Dimorphic expression of cerebrosides in the mycopathogen *Sporothrix schenckii*

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Abstract **Major neutral glycosphingolipid components were extracted from *Sporothrix schenckii*, a dimorphic fungus exhibiting a hyphal saprophytic phase and a yeast parasitic phase responsible for chronic mycotic infections in mammalian hosts. These components, one from the mycelial form and two from the yeast form, were purified and their structures were elucidated by ^{1}H nuclear magnetic resonance (NMR) spectroscopy, electrospray ionization mass spectrometry (ESI-MS), and tandem ESI-MS/MS. All three were characterized as cerebrosides (monohexosylceramides) containing (4*E*, 8*E*)-9-methyl-4,8-sphingadienine as the long-chain base attached to *N*-2′-hydroxyoctadecanoate and *N*-2′-hydroxy-(*E*)-Δ^3-octadecenoate as the fatty acyl components. However, while the mycelial form expressed only β-glucopyranosylceramide, the yeast form expressed both β-gluco- and β-galactopyranosylceramides in approximately equal amounts. In addition, while the glucosylceramides of both mycelial and yeast forms had similar proportions of saturated and (*E*)-Δ^3 unsaturated 2-hydroxy fatty acid, the galactocerebroside of the yeast form had significantly higher levels of (*E*)-Δ^3 unsaturation. The differences in cerebroside hexose structure represent a novel type of glycosphingolipid dimorphism not previously reported in fungi. Possible implications of these findings with respect to regulation of morphological transitions in *S. schenckii* and other dimorphic fungi are discussed.**—Toledo, M. S., S. B. Levery, A. H. Straus, and H. K. Takahashi. **Dimorphic expression of cerebrosides in the mycopathogen *Sporothrix schenckii*. *J. Lipid Res.* 2000.** 41: **797–806.**

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Sporothrix schenckii, the causative agent of sporotrichosis, is a dimorphic fungus that exhibits a hyphal morphology in its low temperature saprophytic phase, but is found primarily as a budding yeast in host tissues. Temperature is an important, but not exclusive, determinant of morphology, CO_2 concentration and nutritional factors also being key contributors to maintenance of a particular form in vitro (1, 2). Sporotrichosis is normally encountered in tropical areas as a localized chronic lymphocutaneous infection after skin injury, and in this form is considered benign and highly reponsive to conventional therapeutics (1, 3–6); however, in immunosuppressed or -compromised hosts, such as transplant recipients or patients with AIDS, development of a disseminated mycosis can be life-threatening and difficult to treat (7–11). In its preference for a yeast morphology in the parasitic phase, *S. schenckii* superficially belongs to the group of thermally dimorphic mycopathogens which includes *Blastomyces dermatitidis* and *Histoplasma capsulatum* (*Ajellomyces dermatitidis* and *A. capsulatus*, respectively), and *Paracoccidioides brasiliensis*; however, the *Ajellomyces* and *Paracoccidioides* species appear to form a distinct taxon from which *S. schenckii* is somewhat distant (2, 12). Within the *Ajellomyces*/*Paracoccidioides* group, temperature is clearly the dominant factor in promoting morphological transitions and, unlike *S. schenckii*, these fungi can cause severe systemic infections even in immunocompetent hosts (7, 9).

Many attempts to understand the phenomena of thermal dimorphism and their relationship to infection processes have focused on components of the fungal cell wall, as these are thought to be the primary determinants of both fungal morphology and host–pathogen interactions (13). The possible functional roles of some other components, such as cell membrane lipids, are more poorly understood. Recently, the involvement of glycosphingolipids (GSLs) in fungal life processes has been subjected to increased scrutiny, particularly as progress has been made in elucidating the molecular basis for sphingolipid biosynthesis in *S. cerevisiae* (14–16). Sphingolipids are being increasingly appreciated as key components of intracellular

Abbreviations: CID, collision-induced dissociation; CMH, ceramide monohexoside (≡ cerebroside); ESI, electrospray ionization; GalCer, β-galactopyranosylceramide (≡ galactocerebroside); GlcCer, β-glucopyranosylceramide (≡ glucocerebroside); GSL, glycosphingolipid; GIPC, glycosylinositol phosphorylceramide; HPTLC, high performance thin-layer chromatography; Fa, fatty acyl; Sph, sphingoid.

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regulatory pathways (17–20) and, in addition, GSLs have been shown to possess immunomodulatory properties in a variety of mammalian systems (21–24). A number of reports have described in vitro physiological activities for fungal GSLs, particularly cerebrosides (ceramide monohexosides, CMHs) (25–32). Kawai and Ikeda (25–28, 30) demonstrated repeatedly that fungal cerebrosides or structurally similar analogs exhibited fruiting-inducing activity in bioassays with *Schizophyllum commune*. Subsequently it was reported (31) that *Pachybasium* cerebrosides potentiated the antifungal activity of aculeacin against *Candida albicans*. More recently, fruiting body-inducing cerebrosides of *Ganoderma lucidum* were found to inhibit the activity of α-type replicative DNA polymerases from calf thymus, cauliflower inflorescence, and a test strain of *Coprinus cinereus*, a mushroom used for bioassays of fruiting body-induction in the study (29). Cerebrosides extracted from the rice pathogen *Magnaporthe grisea* were found to be highly active elicitors of defence responses when applied to rice leaves; these responses included accumulation of phytoalexins and hypersensitive cell death (32). In all of these cases, the active compounds were glucocerebrosides incorporating a characteristic 2-hydroxy fatty *N*-acyl-(4*E*,8*E*)-9-methyl-4,8-sphingadienine ceramide moiety; neither gluco- nor galactocerebrosides from mammalian tissues showed significant activity in the fruiting body and rice plant response assays (27, 32).

