Preparation of deacetyl-, lyso-, and deacetyl-lyso-GM_3 by selective alkaline hydrolysis of GM_3 ganglioside

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Abstract Three methods (using GM_3 quantities ranging from a few milligrams to grams) have been developed to prepare, in high yield, the three derivatives of ganglioside GM_3 [α-Neu5Ac-(2-3)-β-Gal-(1-4)-β-Glc-(1-1)-ceramide]: deacetyl-GM_3 [α-Neu-(2-3)-β-Gal-(1-4)-β-Glc-(1-1)-ceramide], lyso-GM_3 [α-Neu5Ac-(2-3)-β-Gal-(1-4)-β-Glc-(1-1)-sphingosine], and deacetyl-lyso-GM_3 [α-Neu-(2-3)-β-Gal-(1-4)-β-Glc-(1-1)-sphingosine]. This is the first report of the preparation of lyso-GM_3 by a one-pot reaction. We can now define the optimal conditions for the different preparations. Preparation of deacetyl-GM_3: alkaline reagent, 2 M KOH in water; GM_3 concentration, 33 mg/ml; reaction temperature, 90°C; reaction time, 3.5 h; nitrogen atmosphere. Preparation of deacetyl-lyso-GM_3: alkaline reagent, 8 M KOH in water; GM_3 concentration, 10 mg/ml; reaction temperature, 90°C; reaction time, 18 h; nitrogen atmosphere. Preparation of lyso-GM_3: alkaline reagent, 1 M sodium *tert*-butoxide in methanol; GM_3 concentration, 10 mg/ml; reaction temperature, 80°C; reaction time, 18 h; anhydrous conditions. The percentage yield of deacetyl-GM_3 was 70–75%, that of deacetyl-lyso-GM_3 100%, and of lyso-GM_3 36–40%. Deacetyl-GM_3, deacetyl-lyso-GM_3, and lyso-GM_3 were purified by column chromatography, and chemical structures were confirmed by electron spray-mass spectrometry.—Valiente, O., L. Mauri, R. Casellato, L. E. Fernandez, and S. Sonnino. **Preparation of deacetyl-, lyso-, and deacetyl-lyso-GM_3 by selective alkaline hydrolysis of GM_3 ganglioside. *J. Lipid Res.* 2001.** 42: **1318–1324.**

Supplementary key words TLC staining • tritium • *tert*-butoxide • ESI-MS

Gangliosides comprise a family of glycosphingolipids, widely distributed on the vertebrate cell membrane, in which the hydrophilic sugar moiety points directly outside the membrane while the hydrophobic lipid tail is anchored deeply in the membrane lipid layer. Gangliosides are associated with different cell physiological events and are considered to play important roles in recognition and signal transduction phenomena (1–3).

GM_3 ganglioside appears to be the most ubiquitous and abundant ganglioside in extraneural tissues, and is generally recognized as being closely related to several membrane and cellular processes including the modulation of the immune system (4–7). Moreover, its particular abundance in human tumor tissues (8–13) and its special arrangement in human tumor membranes (14–16) make GM_3 ganglioside an attractive option for active specific immunotherapy. In fact, experimental evidence of the immunogenic and antitumor activity of some GM_3-containing vaccines in several species has already been published (17, 18). GM_3 derivatives lacking the acetyl moiety (deacetyl-GM_3), acyl moiety (lyso-GM_3), or even both moieties (deacetyl-lyso-GM_3) could also be important tools for immunological (19) purposes. In fact, they are ideal for obtaining synthetic neoganglioproteins in which the primitive structure of the ganglioside remains quasi-intact, a relevant factor in promoting an efficient immune response against the native ganglioside. Moreover, deacylated GM_3 molecules have also been shown to be important biochemical tools (20).

In the 1970s there appeared the first reports of the preparation of deacylated gangliosides (21) and, subsequently, the preparation of deacetyl-lyso-GM_3 by alkaline reaction. In the following years, other groups extended the procedure, and in some cases improved on it, to the preparation of more complex deacylated gangliosides (22–27).

We developed a procedure for the chemical preparation of GM_3 from GM_1, a ganglioside that is available in large amount and at low cost (28). Through this procedure we were able to use grams of ganglioside GM_3 to develop a study of the stability of GM_3 amide linkages under alkaline conditions. We now present methods for obtaining deacetyl-GM_3, deacetyl-lyso-GM_3, and lyso-GM_3 in high yields. This is the first report of the preparation of lyso-GM_3 by a one-pot reaction.

