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LACTONIZATION OF GD1b GANGLIOSIDE UNDER ACIDIC CONDITIONS

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ABSTRACT

Gangliosides that contain the disialosyl residue α-Neu5Ac-(2→8)-α-Neu5Ac-(2→3)- can lactonize in the presence of traces of acid and this reaction has been studied in detail on GD1b {β-Gal-(1→3)-β-GalNAc-(1→4)-[α-Neu5Ac-(2→8)-α-Neu5Ac-(2→3)]-β-Gal-(1→4)-β-Glc-(1→1)-Cer}. Lactonization occurs rapidly at a proton–ganglioside molar ratio of <1. At equilibrium, the ratio of GD1b to its lactone is 3:7. The data suggest the possibility that a proton-driven lactonization of gangliosides may occur *in vivo*.

INTRODUCTION

Gangliosides, glycosphingolipids characterized by the presence of sialic acid residues, are components of the external layer of plasma membranes and are abundant in the nervous system where they comprise about one fifth of the lipids of the external layer[1] and where they possibly participate in the process of signal transduction[2]. Gangliosides also occur as lactones[3] in small amounts in the brains of rodents[4,5] and in relatively abundant quantities in some cerebral areas of aged people[3]. Furthermore, small amounts of lactonized gangliosides have been found in melanoma cells[6]. Since the pK_a of ganglioside sialic acid is 2.2–2.5, the carboxyl groups should be dissociated at physiological pH, thus providing the negative charges possibly essential for binding cations[7] and interacting with extra-membrane ligands or intra-membrane components. Therefore, lactonization of gangliosides may serve to modulate the biological functions. Moreover, lactonization makes the oligosaccharide chain more rigid[8–11], which also may have important functional implications[6].

We now report that slight modifications of the H^+ concentration modulates the equilibrium between the disialoganglioside GD1b, and its lactone GD1b-L[3,9] {β-Gal-(1→3)-β-GalNAc-(1→4)-[α-Neu5Ac-(2→8,1→9)-α-Neu5Ac-(2→3)]-β-Gal-(1→4)-β-Glc-(1→1)-Cer}.

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EXPERIMENTAL

Solvents were redistilled before use. Kieselgel 60 plates (Merck) were used for h.p.t.l.c. Gangliosides were extracted from calf brain[12], and GD1b was purified (to 99%) and characterized as described[13]. The sodium salts of mixed gangliosides and of purified GD1b were prepared by dialysis against 0.01M sodium hydroxide and then against several changes of distilled water. [^{3}H]-GD1b, labelled at C-6 of the terminal galactose, was prepared by the galactose oxidase–sodium borotritide procedure[14].

Formation of GD1b-L from GD1b. — Aliquots of a solution of GD1b (200 mg) in 2:1 chloroform–methanol (10 mL) were dried under high vacuum in glass tubes and each residue was dissolved in freshly prepared, carbon-dioxide free, bidistilled water, or 10^{-7}–10^{-3}M HCl (1 mL). The final concentrations of GD1b were in the range from 10^{-5}–10^{-3}M. Samples of GD1b (10^{-7}–10^{-6}M) were prepared using [^{3}H]-GD1b. The solutions were kept in sealed tubes at room temperature for 1 h to 14 days.

Reduction of GD1b-L. — Each of the above solutions was mixed with aqueous sodium borohydride (1.75 mL, 60 mg/mL)[11]. After storage for 10 min at room temperature, each solution was dialyzed, frozen, and lyophilized to give a mixture of GD1b and the GD1b-L reduction product, GD1b-ol, R_{GD1b} 1.6 (t.l.c.; chloroform–methanol–aqueous 0.2% $CaCl_2$, 50:42:11). In control experiments, GD1b-ol was isolated and characterized[15,16].

Experiments on the total ganglioside mixture from calf brain. — The major gangliosides in this mixture are GM1 {β-Gal-(1→3)-β-GalNAc-(1→4)-[α-Neu5Ac-(2→3)]-β-Gal-(1→4)-β-Glc-(1→1)-Cer}, GD1a {α-Neu5Ac-(2→3)-β-Gal-(1→3)-β-GalNAc-(1→4)-[α-Neu5Ac-(2→3)]-β-Gal-(1→4)-β-Glc-(1→1)-Cer}, GD1b {β-Gal-(1→3)-β-GalNAc-(1→4)-[α-Neu5Ac-(2→8)-α-Neu5Ac-(2→3)]-β-Gal-(1→4)-β-Glc-(1→1)-Cer}, GT1b {α-Neu5Ac-(2→3)-β-Gal-(1→3)-β-GalNAc-(1→4)-[α-Neu5Ac-(2→8)-α-Neu5Ac-(2→3)]-β-Gal-(1→4)-β-Glc-(1→1)-Cer}.