In preliminary studies of the possible involvement of GSLs in fungal dimorphism and infectivity, the structures of the major GSL components of both forms of *P. brasiliensis* were elucidated (33, 34). An interesting finding was that the cerebrosides of yeast and mycelial forms of *P. brasiliensis* differed significantly in the level of a characteristic (*E*)-Δ^3-unsaturation of the ceramide 2-hydroxy fatty *N*-acyl moiety. To ascertain whether this might be a more generally distributed property of dimorphic mycopathogens, the cerebrosides of both forms of *S. schenckii* were similarly investigated. The results, as described in this paper, were also striking, but in a completely different way: although the average levels of ceramide (*E*)-Δ^3-unsaturation were similar in cerebrosides of both forms of *S. schenckii*, a chemical dimorphism was observed instead with respect to the sugar moieties.

MATERIALS AND METHODS

Fungal isolate and growth conditions

The culture of *Sporothrix schenckii*, strain 65 (originally obtained from cutaneous footpad lesion of an otherwise healthy individual), was provided by Dr. Olga Gompertz, Department of Cellular Biology, Universidade Federal de São Paulo/Escola Paulista de Medicina, São Paulo, SP, Brasil. Mycelium and yeast forms of *S. schenckii* strain 65 were grown in brain–heart infusion (BHI; 37 g/L water), incubated at 25°C and 37°C, respectively, using 2.5 L Fernbach flasks in a shaker at 150 rpm. Both forms were inactivated with 0.1% of thimerosal, and after 48 h mycelium forms were collected by filtration on Whatman no. 1 filter paper, while yeast forms were harvested by centrifugation at 5,000 rpm for 20 min (35).

Solvents for extraction and anion exchange chromatography

Solvent A, isopropanol–hexane–water 55:20:25 (v/v/v, upper phase discarded); solvent B, chloroform–methanol 2:1 (v/v); solvent C, chloroform–methanol–water 30:60:8 (v/v/v).

High performance thin-layer chromatography

Both analytical and preparative HPTLC were performed on silica gel 60 plates (E. Merck, Darmstadt, Germany) using chloroform–methanol–water 60:40:9 (v/v/v, containing 0.002% w/v $CaCl_2$; solvent D) as mobile phase. Lipid samples were dissolved in solvent B and applied by streaking from 5 μL Microcaps (Drummond, Broomall, PA). For analytical HPTLC, detection was made by Bial's orcinol reagent (orcinol 0.55% [w/v] and H_2SO_4 5.5% [v/v] in ethanol–water 9:1 [v/v]; the plate is sprayed and heated briefly to ~200–250°C). For preparative HPTLC, samples were streaked lengthwise on 10 × 20 cm plates; separated glycosphingolipid bands were visualized under UV after spraying with primulin (Aldrich; 0.01% in 80% aqueous acetone). Bands were marked by pencil and individually scraped from the plate. Glycosphingolipids were then isolated from the silica gel by repeated sonication in solvent A followed by centrifugation. After concentration of the extract, primulin was removed by passage through a short column of DEAE-Sephadex A-25 in solvent C.

Extraction and purification of glycosphingolipids

Extraction and purification of glycosphingolipids were carried out as described previously (33, 35). Briefly, glycosphingolipids were extracted by homogenizing yeast or mycelium forms (25–35 g wet weight) in an Omni-mixer (Sorvall Inc. Wilmington, DE), three times with 200 mL of solvent A, and twice with 200 mL of solvent B. The five extracts were pooled, dried on a rotary evaporator, dialyzed against water, lyophilized, resuspended in solvent C, and applied to a column of DEAE-Sephadex A-25 (Ac^- form). Neutral glycosphingolipids were eluted with 5 volumes of solvent C. The neutral glycosphingolipid fraction was further purified from other contaminants by column chromatography on silica gel 60 using a step-wise gradient of chloroform–methanol from 9:1 to 1:1 (v/v) (36). Fractions containing ceramide monohexosides (CMHs), as assessed by analytical HPTLC, were pooled, dried, and further purified by preparative-scale HPTLC as described above. The purity of each fraction was assessed by analytical HPTLC.

^{1}H-nuclear magnetic resonance spectroscopy

Samples of underivatized CMH (~0.5–1.0 mg) were deuterium exchanged by repeated evaporation from $CDCl_3/CD_3OD$ 2:1 (v/v) under a N_2 stream at 37°C, and then dissolved in 0.5 mL DMSO–d_6/2% D_2O (37–39) for NMR analysis. 1-D ^{1}H-NMR spectra were acquired at 35°C on a Varian Unity Inova 600 MHz spectrometer using standard acquisition software available in the Varian VNMR software package. Proton chemical shifts are referenced to internal tetramethylsilane (δ = 0.000 ppm).

The percentage of (*E*)-Δ^3 unsaturation was calculated from the integrated ratios of the vinyl proton resonances corresponding to H-4″ of (*E*)-Δ^3 unsaturated fatty acid and H-5 of the sphingosine moiety. These resonances were chosen as they have similar splitting patterns and chemical shifts, but are completely resolved from each other in all spectra; although the chemical shift of H-5 is slightly affected by the presence or absence of (*E*)-Δ^3 unsaturation, the total integral for this resonance was assumed to represent 1.00 mole, regardless of fatty acyl distribution.

