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G_{M1} GANGLIOSIDE-TRITON X-100 MIXED MICELLES

TRANSITIONS AMONG DIFFERENT MICELLAR SPECIES MONITORED BY PHYSICOCHEMICAL AND ENZYMATIC METHODS

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Summary

Aqueous dispersions of two amphiphiles, G_{M1} ganglioside and Triton X-100, in different proportions, were analysed for some physicochemical properties (surface tension, viscosity, consolution temperature) and for susceptibility to the action of galactose oxidase. By varying the molar ratio between the two components, well defined transitions among different micellar species were recorded by physicochemical measurements. Galactose oxidase was able to recognize the different species of mixed micelles, its kinetics displaying break points which exactly superimposed on those recorded, under the same conditions, by physicochemical methods.

Introduction

Gangliosides are amphiphilic lipids occurring in large amounts in the neuronal plasma membranes [1]. They form, above a certain concentration, micelles of large molecular weight (over 250 000) [2—6]. Whether the tendency of gangliosides to micellize influences the lipid environment of the membranes is not known. It is also uncertain whether or not micellar gangliosides are capable of influencing enzymes and whether enzymes are able to recognize gangliosides in different forms of aggregation. Efforts were made to provide a theoretical approach to these problems [7,8]: however, well defined experi-

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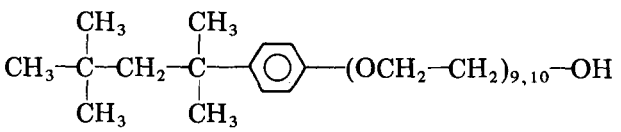
mental models for such studies have not yet been constructed.

Relying on previous experience [9], we worked out a simple model: mixed micelles made up by two amphiphilic substances, G_{M1} ganglioside and Triton X-100, at different proportions. The changes of micellar physicochemical features were checked by viscosity, surface tension and consolution temperature measurements. Galactose oxidase was chosen for relating enzyme action to physicochemical modifications. The enzyme catalyzes the oxidation to aldehyde of the primary alcoholic group on C-6 carried by the galactose residue terminally located in G_{M1} [10]. This slight modification minimizes perturbations of the micellar substrate structure during the enzyme action.

Experimental

Materials

Common chemicals were of analytical grade; solvents were redistilled before use; water was double-distilled in glass apparatus. G_{M1} ganglioside was prepared by sialidase treatment, as described by Ghidoni et al. [11], from a mixture of gangliosides extracted from calf brain by the method of Tettamanti et al. [12]. The purity of the G_{M1} gangliosides obtained, assessed according to Sonnino et al. [13], was over 99%. The lipid composition of G_{M1}, determined by gas-liquid chromatographic methods (see Sonnino et al. [13]) was the following: (a) long chain bases: 2-D-amino-eicosan-1,3-D-diol, 5.4%; 2-D-amino-eicos-4-ene-1,3-D-diol, 54.1%; 2-D-amino-octadecan-1,3-D-diol, 8.1%; 2-D-amino-octadec-4-ene-1,3-D-diol, 32.4%; (b) fatty acids: stearic acid, 98%. Galactose oxidase (EC 1.1.3.9) (from Polyporus circinatus, 98 I. U./mg protein) was purchased from KABI (Stockholm, Sweden); Triton X-100 (the structure of Triton X-100 is the following:



where the average number of oxyethylene units is 9.5) from Rohm and Haas (Philadelphia, PA, U.S.A.); horseradish peroxidase (EC 1.11.1.7) from Boehringer GmbH (Mannheim, F.R.G.); *o*-dianisidine from Merck GmbH (Darmstadt, F.R.G.).

Methods

Preparation of G_{M1} and Triton X-100 solutions. The paper follows the ganglioside nomenclature of Svennerholm [22] and the IUPAC-IUB recommendations. G_{M1} = II^3-NeuAc-GgOse$_4$Cer; Gal(β,1 → 3)GalNAc(β,1 → 4)(NeuAc, α2 → 3)Gal(β,1 → 4)Glc(β,1 → 1′)ceramide. G_{M1} and Triton X-100 were separately dissolved in $CHCl_3/CH_3OH$, 2 : 1 v/v. Known aliquots of each mixture were transferred into a glass tube and the solvent was completely removed by a gentle flow of nitrogen. The residue was dissolved with a proper volume of 25 mM sodium phosphate buffer/5 mM disodium EDTA, pH 7.0 at 37° C, and briefly mixed in a vortex mixer.