Abbreviations: Cer, ceramide, (2*S*,3*R*,4*E*)-2-(octadecanoyl)amino-1,3-dihydroxy-octadec/eicos-4-ene; Neu, neuraminic acid, 5-amino-3,5-dideoxy-D-glycero-D-galacto-nonulosonic acid; Neu5Ac, *N*-acetylneuraminic acid; Sph, sphingosine, (2*S*,3*R*,4*E*)-2-amino-1,3-dihydroxy-octadec/eicos-4-ene; [3-^{3}H(*sphingosine*)]GM_3, α-Neu5Ac-(2-3)-β-Gal-(1-4)-β-Glc-(1-1)-(2*S*,3*R*,4*E*)-2-(octadecanoyl)amino-3-hydroxy-[3-^{3}H]octadec-4-ene.

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MATERIALS AND METHODS

Materials

LiChroprep RP18 for column chromatography (particle size, 40–63 μm) and high performance silica gel-precoated thin-layer plates (HPTLC, Kieselgel 60) were obtained from Merck (Darmstadt, Germany). All the chemicals were of the highest purity available. The solvents were distilled before use and deionized water was freshly distilled in a glass apparatus. Methanol was dehydrated by refluxing over and distilling from magnesium. GM_3 ganglioside was obtained by acid hydrolysis of GM_1-lactone (28). Standard gangliosides and ganglioside derivatives were available in the laboratory.

Isotopically labeled GM_3 containing tritium at position 3 of the *erythro* C_{18}-sphingosine, [3-^{3}H(*sphingosine*)]GM_3 (homogeneity over 99%; specific radioactivity, 2.0 Ci/mmol), was prepared by the dichloro-dicyano-benzoquinone/sodium boro[^{3}H]hydride method followed by reversed-phase HPLC purification (29–32).

Preparation of deacetyl-GM_3

In a round-bottom flask, GM_3 ganglioside, in quantities of 50 mg to 1 g, was dissolved in water (66 mg/ml) under stirring and warmed to 60°C. An equal volume of 4 M KOH, prewarmed to 60°C, was then added under strong stirring. A reflux apparatus, fitted with a connection tube for nitrogen fluxing, was adapted to the flask and the reaction was allowed to proceed at 90°C under vigorous stirring. After 3.5 h, the solution was chilled, neutralized with 6 M HCl, dialyzed, and freeze-dried. The residue was dissolved in the minimum amount of chloroform–methanol 2:1 (v/v) possible, precipitated by the addition of 5 volumes of cold acetone and stored overnight at 4°C. After centrifugation at 5,000 rpm the acetone was discarded and the pellet was dried under high vacuum. Under these experimental conditions the yield of deacetyl-GM_3 was 70–75%.

Deacetyl-GM_3 was purified from the total hydrolysis reaction mixture by silica gel column chromatography, using the solvent system chloroform–methanol–formic acid 30:50:6 (v/v/v). The purification was carried out with 150 mg of the dried pellet obtained by the hydrolysis of 1 g of GM_3 and a 1 × 60 cm column. The yield of the purification process was 89%.

Preparation of deacetyl-lyso-GM_3

The preparation of deacetyl-lyso-GM_3 was performed according to the scheme developed for the preparation of deacetyl-GM_3 with the following optimal conditions: GM_3 concentration, 10 mg/ml; KOH concentration, 8 M; reaction time, 18 h. The reaction yield was quantitative.

After neutralization and dialysis, deacetyl-lyso-GM_3 was precipitated from the reaction mixture as reported above. Deacetyl-lyso-GM_3 was purified by silica gel column (100 mg on a 1 × 20 cm column) chromatography, using the solvent system chloroform–methanol–5 M ammonium hydroxide 30:50:10 (v/v/v). Purification was carried out with 120 mg of the dried pellet obtained by the hydrolysis of 1 g of GM_3 and a 1 × 20 cm column. The yield of the purification process was 91%.

Preparation of lyso-GM_3

In a screw-capped round-bottom flask, GM_3 ganglioside, in quantities between 10 mg and 1 g, was dissolved in anhydrous methanol (12.5 mg/ml) with stirring and warming at 60°C. After 15 min, a 5 M sodium *tert*-butoxide anhydrous methanol solution, prewarmed at 60°C, was added to a 1 M final concentration. The solution was maintained for a further 15 min at 60°C under vigorous stirring, and then the temperature was brought to 80°C and the reaction was allowed to proceed 18 h under vigorous stirring. The reaction mixture was then chilled and dried under vacuum, and the residue, dissolved in the minimum volume of water, was dialyzed. The reaction yield was 36–40%.