T.l.c. — The mixtures of GD1b and GD1b-ol were analyzed, using chloroform–methanol–aqueous 0.2% $CaCl_2$ (50:42:11). The mixture of calf-brain gangliosides, after lactonization and reduction, was analyzed by 2D-t.l.c., using chloroform–methanol–aqueous 0.2% $CaCl_2$ (50:42:11) and chloroform–methanol–aqueous 0.2% $CaCl_2$–32% NH_4OH (50:50:9:1.5) and detection with *p*-dimethylaminobenzaldehyde[17]. Quantification was effected by densitometry[11,18] or, for radioactive GD1b and GD1b-ol, by radiochromatography or liquid scintillation counting[19].

Gangliosides and reduced gangliosides were routinely identified in t.l.c. by comparison with standards and with GD1b-ol and GT1b-ol obtained chemically[15,16] by reduction of GD1b-L and GT1b-L[16] {α-Neu5Ac-(2→3)-β-Gal-(1→3)-β-GalNAc-(1→4)-[α-Neu5Ac-(2→8,1→9)-α-Neu5Ac-(2→3)]-β-Gal-(1→4)-β-Glc-(1→1)-Cer} prepared according to the procedure of McCluer and Evans[20].

Colorimetric procedures. — Ganglioside-bound sialic acid was determined by the resorcinol–HCl method[21,22].

RESULTS AND DISCUSSION

In 1964, Kuhn and Muldner[23] suggested that gangliosides could be present in the membrane as lactones, and Wiegandt[24] reported later that gangliosides that contained the disialyl residue α-Neu5Ac-(2→8)-α-Neu5Ac-(2→3)- could be expected to form lactones. Evans and McCluer[25] postulated the presence of a lactone of GM3 ganglioside extracted from bovine adrenal glands, which is now known to be α-Neu5Ac-(2→3,1→2)-β-Gal-(1→4)-β-Glc-(1→1)-Cer, and, more recently, Gross *et al.*[26], using a borohydride-reduction procedure, demonstrated indirectly the existence of lactones involving the sialic acid carboxyl group in the mixture of gangliosides obtained from rodent brain. Using the same procedure, Nores *et al.*[6] demonstrated the presence of the lactone of GM3 in melanoma cells. GD1b-L, the monolactone of GD1b, has been isolated from rat and human brain and characterized[3,10].

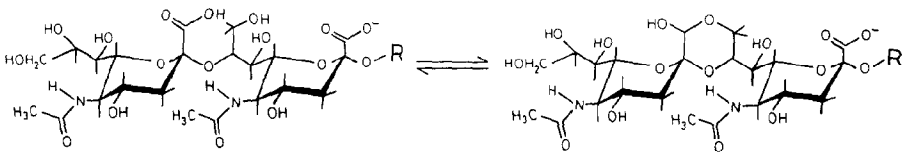
Few data are available on the possible role of ganglioside lactones; they are not substrates of sialidase[3] and are highly immunogenic[6].

For gangliosides, the extent of acid-catalyzed lactonization (**1→2**) greatly depends on the stability of the lactone ring, which may be influenced by changes in the secondary structure of the ganglioside following lactonization.

Lactonization in the presence of HCl was studied using a mixture of gangliosides from calf brain and pure GD1b ganglioside. Since ganglioside lactones are not stable under such laboratory procedures[3,16] as drying, dialysis, and lyophilization, immediate reduction with sodium borohydride[11,15,16] was effected in order to give the stable nonulosamine-containing ganglioside.

When the total mixture of gangliosides from calf brain, which contains mainly GM1, GD1a, GD1b, and GT1b, was treated at room temperature for 1 day with 10^{-3}M HCl and then reduced with sodium borohydride, 2D-t.l.c. revealed (Fig. 1b), in comparison with the control (Fig. 1a), that the proportions of GM1 and GD1a were unchanged and that those of GD1b and GT1b were decreased by ~70%; two new spots (Fig. 1b, 5 and 6), corresponding to the α-nonulosamine-(2→8)-α-Neu5Ac-(2→3)-containing GD1b and GT1b, were identified by co-chromatography with standards.