Electrospray ionization mass–spectrometry

All ESI–MS and tandem ESI–MS/CID-MS experiments were performed in the positive ion mode on a PE-Sciex (Concord, On-

tario, Canada) API-III spectrometer, using direct infusion (5.0 μL/min) of CMH samples dissolved in 100% MeOH (~1–2 ng/μL), under conditions similar to those described previously (33), except that addition of ammonium acetate was omitted. In single analyzer experiments, the mass range from *m/z* 700–850 was scanned in 0.1 u steps, with a dwell time of 5 msec and an orifice potential (OR) of 200 V. For ESI–MS/CID–MS experiments, precursor ions selected in Q1 were subjected to collision-induced dissociation (with argon as collision gas) in Q2 at a "collision gas thickness" (CGT) of ~400, while the mass range in Q3 was scanned from *m/z* 100–800 in 0.2 u steps, with a dwell time of 2 msec.

RESULTS

Detection and isolation of cerebroside fractions from mycelium and yeast forms of *S. schenckii*

Cerebrosides were initially detected in the neutral lipid fractions from *S. schenckii* by analytical HPTLC with comparison to authentic standards (**Fig. 1**). In lipids extracted from the mycelium form, a single putative cerebroside component was observed, having an R_f value identical to that of GlcCer from *A. fumigatus*, and stainable by both primulin and orcinol. In the yeast form extract, two such components, having R_f values identical to those of GlcCer and GalCer from *A. fumigatus*, were detected. Quantitation by densitometry after orcinol staining gave a proportion of 0.52/0.48 for the upper and lower *S. schenckii* yeast form components, respectively. All three *S. schenckii* components were isolated by normal phase column chromatography and preparative-scale HPTLC, and characterized by ^{1}H-NMR spectroscopy and ESI-MS.

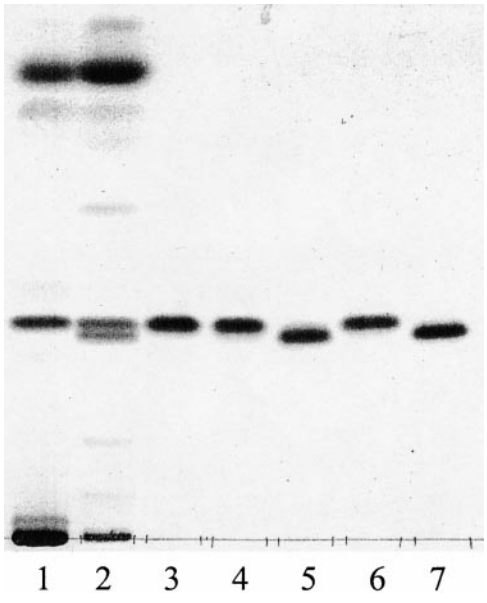


Fig. 1. HPTLC analysis of crude lipid fractions from *S. schenckii* mycelium and yeast form (lanes 1,2); purified CMH component from mycelium form (lane 3); purified higher and lower R_f CMH components from yeast form (lanes 4,5); and purified higher and lower R_f CMH components from *A. fumigatus* (lanes 6,7), previously characterized as GlcCer and GalCer, respectively. Approximately 3–5 μg total cerebroside was applied as a 5 mm streak in each lane; development and visualization were performed as described in Materials and Methods.

^{1}H-NMR spectroscopic analysis of *S. schenckii* cerebrosides

Both ^{1}H- and ^{13}C-NMR spectra for several fungal cerebrosides have been previously acquired in DMSO-d_6/2% D_2O at 35°C and all resonances assigned by homonuclear and heteronuclear 2-D correlation methods (33). It was therefore sufficient for the present work to obtain 1-D ^{1}H-NMR spectra on the three *S. schenckii* cerebrosides under identical conditions in order to characterize them with respect to monosaccharide identity and key ceramide structural features, including the presence or absence of (*E*)-Δ^3-unsaturation of the 2-hydroxy fatty *N*-acyl moiety.

The spectra of the high R_f components from both mycelium and yeast forms were almost identical (**Fig. 2**, panels A and B), each exhibiting a set of resonances with chemical shifts and coupling patterns characteristic for the 7-proton β-*gluco*pyranosyl spin system (38) (see Scheme 1 for structures and numbering), additional resonances identifying (4*E*,8*E*)-9-methyl-4,8-sphingadienine, and resonances for both *N*-2′-hydroxyalkanoate and *N*-2′-hydroxy-(*E*)-3′-alkenoate. The proportions of (*E*)-Δ^3-unsaturation, calculated from the relative integrals of the Fa-4 and Sph-5 resonances, were similar in both mycelium and yeast GlcCer fractions (39% and 37%, respectively, ±2%). Interestingly, although the differences in fatty acyl structure appear to have no effect on the chemical shift of β-Glc H-1, two resonances are observed at slightly different chemical shifts for the β-Glc H-2 in both spectra, due to the long-range influence of the (*E*)-Δ^3-unsaturation in ~40% of the ceramide in each fraction.