Lyso-GM_3 was partially purified by LiChroprep reversed-phase column chromatography followed by silica gel column chromatography. The dried residue obtained from the hydrolysis of 1 g of GM_3 was dissolved in the minimum volume of methanol–water 5:1 (v/v), containing a few drops of diluted sulfuric acid to achieve a pH of about 1, and applied on a 3 × 10 cm LiChroprep column; elution was carried out with the methanol-water solvent system, the first 250 ml at a ratio of 5:1 by volume, the remaining at a ratio of 15:1 by volume; chromatography was performed at 45°C. Fractions containing lyso-GM_3 were collected, dried, and applied on a 2 × 50 cm silica gel column; elution was carried out with a solvent system of chloroform–methanol–water 50:40:10 (v/v/v). The yield of the purification process was 82%.

Determination of the optimal experimental conditions and reaction yields

Ganglioside GM_3 dissolved in propan-1-ol–water 7:3 (v/v), was mixed with 10^7–10^8 dpm of [3-^{3}H(*sphingosine*)]GM_3 dissolved in the same solvent system. The solution was dried and the residue was treated for the preparation of deacetyl-GM_3, deacetyl-lyso-GM_3, or lyso-GM_3, as reported above. Samples of the reaction mixtures were taken every half-hour, chilled, neutralized, and subjected to HPTLC analysis followed by radioimaging to determine the extent of the reaction.

Analytical procedures

Glycolipid-bound sialic acid was measured by the HCl-resorcinol method (33, 34), using pure *N*-acetylneuraminic acid (Neu5Ac) as reference.

The reaction mixtures were analyzed by HPTLC, using the solvent systems chloroform–methanol–100 mM aqueous KCl–5 M formic acid 30:50:3:3 (v/v/v/v), and chloroform–methanol–0.2% aqueous $CaCl_2$ 30:50:9 (v/v/v).

Ganglioside GM_3 and ganglioside derivatives were visualized on the HPTLC plates by treatment with anisaldehyde (35), *p*-dimethylaminobenzaldehyde (36), and orcinol (37) spray reagents; amine-containing GM_3 derivatives were visualized by treatment with 20% methanolic ninhydrin followed by heating at 80°C. The ganglioside and ganglioside derivative spots were quantified with a GS-700 imaging densitometer (Bio-Rad, Hercules, CA). Radioactive compounds were recognized and quantified after HPTLC by radioimaging with a β-imager instrument (Biospace, Paris, France).

The radioactivity associated with GM_3, the reaction mixtures, and the purified compounds was determined by liquid scintillation counting.

Structural analysis of GM_3 derivatives was made by mass spectrometry (MS) (28). Electrospray ionization (ESI)-MS of the GM_3 derivatives was carried out on a ThermoQuest Finnigan LCQdeca mass spectrometer equipped with an electrospray ion source and an Xcalibur™ data system (Thermo Finnigan, San Jose, CA). Samples were dissolved in methanol at a concentration of 2–3 ng/μl and introduced into the electrospray needle by mechanical infusion through a microsyringe at a flow rate of 3 μl/min.

RESULTS

Aqueous base hydrolysis of GM_3 with KOH solution afforded deacetyl-GM_3 and deacetyl-lyso-GM_3. **Figure 1** shows the time course of the reactions performed with 2 and 8 M KOH. Under both experimental conditions the hydrolysis of GM_3 took place in a step-by-step reaction:

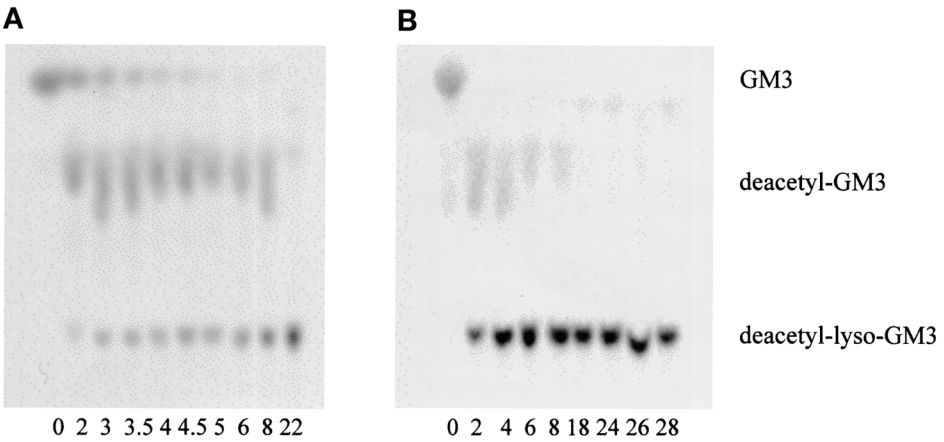

Fig. 1. Alkaline hydrolysis of radioactive GM_3 in KOH aqueous solutions. Time course of the reaction for the preparation of deacetyl-GM_3 (A) and deacetyl-lyso-GM_3 (B), as determined by normal-phase TLC followed by radioimaging; 2,000 dpm applied on a 3-mm line, time of acquisition: 4 h. The number under each lane indicates the reaction time in hours. Solvent system: chloroform–methanol–0.2% aqueous $CaCl_2$ 30:50:9 (v/v/v).

first the deacetylation of sialic acid and then the deacylation of ceramide. Prolonging the reaction time under both experimental conditions led to a quantitative yield of deacetyl-lyso-GM_3. Nevertheless, the optimal reaction time for hydrolyzing GM_3 in aqueous 2 M KOH was found to be 3.5 h. For shorter times most of the GM_3 was consumed and only a minor quantity of deacetyl-lyso-GM_3 formed; under these experimental conditions we had the higher yield of deacetyl-GM_3, determined to be 70–75%, as reported in **Table 1**.

In spite of our many combinations of experimental conditions, no lyso-GM_3 could be formed by hydrolysis of GM_3 in aqueous solutions of KOH. Likewise, no lyso-GM_3 was formed by solubilizing GM_3 in mixtures of water and alcohols of different carbon content or by solubilizing GM_3 in anhydrous solvents.

Lyso-GM_3 was obtained with sodium *tert*-butoxide in anhydrous methanol. **Figure 2** shows the time course of the reaction with sodium *tert*-butoxide. The consumption of GM_3 occurred slowly, but under more drastic conditions the sialic acid of lyso-GM_3 lost the acetyl group rapidly. Under the optimal experimental conditions there remained a large amount of GM_3 in the reaction mixture alongside the three formed GM_3 derivatives, deacetyl-GM_3, lyso-GM_3, and deacetyl-lyso-GM_3. We calculated the yield of lyso-GM_3 to be 36–40%.

Determining the reaction yield by TLC separation of the reaction mixture followed by colorimetric staining was

TABLE 1. Percent distribution between the reaction products obtained by alkaline hydrolysis of GM_3 performed under optimal conditions for the preparation of deacetyl-GM_3 (A), deacetyl-lyso-GM_3, (B) and lyso-GM_3 (C), as determined by radioactive and colorimetric methods

	Radioactivity	*p*-Dimethylamino -benzaldehyde	Anisaldehyde	Orcinol	Ninhydrin
A. Experimental conditions: solvent, 2 M KOH in water; GM_3 concentration, 33 mg/ml; reaction temperature, 90°C; reaction time, 3.5 h					
GM_3	9–10	12–15	13–17	10–12	—
Deacetyl-GM_3	71–73	69–70	60–62	62–64	80–83
Lyso-GM_3	—	—	—	—	—
Deacetyl-lyso-GM_3	17–20	16–18	23–25	24–28	17–20
B. Experimental conditions: solvent, 8 M KOH in water; GM_3 concentration, 10 mg/ml; reaction temperature, 90°C; reaction time, 18 h					
GM_3	—	—	—	—	—
Deacetyl-GM_3	—	—	—	—	—
Lyso-GM_3	—	—	—	—	—
Deacetyl-lyso-GM_3	100	100	100	100	100
C. Experimental conditions: solvent, 1 M sodium *tert*-butoxide in methanol; GM_3 concentration, 10 mg/ml; reaction temperature, 80°C; reaction time, 18 h					
GM_3	32–38	52–57	62–67	50–54	—
Deacetyl-GM_3	19–21	12–14	9–11	21–24	15–17
Lyso-GM_3	32–37	19–21	11–13	15–17	73–78
Deacetyl-lyso-GM_3	10–12	10–12	12–14	8–10	7–9