Galactose oxidase assay. This assay was carried out according to the coupled

peroxidase-dianisidine method perfected by Ghidoni et al. [14], the only modification being that methanol was substituted by ethanol, which minimizes autoxidation. The buffer used was 25 mM sodium phosphate/5 mM disodium EDTA (pH 7.0) and the incubations performed at 37°C for 10 min.

Detection of oxidized G_{M1}. Oxidized G_{M1} was separated from G_{M1} by thin-layer chromatography using silica gel plates (HPTLC, Merck GmbH), and $CHCl_3/CH_3OH/0.3\%$ aq. $CaCl_2$, (60 : 35 : 8, v/v), (1 h run at 20°C) as solvent. The oxidized form of G_{M1} runs faster than G_{M1}. Oxidized G_{M1}, eluted from the plate and desiccated, was reduced back to G_{M1} by treatment (20°C for 2 h) with 0.1 ml 0.3% $NaBH_4$ in 0.1 M NaOH.

Physicochemical measurements. These were done in the same buffer used for galactose oxidase assay. Viscosity determinations were performed at 37°C with a capillary viscometer apparatus; surface tension determinations were accomplished by the method reported by Shinoda et al. [15]. Consolution temperatures were determined by checking the sudden increase of turbidity occurring at the consolution temperature, both visually and by measuring absorbances at 360 nm [16]; the mixture under examination was heated linearly in order to obtain a temperature rise of 1 K/5 min.

Results

It was confirmed (see Fig. 1) that Triton X-100, in the monomeric form, decreases surface tension, as previously shown [17]; conversely, G_{M1} ganglioside in the concentration range 0.1—2 mM does not cause any measurable change in surface tension. The observed decrease in surface tension (see Fig. 1A), due to monomeric Triton X-100, reaches a minimum at a concentration of 0.3 mM. This corresponds to its critical micellar concentration [17]. In the presence of 0.8 mM G_{M1} (a concentration well in excess of ganglioside critical micellar concentration [5,6]), the decrease of surface tension is less sharp and the minimum value is reached at a concentration of 3 mM Triton X-100. In this system a portion of monomeric Triton X-100 is involved in the formation of mixed G_{M1}-Triton X-100 micelles; therefore the actual concentration of monomeric detergent is lower than that of total detergent. Only at 3 mM does the concentration of monomeric detergent become constant, and the surface tension thus levels at its minimum value.

Pure Triton X-100 solutions exhibit (see Fig. 1B) the lowest value of relative viscosity at 0.3 mM — that is, at the critical micellar concentration — and then the relative viscosity rises by increasing concentration of the detergent (in micellar form). In the presence of 0.8 mM G_{M1} the concentration of Triton X-100 giving the lowest value of relative viscosity shifts from 0.3 to 3 mM, the increase of relative viscosity being thereafter much sharper than in the absence of ganglioside. The relative viscosity of pure G_{M1} solutions remains unchanged in the examined concentration range (0.1—2 mM). The calculated intrinsic viscosity of micellar Triton X-100 is 6.1 cm^3/g, which is consistent with an hydrated, non-spherical micelle [17]. In the presence of 0.8 mM G_{M1}, the intrinsic viscosity value becomes 9.0 cm^3/g, indicating a much higher asymmetry, or hydration, of the particles.