The spectrum of the low R_f component from the yeast form (Fig. 2, panel C), on the other hand, exhibited a set of resonances with chemical shifts and coupling patterns characteristic for the 7-proton β-*galacto*pyranosyl spin system (39) (**Scheme 1**). Additional resonances identifying the (4*E*,8*E*)-9-methyl-4,8-sphingadienine, along with both *N*-2′-hydroxyalkanoate and *N*-2′-hydroxy-(*E*)-3′-alkenoate, were again observed. The proportion of (*E*)-Δ^3-unsaturation in the yeast GalCer fraction was somewhat higher (54 ± 2%) than in either of the GlcCer fractions. Minor resonances corresponding to a small amount of GlcCer were also present, due to slight overlap of the two bands during the preparative HPTLC step.

An additional key upfield resonance for these compounds is that for the sphingadienine 9-methyl group, which was observed in all three spectra as a singlet at 1.545 ± 0.002 ppm (not shown). Remaining GSL structural features, not conveniently assessed by NMR analysis, are the chain lengths of the fatty acyl and sphingosine moieties. These were determined unambiguously by mass spectrometric methods, which in addition provided confirmation of most of the features discussed above.

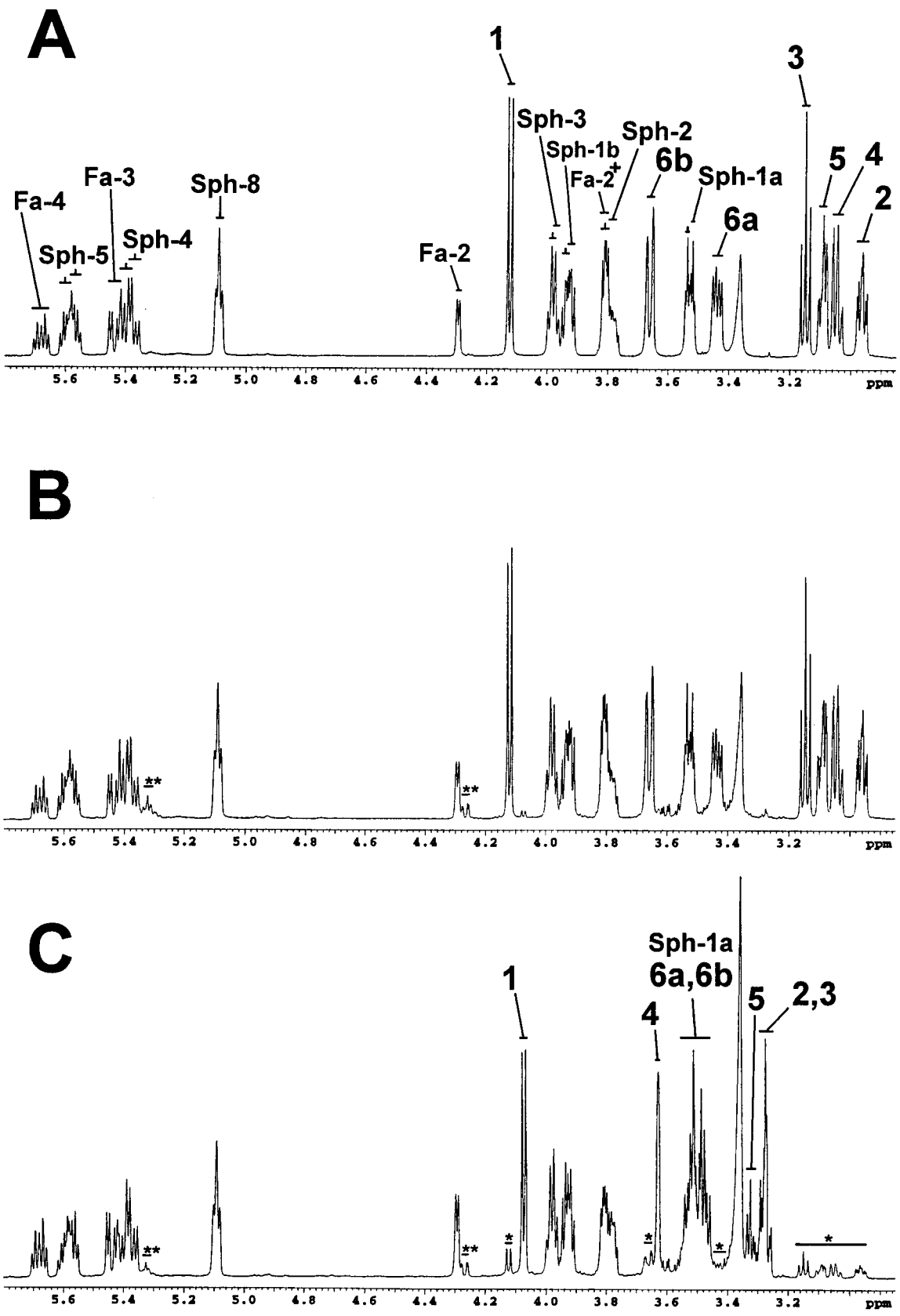
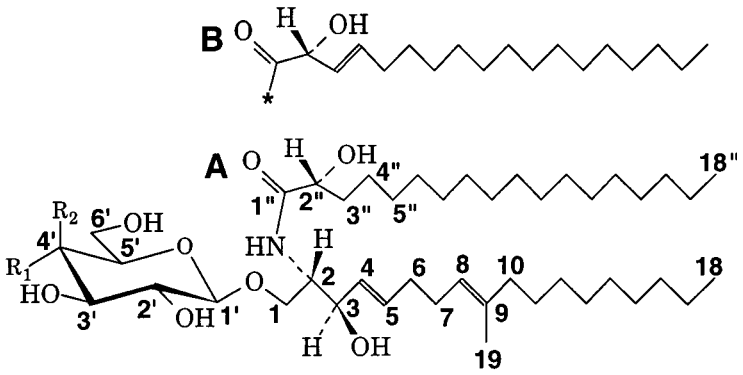