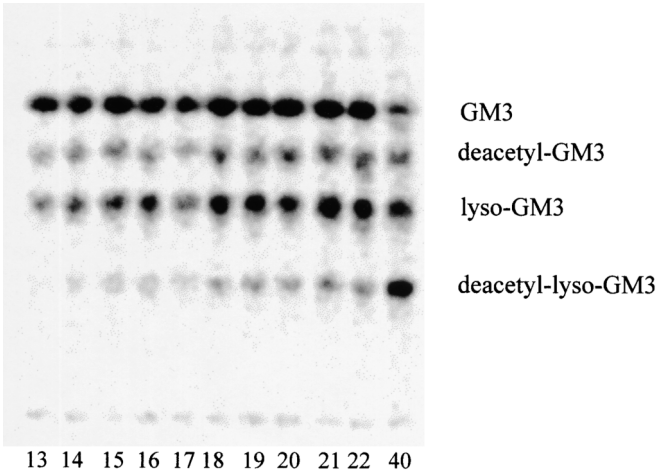

Fig. 2. Alkaline hydrolysis of radioactive GM_3 in *tert*-butoxide solution. Time course of the reaction for the preparation of lyso-GM_3, as determined by normal-phase TLC followed by radioimaging; 2,000 dpm applied on a 3-mm line, time of acquisition: 4 h. Solvent system: chloroform–methanol–100 mM aqueous KCl–5 M formic acid 30:50:3:3 (v/v/v/v). The number under each lane indicates the reaction time in hours.

not satisfactory. In fact, we obtained different results with the four colorimetric staining systems used. Orcinol, anisaldehyde, and *p*-dimethylaminobenzaldehyde reagents are used mostly to stain glycolipids. Nevertheless, neuraminic acid and the sphingosine amino group partly modified, but in different ways, the spot color, so that a correct analysis of the reaction mixture became difficult. The use of ninhydrin was useful to characterize the formed amine-containing compounds, but the amino groups of neuraminic acid and the sphingosine moieties reacted differently with the reagent. Moreover, ninhydrin staining resulted in no information about the remaining GM_3. Thus, the reaction yields were determined by adding radioactive GM_3 with isotopic tritium in the sphingosine moiety to the natural, unlabeled GM_3. The radioactive products were analyzed by radioimaging after TLC separation. Table 1 shows the reaction yields determined by radiochemical and colorimetric procedures.

Purification of lyso-GM_3 was carried out by C_{18} reversed-phase silica gel (LiChroprep RP18) column chromatography. **Figure 3** shows that lyso-GM_3, GM_3, and deacetyl-GM_3 were separated from each other but were still contaminated by deacetyl-lyso-GM_3. Thus, lyso-GM_3 and deacetyl-lyso-GM_3 were then separated by normal-phase silica gel column chromatography.

Deacetyl-GM_3, lyso-GM_3, and deacetyl-lyso-GM_3, prepared and purified as reported above, were characterized by mass spectrometric analyses. **Figures 4**, **5**, and **6** show the ESI-MS and the derived MS/MS spectra of the three compounds. Each product was represented mainly by two [M − 1] ions differing by 28 *m/z* units and corresponding to the molecular species containing C_{18}-sphingosine and C_{20}-sphingosine. This is in agreement with the a priori knowledge that the lipid moiety of the GM_3 ganglioside used for the synthesis contained mainly ceramides, the stearic acid being linked to C_{18}- and C_{20}-sphingosine (28). The presence in each GM_3 derivative of two molecular species, differing in the lipid moiety but having the same oligosaccharide species, was confirmed by the MS spectra derived by each separated [M − 1] ion, where all the fragments differ by 28 *m/z* units.

The values of the [M − 1] ions confirmed the structure of the three GM_3 derivatives. The [M − 1] ions at *m/z* 1138 and 1166 of deacetyl-GM_3, those at *m/z* 914 and 942 of lyso-GM_3, and those at *m/z* 872 and 900 of deacetyl-lyso-GM_3 differed from those of GM_3, of 42, 266, and 308 *m/z* units, respectively, that is, to the lack of the acetyl group, the stearoyl group, and both together.

The MS/MS spectra of the isolated [M − 1] ions, with *m/z* 1138 and 1166, of deacetyl-GM_3 (Fig. 4B and C) contained the three ions at *m/z* 889, 727, and 565 and at *m/z* 917, 755, and 593, corresponding to lactosylceramide, glucosylceramide, and ceramide containing stearic acid linked to C_{18}-sphingosine and C_{20}-sphingosine, respectively. This fragmentation pattern indicates, as previously reported (28), a sequential detachment of sugar units from the ceramide moiety. The ions at *m/z* 1094 and 1122 (Fig. 4B), and at *m/z* 1120 and 1148 (Fig. 4C), should derive from the loss of the carbon dioxide and ammonia from the two [M − 1] ions. The MS/MS spectra derived from deacetyl-lyso-GM_3 and lyso-GM_3 (Figs. 5 and 6) contained fragments corresponding to the sequential detachment of sialic acid and galactose, whereas