Triton X-100 exhibits the 'consolution' phenomenon, that is the aggregation

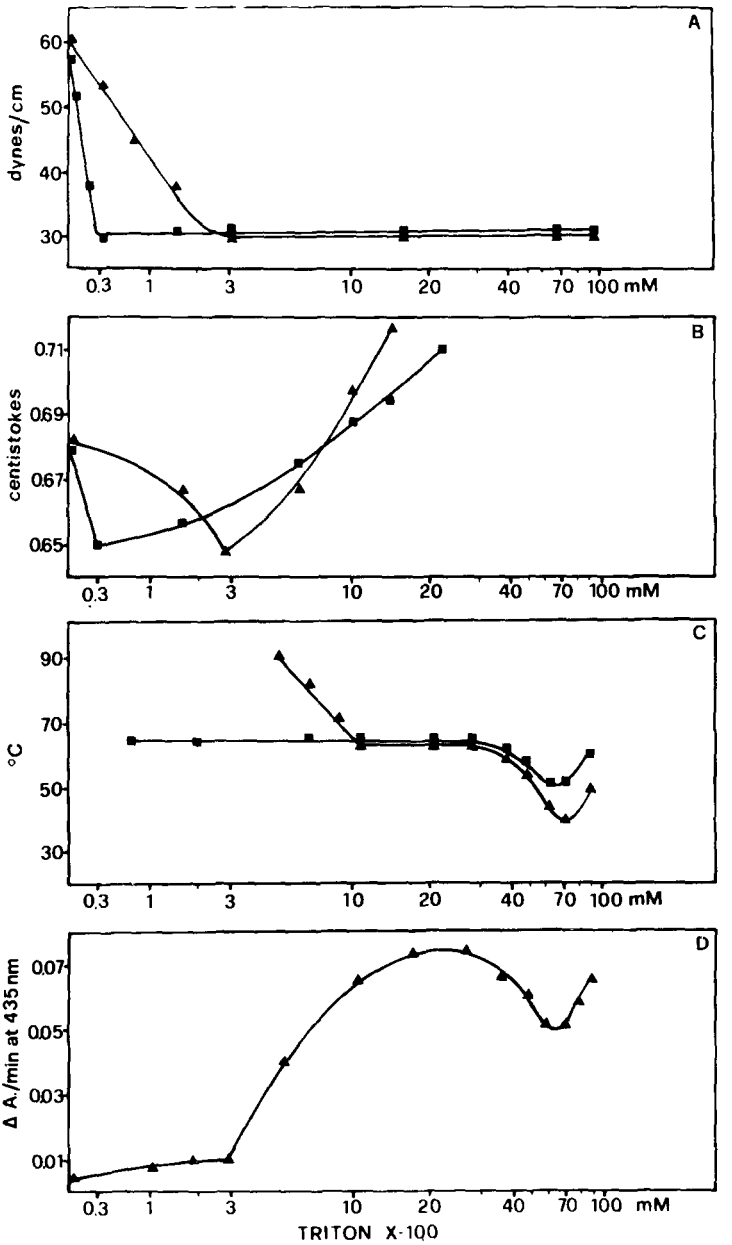


Fig. 1. Behaviour of surface tension (A), relative viscosity (B), consolution temperature (C), and galactose oxidase activity (D), in aqueous solutions of G_{M1} ganglioside (0.8 mM) containing increasing amounts of Triton X-100 (▲———▲). The activity of galactose oxidase is expressed as ΔA at 435 nm (min^{-1} per μg protein). ■———■, absence of G_{M1} ganglioside.

of micelles into large complexes above a certain temperature (consolution temperature). Over this critical temperature two isotropic immiscible phases having different surfactant content are formed [18]. The consolution properties of Triton X-100, studied under our experimental conditions, and in the presence or absence of 0.8 mM G_{M1}, are illustrated in Fig. 1C. Whilst pure

G_{M1} does not show the consolution phenomenon, the presence of G_{M1} markedly influences the consolution properties of Triton X-100. Below 10 mM Triton X-100, the values of consolution temperature increase, whilst between 60 and 70 mM the consolution temperature falls from 54°C to 39°C.

