, during a first temperature scan, only a partial reduction of the micellar average

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aggregation number occurs, to the value  $N_m$ , a number which stays practically unchanged for any subsequent intermediate cycle throughout which the temperature does not exceed 40 °C, path b' in figure 1.



**Figure 1.** Typical behaviour of the aggregation number  $N$  of ganglioside micelles submitted to the thermal treatment as described in the text. The initial value  $N_i$  is reduced to  $N_f$  after a temperature scan to higher than  $T_c = 55$  °C. For a complete description see the text.

Such hysteresis effects require the existence of—at least—two stable states for the ganglioside micelle. The transition between the two states is progressively induced by a temperature rise, but is irreversible with respect to temperature and does not involve any chemical denaturation of the ganglioside monomers.

According to the existing geometrical models for the aggregation of amphiphiles by imposing the constraint of micellar incompressibility [7], the aggregation number  $N$  is strictly connected to the monomer hindrances. Then, a variation of  $N$  which does not involve chemical denaturation has to be explained by postulating two—or more—internal conformations for the monomer with different surface area, in particular for the bulky saccharidic head group of gangliosides, as in fact suggested by NMR modelling studies [8–10] and also further discussed on the basis of recent calorimetric data [5]. Moreover, the observed thermal hysteresis effects and the presence of a critical temperature—55 °C—at which hysteresis disappears is a clear indication of a strong cooperativity in the conformational transition of the headgroups. This implies that preferential short range interactions occur between similar conformers, which can of course show up once the monomers are close to one another, packed in a micelle-like aggregate or, eventually, in a domain on a membrane, then behaving as a micro-phase.

The described behaviour has been observed for most gangliosides: the general features of figure 1 are the same, in particular the threshold value of 55 °C. In addition, it is quite interesting to notice that, although the extent of the average aggregation number variation is different for different gangliosides, being smaller for smaller micelles, it can nevertheless be attributed to an increase of 4% in average monomer hindrance in all cases [1].

For large micelles, like those containing gangliosides, even such small hindrance changes can result in huge reductions of the average aggregation number. Hence the geometrical variations of the micelle, carefully probed by light scattering, become a powerful tool to follow conformational transitions at the micelle surface which may be induced by temperature variation [1, 3–5] or intermicellar interactions [4].

In order to give further evidence for the cooperative nature of the surface ganglioside headgroup transition, we investigated the effect of the addition of a foreign substance in modulating the hysteretic behaviour. If cooperative, the dilution of the GM1 ganglioside

by an inert amphiphile—dodecylphosphocholine—mixed in the same micelle would cause a decrease of the extent of the aggregation number variations, and then a sudden drop to zero of the hysteretic phenomenon for a critical ganglioside/spacer ratio. This is suggested by the well known behaviour of diluted magnetic alloys where a magnetic atom—or molecule—can assume two orientations—spin-up and spin-down—which depend both on the orientations of the surrounding magnetic atoms and on the distribution of the inert atoms—spacers—around each magnetic atom [11].

## 2. Results and discussion

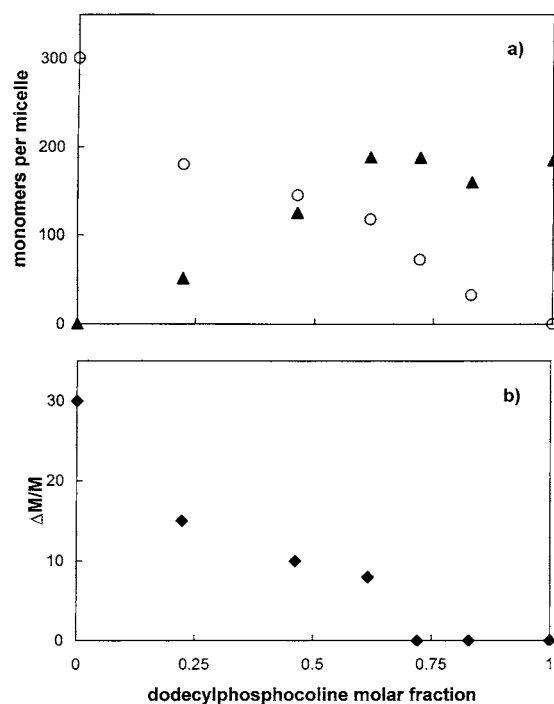
Dodecylphosphocholine is a single tail amphiphile with an headgroup similar to that of phospholipids which instead have a double chained tail. This is one of the reasons because it was chosen as a spacer for the GM1 monomers, as it mimics the more abundant surroundings of ganglioside headgroups on a biological membrane. Moreover, it forms micelles in solution, and mixed micelles with GM1, a structure where the hysteretic behaviour of the geometrical variations is easily seen by light scattering.