The lactonization process was studied in detail with GD1b. Fig. 2 shows the behaviour of 0.001, 0.451, and 0.904mM GD1b in 3.092–0.001mM HCl. Lactonization occurred very rapidly during the first hour and then proceeded slowly. The maximum rate of the lactonization reaction was achieved with an H^+–GD1b ratio



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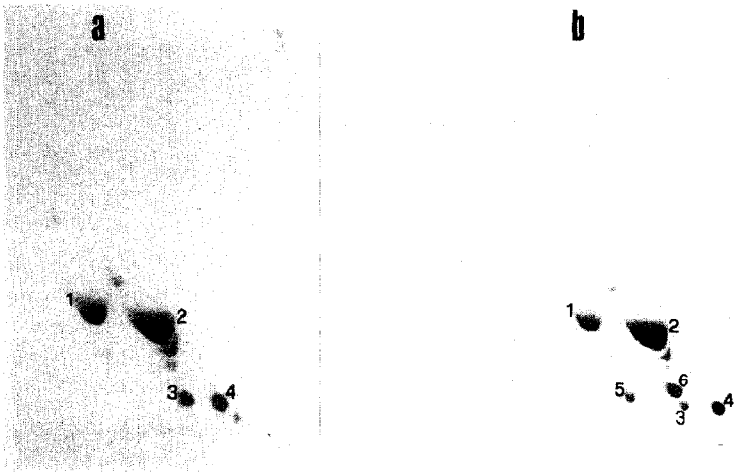
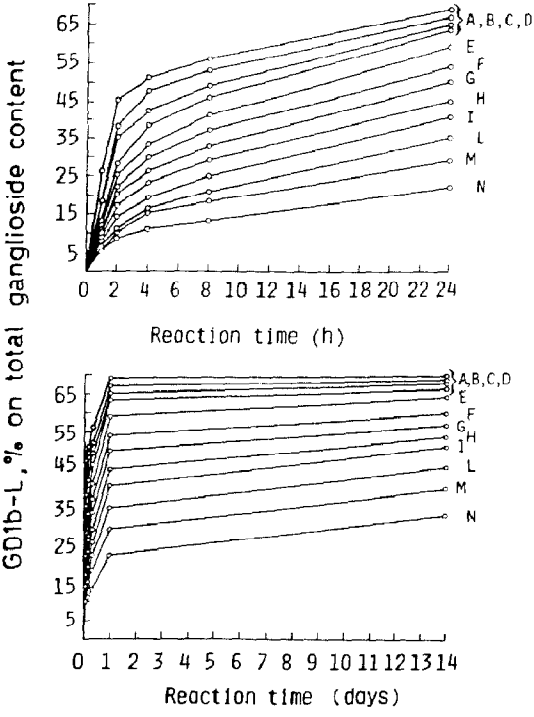


Fig. 1. 2D-T.l.c. of the mixture of gangliosides from calf brain after storage of solutions for 24 h in (a) water, and (b) mM HCl (2 mg/mL), followed by borohydride reduction: 1, GM1; 2, GD1a; 3, GD1b; 4, GT1b; 5, GD1b-ol; 6, GT1b-ol.



CONCENTRATIONS (mM)

	GD1b	H^+
A =	0.451	3.09
B =	0.451	2.09
C =	0.904	3.09
D =	0.451	1.32
E =	0.904	2.09
F =	0.451	0.794
G =	0.001	0.0015
H =	0.451	0.501
I =	0.904	0.794
L =	0.451	0.323
M =	0.904	0.501
N =	0.010	0.0036

Fig. 2. Lactonization of GD1b in the presence of hydrochloric acid at room temperature.

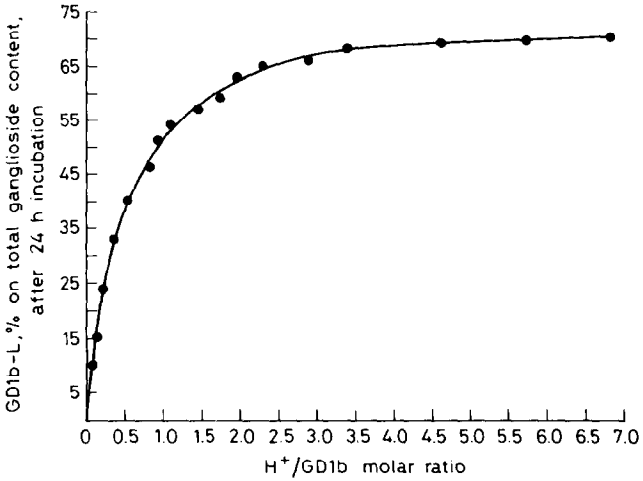


Fig. 3. Lactonization of GD1b after 24 h as a function of H^+ ganglioside ratio.

<1 (Fig. 3); the rate then decreased exponentially. At equilibrium, the molar ratio of GD1b and GD1b-L was 3:7.

The data reported here suggst the possibility of lactonization of gangliosides *in vivo*. The cytoplasmatic pH is regulated strictly, and the removal of acid from the cytosol by Na^+–H^+ exchange or by proton-translocating ATP-ases occurs in modulating cellular events. Thus, the lactonization of ganglioside molecules should parallel the increase in $[H^+]$.