Fig. 2. Downfield sections of 1-D ^{1}H-NMR spectra of CMH fractions from *S. schenckii* (panel A, mycelium form CMH; panel B, high R_f yeast form CMH; panel C, low R_f yeast form component). Resonances from non-exchangeable protons of sphingosine (Sph), fatty acyl (Fa), and Hexose (prefix omitted) are designated by Arabic numerals. Resonances marked by * in panel C are from hexose moiety of high R_f CMH component incompletely separated in preparative HPTLC (see panel B). Resonances marked by ** in panels B and C are from an unknown impurity. Two locations each are shown for Sph-1a,1b to Sph-5 corresponding to components having saturated and unsaturated 2-hydroxy fatty acid. Fa-2$^+$ is H-2 from saturated 2-hydroxy fatty acid, isochronous with the upfield Sph-2 resonance.



Scheme 1. Structures of (4*E*,8*E*)-*N*-2′-hydroxyoctadecanoyl-1-β-d-glucopyranosyl- (fatty acid **A**, R_1 = OH, R_2 = H) (4*E*,8*E*)-*N*-2′-hydroxyoctadecanoyl-1-β-d-galactopyranosyl- (fatty acid **A**, R_1 = H, R_2 = OH) (4*E*,8*E*)-*N*-2′-hydroxyoctadec-(*E*)-3-enoyl-1-β-d-glucopyranosyl- (fatty acid **B**, R_1 = OH, R_2 = H), and (4*E*,8*E*)-*N*-2′-hydroxyoctadec-(*E*)-3-enoyl-1-β-d-galactopyranosyl- (fatty acid **B**, R_1 = H, R_2 = OH), -9-methyl-4,8-sphingadienines, with numbering of sphingosine, hexose, and fatty acid moieties.

Analysis of *S. schenckii* cerebrosides by electrospray ionization MS and tandem MS/MS

In positive ion mode ESI–MS, monosodiated pseudomolecular ions were observed at both *m/z* 776 and *m/z* 778 for all three cerebroside fractions (**Fig. 3,** panels A–C), corresponding to nominal molecular masses of 753 and 755 daltons, respectively. These molecular masses are consistent with monohexosylceramides containing (4*E*,8*E*)-9-methyl-4,8-sphingadienine attached to either *N*-2′-hydroxy-(*E*)-3′-octadecenoate or *N*-2′-hydroxyoctadecanoate, respectively. Confirmation that the observed differences of *m/z* 2 are in each case due to variation in the fatty acid moiety was provided by tandem ESI–MS/CID–MS experiments. In these experiments, product ion spectra were obtained from pseudomolecular ions, either *m/z* 776 or *m/z* 778, selected in Q1 (**Fig. 4,** panels A and B). All spectra were characterized by highly abundant [M + Na–acyl]$^+$ (O; we previously referred to this ion as "Ω" (33)) and [M + Na–HexOH–Sph-C_3-C_{19}]$^+$ (T) fragments, as observed previously under these conditions for fungal cerebrosides from *P. brasiliensis* and *A. fumigatus* (33). In these spectra, the O ion is always observed at *m/z* 496, while the *m/z* 2 difference is carried by the T fragment, containing the fatty acid moiety, observed at either *m/z* 346 or *m/z* 348 depending on the mass of the pseudomolecular ion selected.

These experiments provided the information lacking from the NMR spectra; in addition, the relative abundances of the pseudomolecular ions at *m/z* 776 and 778 (Fig. 3, panels A–C) confirmed that the GlcCer fractions from both mycelium and yeast forms of *S. schenckii* exhib-