Galactose oxidase works poorly upon homogeneous micelles of G_{M1}, while attacking dispersions of G_{M1} and Triton X-100 [14]. The profile of its activity (see Fig. 1D) versus increasing Triton X-100 and constant (0.8 mM) G_{M1} concentrations follows a triphasic curve, with two well defined breaks. The former occurs at 3 mM, the latter at 66 mM Triton X-100. In the presence of a fixed (23 mM) Triton X-100 concentration the kinetics of the enzyme action versus increasing G_{M1} concentrations (see Fig. 2B) follow biphasic behaviour with a break point at 0.2 mM ganglioside. As shown in Fig. 2A the break point in the enzyme kinetic curve has an equivalent 'break' in the consolution behaviour of the same system.

Surface tension and viscosity measurements at 0.8 mM G_{M1} and increasing Triton X-100 concentrations show a shift in the critical value from 0.3 to 3 mM of the detergent. Also, galactose oxidase action displays a critical increase at 3 mM Triton X-100. As shown by laser light scattering experiments, not reported here, in a system containing 0.8 mM G_{M1} and Triton X-100, most of the Triton X-100 is not free but incorporated in a mixed micelle. Assuming that in the same system the critical micellar concentration, and therefore the maximum monomer concentration, remains 0.3 mM, the composition of the mixed micelle is about 0.8 mM G_{M1} and 2.7 mM Triton X-100. This appears to be a critical ratio; in fact, upon changing G_{M1} concentration, the critical points of surface tension and viscosity minima as well as initiation

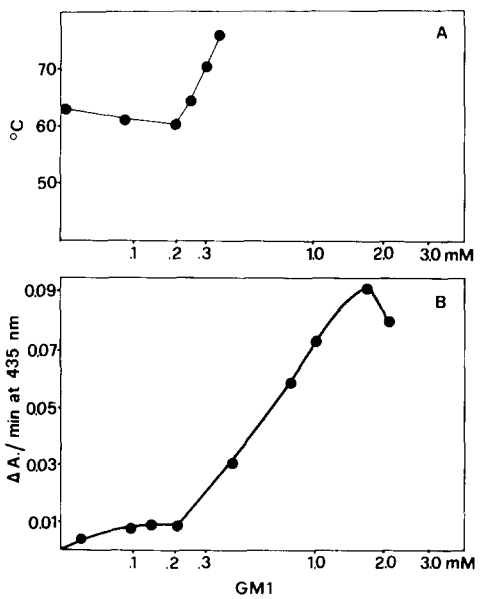


Fig. 2. Behaviour of consolution temperature (A) and of galactose oxidase activity (B) in aqueous solutions of Triton X-100 (23 mM) containing increasing amounts of G_{M1} ganglioside. Galactose oxidase activity: see legend to Fig. 1.

of galactose oxidase action proportionately shift. For instance, with 0.4 and 1.6 mM G_{M1} these values are 1.65 and 5.7 mM Triton X-100, respectively.

In the whole range of experimental conditions used the action of galactose oxidase was accompanied by the formation of oxidized G_{M1} which, after $NaBH_4$ treatment, turned back to G_{M1}. The presence of Triton X-100, in the range of used concentrations, did not significantly influence the action of galactose oxidase, assayed on pure galactose.

Discussion

Laser light scattering studies [19] showed that G_{M1} ganglioside, of the same batch and under the same conditions used in the present work, is present, from 10^{-6} M to 10^{-3} M, as disk-like micelles of M_r about 500 000 [19]. With regards to Triton X-100, it becomes micellar at 0.3 mM [17], and these micelles, reported to be ellipsoid in shape [20], have a M_r of approx. 100 000.

Combinations of soluble amphiphiles such as G_{M1} ganglioside and Triton X-100 are assumed to form mixed micelles [21]. The data presented in this paper provide experimental support for the formation of mixed micelles of G_{M1} ganglioside and Triton X-100 at different molar proportions. The variation of the G_{M1} ganglioside/Triton X-100 molar ratio is accompanied by a physicochemical differentiation which characterizes individual species of mixed micelles. This is suggested by the occurrence of defined points of transition ('breaks') from one species to another. One of these breaks, detected by surface tension determinations, occurs when mixed micelles rich in ganglioside become micelles enriched in Triton X-100. The critical G_{M1} ganglioside/Triton X-100 molar ratio is, in this respect, 1 : 3.25. A second transition, observed by consolution point determinations, corresponds to the formation of mixed micelles very rich in Triton X-100 (G_{M1}/Triton X-100 molar ratio around 1 : 100). These latter micelles display an enhanced tendency to aggregate ('sticky micelles') and exhibit a consolution temperature as low as 39°C. Their increased tendency to aggregate should reflect a particular distribution of G_{M1} molecules on the micelle surface which facilitates mutual adhesion.