The stabilities of GM1 and GD1a confirm the hypothesis that only gangliosides which contain the disialosyl residue α-Neu5Ac-(2→8)-α-Neu5Ac-(2→3)- can lactonize, and that this process involves only the terminal Neu5Ac residue.

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REFERENCES

1 A. NEUBERGER AND L. L. M. VAN DEENEN (Eds.), *New Comprehensive Biochemistry*, Vol. 10, Elsevier, Amsterdam, 1985, pp. 199–260.
2 P. H. FISHMAN, *J. Membr. Biol.*, 69 (1982) 85–98.
3 L. RIBONI, S. SONNINO, D. ACQUOTTI, A. MALESCI, R. GHIDONI, H. EGGE, S. MINGRINO, AND G. TETTAMANTI, *J. Biol. Chem.*, 261 (1986) 8514–8519.
4 S. K. GROSS, M. A. WILLIAMS, AND R. H. MCCLUER, *J. Neurochem.*, 34 (1980) 1351–1361.
5 S. SONNINO, R. GHIDONI, V. CHIGORNO, M. MASSERINI, AND G. TETTAMANTI, *Anal. Biochem.*, 128 (1983) 104–114.
6 G. A. NORES, T. DOHI, M. TANIGUCHI, AND S. HAKOMORI, *J. Immunol.*, 139 (1987) 3171–3176.

7 J. P. BEHR AND J.-M. LEHN, *FEBS Lett.*, 31 (1973) 297–300.
8 S. SONNINO, G. KIRSCHNER, G. FRONZA, H. EGGE, R. GHIDONI, D. ACQUOTTI, AND G. TETTAMANTI, *Glycoconjugate J.*, 2 (1985) 343–354.
9 R. K. YU, T. A. W. KOERNER, S. ANDO, H. C. YOHE, AND H. J. PRESTEGARD, *J. Biochem. (Tokyo)*, 98 (1985) 1367–1372.
10 D. ACQUOTTI, G. FRONZA, L. RIBONI, S. SONNINO, AND G. TETTAMANTI, *Glycoconjugate J.*, 4 (1987) 119–127.
11 G. FRONZA, G. KIRSCHNER, D. ACQUOTTI, R. BASSI, L. TAGLIAVACCA, AND S. SONNINO, *Carbohydr. Res.*, 182 (1988) 31–40.
12 G. TETTAMANTI, F. BONALI, S. MARCHESINI, AND V. ZAMBOTTI, *Biochim. Biophys. Acta*, 296 (1973) 160–170.
13 R. GHIDONI, S. SONNINO, G. TETTAMANTI, N. BAUMANN, G. REUTER, AND R. SCHAUER, *J. Biol. Chem.*, 255 (1980) 6990–6995.
14 G. GAZZOTTI, S. SONNINO, R. GHIDONI, P. ORLANDO, AND G. TETTAMANTI, *Glycoconjugate J.*, 1 (1984) 111–121.
15 S. K. GROSS, J. E. EVANS, V. N. REINHOLD, AND R. H. MCCLUER, *Carbohydr. Res.*, 41 (1975) 344–350.
16 R. W. LEDEEN, E. L. HOGAN, G. TETTAMANTI, A. J. YATES, AND R. K. YU (Eds.), *Fidia Research Series*, Vol. 16, Liviana Press, Padova, 1988, pp. 47–61.
17 S. M. PARTRIDGE, *Biochem. J.*, 42 (1948) 238–248.
18 V. CHIGORNO, S. SONNINO, R. GHIDONI, AND G. TETTAMANTI, *Neurochem. Int.*, 5 (1982) 397–403.
19 R. GHIDONI, S. SONNINO, V. CHIGORNO, B. VENERANDO, AND G. TETTAMANTI, *Biochem. J.*, 213 (1983) 321–329.
20 R. H. MCCLUER AND J. E. EVANS, *Adv. Exp. Med. Biol.*, 19 (1972) 95–102.
21 L. SVENNERHOLM, *Biochim. Biophys. Acta*, 24 (1957) 604–611.
22 J. MIETTINEN AND J. T. TAKKI LUKKAINEN, *Acta Chem. Scand.*, 13 (1959) 856–858.
23 R. KUHN AND J. MULDNER, *Naturwissenschaften*, 51 (1964) 635–636.
24 H. WIEGANDT, *Ergeb Physiol. Biol. Chem. Exp. Pharmakol.*, 57 (1966) 190–222.
25 J. E. EVANS AND R. H. MCCLUER, *Fed. Proc.*, 30 (1971) 1133.
26 S. K. GROSS, M. A. WILLIAMS, AND R. H. MCCLUER, *J. Neurochem.*, 34 (1980) 1351–1361.