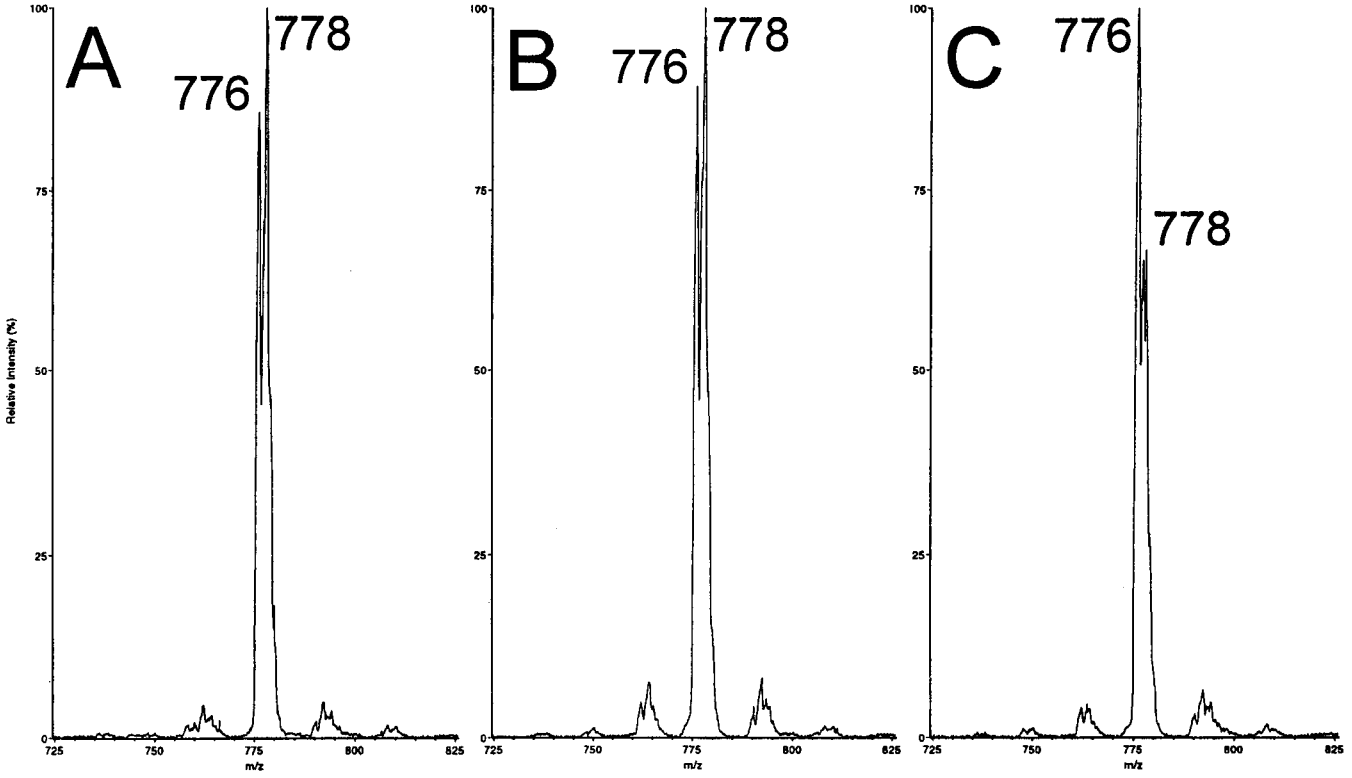


Fig. 3. $^+$ESI mass spectrometry of cerebrosides from *S. schenckii*. Panels A–C, pseudomolecular ion regions of $^+$ESI-MS spectra for mycelium form CMH, high R_f yeast form CMH, and low R_f yeast form CMH, respectively.