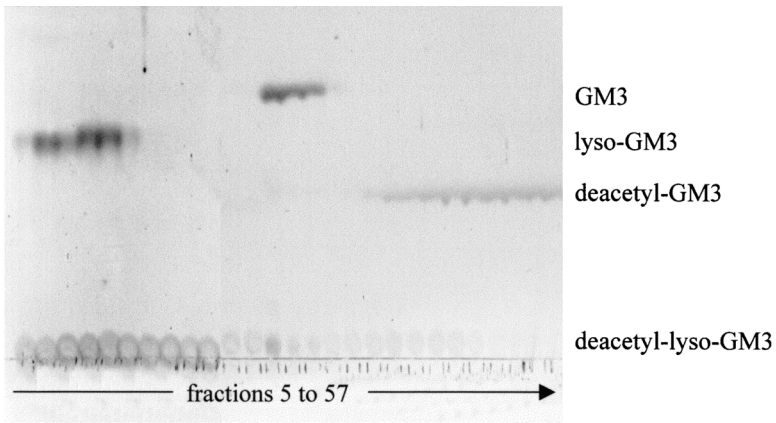

Fig. 3. Alkaline hydrolysis of GM_3 in *tert*-butoxide solution. Reversed-phase column chromatography elution profile of the reaction mixture obtained under optimal experimental conditions. Solvent system: chloroform–methanol–0.2% aqueous $CaCl_2$ 30:50:9 (v/v/v). Colorimetric staining was with *p*-dimethylaminobenzaldehyde reagent.

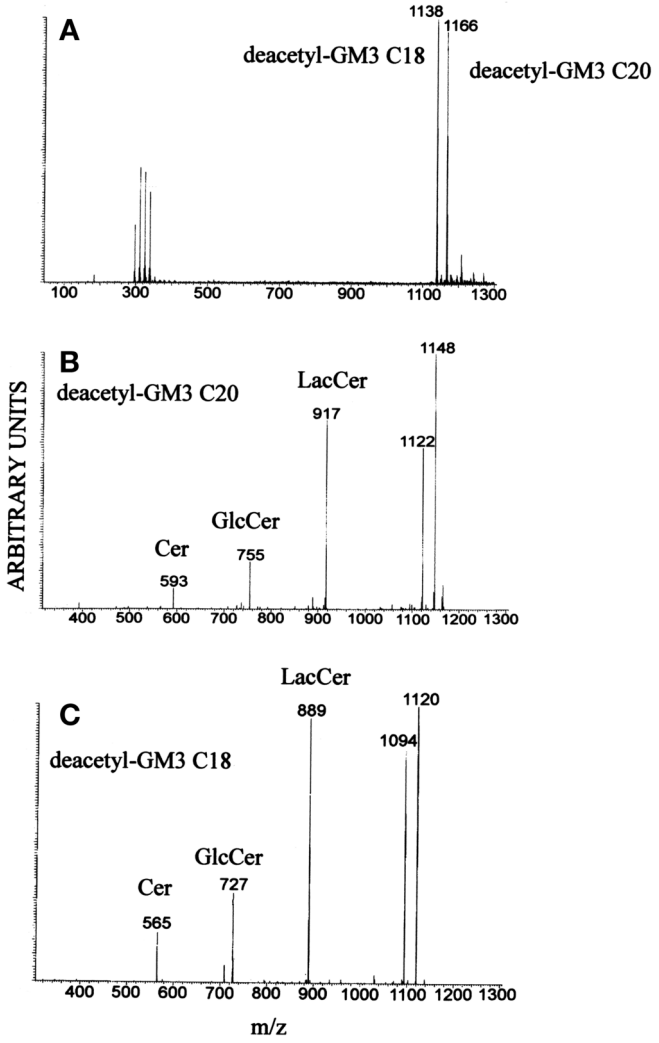

Fig. 4. Electron spray ionization mass spectrometry of the deacetyl-GM_3 prepared from GM_3. A: MS spectrum: the two [M − 1] ions at *m/z* 1138 and 1166 represent the two species of deacetyl-GM_3 with ceramide containing stearic acid linked to C_{18}- and C_{20}-sphingosine, respectively. B: MS/MS spectrum derived from the isolated [M − 1] ion at *m/z* 1166. C: MS/MS spectrum derived from the isolated [M − 1] ion at *m/z* 1138. Cer, Ceramide; GlcCer, glucosylceramide; LacCer, lactosylceramide.