These events have a biological correlate; in fact, galactose oxidase, not only distinguishes between homogeneous micelles of G_{M1} and mixed G_{M1}/Triton X-100 micelles, but also recognizes the various species of mixed micelles. This is doubly meaningful. First, the galactose oxidase-ganglioside interactions should be viewed as largely dependent on the surface location and packing of the ganglioside saccharide chains. Secondly, such interactions may, in general, govern the action of the enzymes acting physiologically on gangliosides.

The results of this work are introductory. It seems now necessary: (a) to establish the physicochemical parameters: hydrodynamic radius, M_r, shape, asymmetry, and surface charge distribution of the various G_{M1}/Triton X-100 mixed micelles; (b) to correlate these to kinetic parameters of the enzyme action, determined on each micellar species; (c) to compare the kinetics of the enzyme action on micellar and other artificial and natural surfaces. Studies along these lines, which would contribute to a better knowledge of the enzymology of natural amphiphiles, are already in progress in our laboratory.

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References

1 Wiegandt, H. (1971) Adv. Lipid Res. 9, 249—289
2 Gammack, D. (1963) Biochem. J. 88, 373—383
3 Howard, R. and Burton, R.M. (1964) Biochim. Biophys. Acta 84, 435—440
4 Yohe, H.C. and Rosenberg, A. (1972) Chem. Phys. Lipids 9, 279—294
5 Formisano, S., Johnson, M.L., Lee, G., Aloj, S.M. and Edelhoch, H. (1979) 18, 1119—1124
6 Rauvala, H. (1979) Eur. J. Biochem. 97, 555—564
7 Gatt, S. and Bartfai, T. (1977) Biochim. Biophys. Acta 488, 1—12
8 Gatt, S. and Bartfai, T. (1977) Biochim. Biophys. Acta 488, 13—24
9 Tettamanti, G., Cestaro, B., Lombardo, A., Preti, A., Venerando, G. and Zambotti, V. (1974) Biochim. Biophys. Acta 350, 415—424
10 Suzuki, Y., and Suzuki, K. (1972) J. Lipid Res. 13, 687—690
11 Ghidoni, R., Sonnino, S., Tettamanti, G., Wiegandt, H. and Zambotti, V. (1976) J. Neurochem. 27, 511—515
12 Tettamanti, G., Bonali, F., Marchesini, S. and Zambotti, V. (1973) Biochim. Biophys. Acta 296, 160—170
13 Sonnino, S., Ghidoni, R., Galli, G. and Tettamanti, G. (1978) J. Neurochem. 31, 947—956
14 Ghidoni, R., Tettamanti, G. and Zambotti, V. (1974) Ital. J. Biochem. 23, 320—328
15 Shinoda, K., Hato, M. and Hayashi, T. (1972) J. Phys. Chem. 76, 909—914
16 Lichtenberg, D., Yedgar, S., Cooper, G. and Gatt, S. (1979) Biochemistry 18, 2574—2582
17 Corti, M. and Degiorgio, V. (1975) Opt. Commun. 14, 358—362
18 Eckert, C.A. (1976) Techniques of Chemistry, Vol. VIII, part. II, pp. 1—63, Wiley, New York
19 Corti, M., Degiorgio, V., Ghidoni, R., Sonnino, S., and Tettamanti, G. (1980) Chem. Phys. Lipids 26, 225—238
20 Robson, R.J. and Dennis, E.A. (1977) J. Phys. Chem. 81, 1975—1978
21 Tanford, C. (1973) The Hydrophobic Effect, pp. 81—85, Wiley, New York
22 Svennerholm, L. (1963) J. Neurochem. 10, 613—623