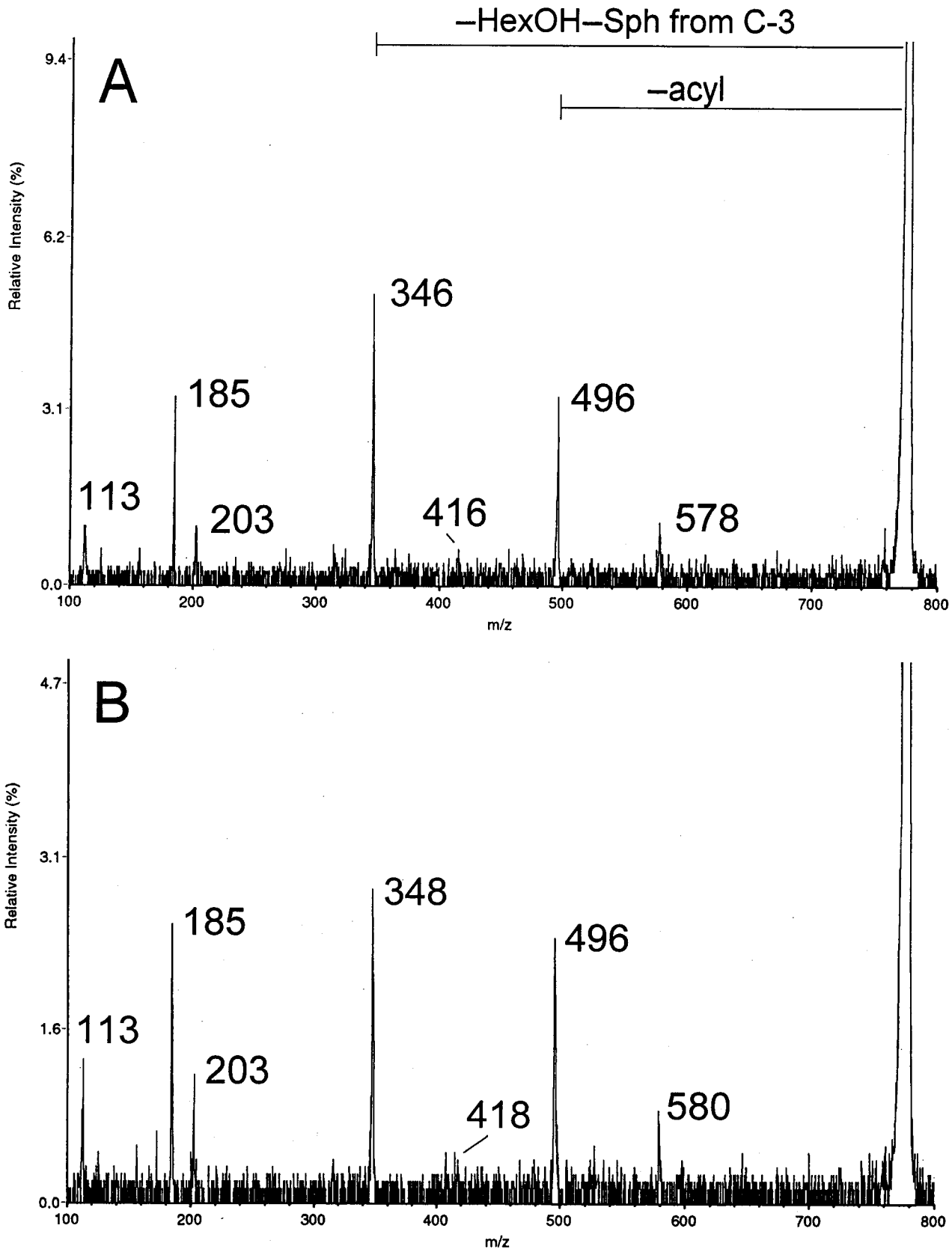


Fig. 4. Tandem $^{+}$ESI-MS/CID-MS product ion spectra of selected $[M+Na]^{+}$ from *S. schenckii* mycelium form cerebroside. Panel A, product ion spectrum from *m/z* 778; panel B, product ion spectrum from *m/z* 776. Similar pairs of CID spectra were obtained from both *S. schenckii* yeast form cerebrosides.

ited similar levels of (*E*)-Δ^3-unsaturation, while somewhat higher levels could be observed in the yeast form GalCer fraction. The apparent proportions of (*E*)-Δ^3-unsaturation differed from those calculated from NMR spectra, probably due to a somewhat higher ionization efficiency of the cerebroside carrying an additional double bond.

DISCUSSION

A careful examination by multiple techniques of cerebrosides from both forms of *S. schenckii* revealed a dimorphism with respect to hexose composition which correlated with the phenotypic dimorphism exhibited by this fungus. Only β-glucosylceramides were detected in the hyphal phase, while approximately equal amounts of β-galactosyl- and β-glucosylceramides were found in the yeast phase. These results suggest that activation of a GalCer synthase accompanies the mycelium→yeast transition, or that suppression of this activity accompanies the yeast →mycelium transition. It is also possible that the disappearance of GalCer in the mycelial phase results from activation of a β-galactose-specific cerebrosidase.

Besides its occurrence in the yeast form of *S. schenckii*, the simultaneous expression of both GlcCer and GalCer has been reported previously only for *Aspergillus fumigatus* (33, 40). The proportion of GalCer to GlcCer in *A. fumigatus* is apparently quite variable (33), although it is unclear at present whether this variability correlates with factors other than strain differences. Interestingly, the level of (*E*)-Δ^3-unsaturation was observed to be higher in GalCer than GlcCer for both *A. fumigatus* and the yeast form of *S. schenckii*, suggesting that homologous GalCer synthases, characterized by a higher activity than the GlcCer synthases for the (*E*)-Δ^3-unsaturated ceramide substrate, may be present in these two fungi.

In contrast to these variable structural features, the major long chain base in all three *S. schenckii* cerebrosides was found to be (4*E*,8*E*)-9-methyl-4,8-sphingadienine, as has been reported for the majority of fungi. Our results thus conflict with those of a previous study, in which it was reported that the yeast form cerebroside of *S. schenckii* consisted exclusively of glucose, sphing-4-enine ("sphingosine"), and 2-hydroxyoctadecanoic acid (41). In that study, the lower R_f component was observed but not analyzed.

Although cerebrosides containing (4*E*,8*E*)-9-methyl-4,8-sphingadienine are widely distributed in the fungal kingdom, little is known about their metabolism or possible functions. With respect to metabolism, it is of particular interest that the ceramides found in fungal cerebrosides are structurally distinct from those found in the glycosylinositol phosphorylceramides (GIPCs) of the same fungi, as has been pointed out independently by Dickson and Lester (16) and by us (33). Unlike the cerebrosides from *Candida albicans* (42), *P. brasiliensis* (33), and *H. capsulatum* (M. S. Toledo, S. B. Levery, A. H. Straus, and H. K. Takahashi, unpublished results), in which the ceramides are of the type reported here (Scheme 1), the GIPCs of these fungi are found predominantly with saturated, longer chain 2-hydroxy fatty acids (h24:0 or h26:0) attached to t18:0 4-hydroxysphinganine (phytosphingosine)(34, 43–45). It is not known how this partitioning of ceramide types into CMH and GIPC biosynthesis is accomplished, but two possibilities which have been suggested (16, 33) are compartmentalization of their respective biosynthetic and/or transport pathways, or selective recognition of ceramide structural elements somewhat remote from the reaction site by the putative IPC synthase (46) and the as yet uncharacterized fungal cerebroside synthases.

With respect to function, a number of reports have described physiological activities for fungal glucocerebrosides or ceramides derived from them in in vitro bioassays (25–32). Such studies are not necessarily demonstrations of true function, but are highly suggestive that significant functional roles exist for these compounds in vivo. In mammalian systems, considerable evidence has accumulated that simple sphingolipids such as cerebrosides, sphingomyelin, ceramide, or other products of sphingolipid catabolism, have definite functional roles, e.g., as activating or modulating elements of membrane-associated signaling cascades controlling major cellular events during development, morphogenesis, apoptosis, or stress response (18–20, 47–52). GSLs appear to be closely associated in caveolae with many of the receptor and transducer components of protein phosphorylation cascades (53), such as adenylyl cyclase, G_α and G_β, Ras, mitogen-activated protein kinase (MAPK), and protein kinase C (PKC), which are implicated in these processes. Because a growing body of evidence now suggests that homologous signal transduction enzymes regulate morphological transitions in fungi (54–61), the change in cerebroside composition associated with morphogenesis of *S. schenckii* may point to a similar functional involvement of GSLs in these processes.