2 mM solutions of GM1 and dodecylphosphocholine were prepared. Two-component micelles were obtained by mixing in different appropriate proportions the solutions of the individual amphiphiles. Two-component micelles readily form, before the mixed solution is poured into the scattering cell, according to the quite high cmc of dodecylphosphocholine— $\sim 0.1$  mM. The mixed solutions were then submitted to temperature variations—as in figure 1—and the evolution of the scattered intensity, connected to the average aggregation number, was followed on appropriate timescales until the equilibrium value was attained. In the case of pure GM1 micelles, equilibration times of the order of few hours are needed after each temperature step [2], due to the slow exchange rates of ganglioside monomers, connected to their low cmc— $\sim 10^{-8}$  M. In the case of GM1–dodecylphosphocholine mixed micelles, the observation of still quite long equilibration times indicates that a similar process, involving ganglioside monomer rearrangement, is taking place.

The obtained results are reported in figure 2. In panel (a) the absolute number of GM1—dots—and dodecylphosphocholine—triangles—monomers in the mixed micelles before the thermal treatment are reported as a function of their mole fraction in the micelles themselves. In panel (b) the extent of the variation of the average aggregation number of the mixed micelles due to the thermal treatment is shown.

The most interesting result is the existence of a critical ganglioside/spacer mole ratio above which no variation of the average aggregation number is observed; that is, cooperative phenomena are prevented. It can be noticed that just below and above the critical ratio the aggregation number is not very different, so that the disappearance of the phenomenon is not at all attributable to a loss of sensitivity. Rather, the number of dodecylphosphocholine monomers varies very slowly in that range, while ganglioside headgroups are progressively smoothly diluted on the mixed micelle surface.

As already said, the phenomenon shows some resemblance to the behaviour of diluted magnetic alloys—e.g. Fe–Cr or Fe–Au alloys—where a magnetic atom—or molecule—can assume two orientations—spin-up and spin-down—which depend both on the internal state of the surrounding magnetic atoms as well as on the distribution of the inert atoms—spacers—around each magnetic atom [11]. The system, therefore, can be described by a conservative order parameter given by the concentration of magnetic and inert atoms—their sum must be constant—which is coupled with a non-conservative order parameter given by the fraction of spin-up (or spin-down) magnetic atoms, belonging to model C in the Halperin–Hohenberg classification of cooperative phenomena [12].



**Figure 2.** (a) Absolute number of GM1—dots—and dodecylphosphocholine—triangles—monomers in the mixed micelles before the thermal treatment, reported as a function of their mole fraction in the mixed micelles themselves. (b) Fractional variation of the average molecular weight of the mixed micelles,  $\Delta M/M$ , calculated with respect to the initial value, subsequent to the thermal treatment, reported as a function of the mole fraction of the two components in the mixed micelles. A progressive reduction of the discrepancy between initial and final values can be appreciated, a discrepancy which finally disappears for a ganglioside/spacer mole ratio close to 1/3.

Now we make the following assumptions: (i) strong short range interactions only among pairs of ganglioside molecules belonging to the same internal conformation; (ii) nearly ideal GM1–phospholipid mixing, where the phospholipid acts as an inert solvent [13, 14]. Within these approximations and neglecting finite size effects, the critical ganglioside concentration (expressed in mole ratio ganglioside/spacer) can be calculated from the mean-field theory of dilute magnetic alloys and is given in this approximation as [11]

$$X_C \sim 1/(z - 1) \quad (1)$$

where  $z$  is the coordination number— $z \sim 4$  in a 2D lattice [15]. It is worth noticing that the critical concentration depends only on the coordination number and not on the strength of the cooperative interactions and temperature, indicating that the phenomenon is of topological nature. The critical concentration  $X_C$  is the composition at which two molecules, separated by a great distance on the micelle surfaces, can be connected by roughly a one-dimensional network of interacting molecules, that is,  $X_C$  is a kind of percolation threshold. Above this limit, the action of one ganglioside molecule can propagate to molecules a great distance away. Although the simple equation (1) is based on drastic approximations, the agreement with experimental data is good.

This kind of phenomenon has been already observed also in nematic liquid crystals where even a small amount of added impurities—less than 10%—may destroy the nematic order

by forming an isotropic phase [16] as well as in phospholipid–cholesterol mixed bilayers where the rigid cholesterol—or other rigid molecules—behaves as a spacer which reduces the strong cooperative melting of the phospholipid hydrocarbon chains. At a certain critical cholesterol concentration, the value of which depends on the temperature and lipid chain length, the transition from ordered to disordered conformation of the lipid chain is no longer cooperative [17] as revealed, e.g. by the characteristic broadening and lowering of the specific heat variations against temperature [18, 19].

However, there are a few important differences between ours and previous systems: (a) in the ganglioside–phospholipid system the inert molecule—the phospholipid—reduces a cooperative transition located in the hydrophilic rather than in the hydrophobic part of the amphiphilic assembly; (b) the studied cooperative transition is not reversible at variance with all the previous examples; (c) the transition occurs in a restricted geometry—the aggregation number of ganglioside micelles is of the order of hundreds [1–5]; (d) the cooperative transition is coupled with the geometrical variation of the micelle via the incompressibility constraint allowing for an easy detection by scattering techniques.

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