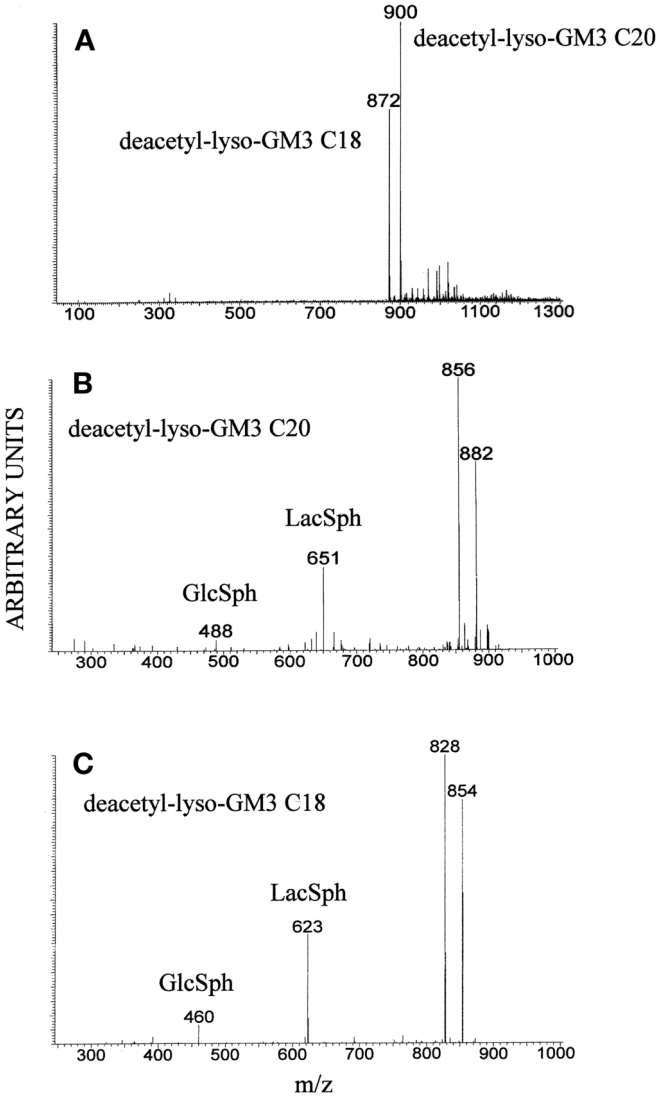

Fig. 5. Electron spray ionization mass spectrometry of the deacetyl-lyso-GM_3 prepared from GM_3. A: MS spectrum: the two [M − 1] ions at *m/z* 872 and 900 represent the two species of deacetyl-lyso-GM_3 with C_{18}- and C_{20}-sphingosine, respectively. B: MS/MS spectrum derived from the isolated [M − 1] ion at *m/z* 900. C: MS/MS spectrum derived from the isolated [M − 1] ion at *m/z* 872. GlcSph, Glucosylsphingosine; LacSph, lactosylsphingosine.

the ion corresponding to C_{18}- or C_{20}-sphingosine was scant or absent.

DISCUSSION

In 1970 Taketomi and Kawamura (21) described the preparation of deacetyl-lyso-GM_3 by alkaline hydrolysis of ganglioside GM_3 in aqueous butanol. In the following years minor and major modifications were introduced to the original protocol to adapt the procedure to other gangliosides and increase the product yield (22–24, 26, 38–41). The deacylation of polysialylated gangliosides is still a problem because of the variety of products formed under alkaline conditions.

The procedures for alkaline hydrolysis of GM_3 were described to give deacetyl-GM_3 and deacetyl-lyso-GM_3 in different yields, together with several by-products. Thus lyso-GM_3 was then synthesized by acetylation of a deacetyl-lyso-GM_3 previously protected at the sphingosine amino group (23, 25, 26).

The objective of this work was to develop simple procedures for the preparation of deacetyl-GM_3, deacetyl-lyso-GM_3, and lyso-GM_3, this last being achieved, for the first time, by a one-pot reaction. The search for optimal experimental conditions was simplified by using a GM_3 containing tritium at position 3 of *erythro* sphingosine, and by TLC analysis of the reaction mixtures followed by radioimaging.

Deacylation of gangliosides has always been carried out under alkaline conditions, using aqueous or anhydrous alcohol solutions. Gangliosides are soluble in water, where they form aggregates, such aggregates having the oligosaccharide chain protruding into the aqueous environment.