Extensive and rapid alterations in GSL glycan expression correlating with induction of differentiation have been observed in some mammalian cells (62) and in parasites (63). Regarding dimorphic fungi, Barr et al. (44, 45) earlier reported significant qualitative changes in expression of GIPC glycans associated with morphogenesis in *H. capsulatum*, and quantitative differences in GIPC expression were observed between the yeast and mycelial phases of *P. brasiliensis* (34, 35, 64). GIPCs have been isolated from hyphae of *C. albicans* and shown to be similar to those found in *Saccharomyces cerevisiae* (43), but yeast forms of *C. albicans* have not been studied with respect to these compounds, nor have possible correlations of GIPC biosynthesis with dimorphism been examined in this pathogen. A great deal is now known about the control of GIPC biosynthesis in *S. cerevisiae* at the molecular level (reviewed in refs. 14–16), and considerable evidence has accumulated supporting key functional roles for GIPCs and their biosynthetic intermediates in signal transduction pathways governing stress response, calcium ion homeostasis, and regulation of the yeast cell cycle (15, 65, 66). An interesting point, however, is that cerebrosides of the type found in many fungi have not yet been identified in *S. cerevisiae*. Although isolations of putative cerebrosides from *S. cerevisiae* have been reported a number of times (67–

70), their structures were not elucidated unambiguously. The *possible* deletion of this pathway in at least some common strains of *S. cerevisiae* may in part account for the current lack of knowledge about the biosynthesis and in vivo functions of fungal cerebrosides, their catabolic intermediates, or their possible degradation products.

More relevant to possible functional correlations of cerebroside expression with dimorphism, Ghannoum et al. (71) reported quantitative differences in expression of some lipid classes, including ceramide monohexosides, between yeast and mycelial forms of *C. albicans* (ATCC 10231). Thus, while CMH comprised 9–10% of the total lipids extracted from the yeast form (measured by densitometry on two-dimensional thin-layer chromatograms), only trace amounts were detected in the mycelial form, whereas the total lipid content of the mycelial form was considerably higher than that found in the yeast form (3× and 5× in 12 and 96 h cultures, respectively). However, a later report by Matsubara et al. (42), in which the glucocerebrosides from both forms of *C. albicans* (strain 3125) were characterized in considerable detail by chemical and spectrometric methods, failed to note any quantitative difference in CMH content between the two forms. While it is possible that the issue was simply ignored in that work, it seems doubtful that such a significant quantitative difference in the lipid of interest would have drawn no comment had it been observed. It is possible that the discrepancy resulted from the difference in strain; an even more likely source could be differences in the conditions of culture and methods of selection of the two forms: in the first case the fungus was cultured under conditions promoting growth of interconverting yeast and mycelial forms (72), with separation of the two by filtration prior to extraction (71); in the second case the two forms were grown separately after transformation of yeast to mycelial form by a modification of the culture conditions (42).

As far as we are aware, the present report is the first description of altered cerebroside hexose structure in different phases of a dimorphic fungus. Previously, we reported cerebroside dimorphism of a different kind in *P. brasiliensis* (33). In the case of *P. brasiliensis*, both mycelium and yeast forms synthesize GlcCer exclusively; however, the mycelium form expresses (*E*)-Δ^3-unsaturation of the 2-hydroxy fatty *N*-acyl moiety at much higher levels than the yeast form. Preliminary observations with *H. capsulatum* indicate that it exhibits a cerebroside dimorphism similar to that of *P. brasiliensis* (M. S. Toledo, S. B. Levery, E. Suzuki, A. H. Straus, H. K. Takahashi, unpublished results). In contrast, GlcCer of yeast and hyphal forms of *C. albicans* were reported to be structurally identical with respect to their ceramide moieties, both having only saturated 2-hydroxy fatty acid (Scheme 1A; R_1 = OH, R_2 = H) (42).

Cerebroside synthesis in dimorphic mycopathogens thus appears to be a complex and variable phenomenon that should warrant careful further studies of both structure and biosynthesis in these fungi. The consequences of disruption of either gluco- or galactocerebroside synthesis are fatal in mammals (73–75); in the mouse a knockout of the UDP-Glc:ceramide β-glucosyltransferase resulted in lethality and resorption of embryos between 7.5 and 9.5 days post-coitum (73), while a knockout of the UDP-Gal:ceramide β-galactosyltransferase resulted in dismyelinosis leading to a progressive and ultimately fatal deficit in nerve conduction (74, 75). The possible functions of GlcCer in fungi may be unrelated to those in mammals, especially considering that this compound is an essential intermediate in the biosynthesis of most complex GSLs in mammals, but appears so far not to be further glycosylated in fungi; nevertheless, we anticipate that disruption of fungal GlcCer synthesis should have significant and interesting consequences. The possibility that products of degradative pathways could be functionally relevant is also worth examining. Although it is believed that ceramide and sphingoids used in signal transduction in mammalian cells are derived from either sphingomyelin degradation or de novo synthesis, but not from the products of cerebroside turnover (17–19, 47, 52, 76), and in *S. cerevisiae* from de novo synthesis, but not from products of GIPC turnover (15, 16), it cannot be excluded that pathways exist in other fungi for selective functional turnover of endogenous gluco- or galactocerebrosides.

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