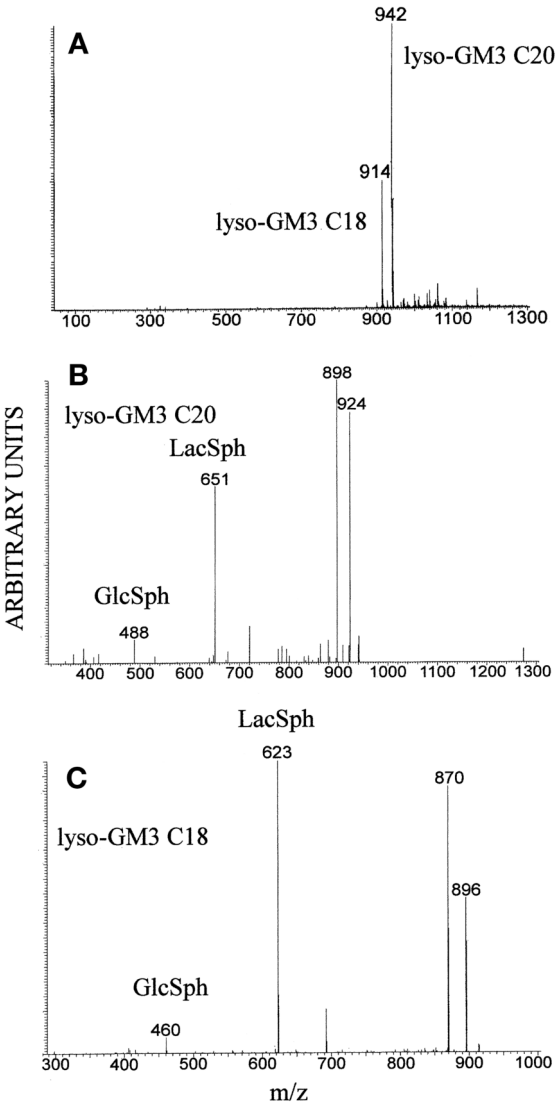

Fig. 6. Electron spray ionization mass spectrometry of the lyso-GM_3 prepared from GM_3. A: MS spectrum: the two [M − 1] ions at *m/z* 914 and 942 represent the two species of lyso-GM_3 with C_{18}- and C_{20}-sphingosine, respectively. B: MS/MS spectrum derived from the isolated [M − 1] ion at *m/z* 942. C: MS/MS spectrum derived from the isolated [M − 1] ion at *m/z* 914. GlcSph, glucosylsphingosine; LacSph, lactosylsphingosine.

Thus, the amide linkage of sialic acid should be much more accessible to the base attack than the ceramide amide linkage located at the water-lipid interface. The results confirmed our hypothesis. In fact, the release of the acetyl group was much faster than that of the fatty acid. The reaction could be controlled well by modulating the experimental parameters, and we were able to establish two sets of experimental conditions yielding 70–75% of deacetyl-GM_3 or 100% of deacetyl-lyso-GM_3. Moreover, the molar recovery of the sphingolipids was quantitative, that is, all the starting radioactivity (GM_3 associated) was found associated with the product(s) at the end of the reaction.

Because of the results indicating that the acetyl group of sialic acid was released rapidly from aggregates of GM_3, we attempted to obtain lyso-GM_3 by dissolving GM_3 in anhydrous alcohols where monomers are present. Several experiments were performed with KOH, NaOH, and tetramethylammoniumhydroxide in methanol, propanol, and butanol, but the yield of lyso-GM_3 was always scant.

Sodium *tert*-butoxide in methanol slowly but effectively yielded lyso-GM_3. The detachment of the acetyl and fatty acyl groups occurred simultaneously, but the latter process was much more important. Thus, lyso-GM_3 was the main product of the reaction mixture. Some GM_3 still remained in the reaction mixture. Nevertheless, any increase in the reaction time, reaction temperature or base concentration corresponded to a decrease in GM_3, deacetyl-GM_3, and lyso-GM_3 in favor of deacetyl-lyso-GM_3. A final problem we met with was the purification of lyso-GM_3. In fact, the resolution by silica gel column chromatography was far less than that of HPTLC, and poor results were also obtained by ion-exchange chromatography. The problem was partially overcome by purification performed at 45°C on a C_{18} reversed-phase silica gel (LiChroprep RP18) column. GM_3, deacetyl-GM_3, and lyso-GM_3 were separated from each other, but the hydrophilic deacetyl-lyso-GM_3 could be completely removed from lyso-GM_3 only by a second chromatographic step involving normal-phase column chromatography.

In conclusion, in this study we present simple procedures for the preparation of deacetyl-GM_3, deacetyl-lyso-GM_3, and lyso-GM_3. Lyso-GM_3 was obtained, for the first time, by a one-pot reaction.

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