The pathogenic yeast *Candida albicans* has the ability to synthesize unique sequences of β-1,2-oligomannosides that act as adhesins, induce cytokine production, and generate protective antibodies. Depending on the growth conditions, β-1,2-oligomannosides are associated with different carrier molecules in the cell wall. Structural evidence has been obtained for the presence of these residues in the polysaccharide moiety of the glycolipid, phospholipomannan (PLM). In this study, the refinement of purification techniques led to large quantities of PLM being extracted from *Candida albicans* cells. A combination of methanolysis, gas chromatography, mass spectrometry, and nuclear magnetic resonance analyses allowed the complete structure of PLM to be deduced. The lipid moiety was shown to consist of a phytoceramide associating a C18/C20 phytosphingosine and C24 hydroxy fatty acids. The spacer linking the glycan part was identified as a unique structure: -Man-P-Man-Ins-P-. Therefore, in contrast to the major class of membranous glycosphingolipids represented by mannose diinositol phosphoceramide, which is derived from mannose inositol phosphoceramide by the addition of inositol phosphate, PLM seems to be derived from mannose inositol phosphoceramide by the addition of mannose phosphate. In relation to a previous study of the glycan part of the molecule, the assignment of the second phosphorus position leads to the definition of PLM β-1,2-oligomannosides as unbranched linear structures that may reach up to 19 residues in length. Therefore, PLM appears to be a new type of glycosphingolipid, which is glycosylated extensively through a unique spacer. The conferred hydrophilic properties allow PLM to diffuse into the cell wall in which together with mannan it presents *C. albicans* β-1,2-oligomannosides to host cells.

Sphingolipids are ubiquitous and essential components of living cells found mainly on the outer leaflet of plasma membranes (1–3). Along with their role in membrane permeability and fluidity, they have also been shown to act as second messengers produced in response to various stress situations, which regulate basic processes such as cell cycle control, apoptosis (4), cell-cell interactions (1), and immune response (5).

Sphingolipids are ceramide structures composed of a long chain base whose amino group is amide-linked to various fatty acids. This basic structure as well as those derived by the addition of polar groups such as phosphocholine or carboxydrates to the ceramide may vary according to the species (6–8). It has been established that sphingolipids from fungal and mammalian cells display important differences in their fine structure and biosynthetic pathways (9). Fungal species incorporate phytosphingosine (PHS) and presumably dihydrosphingosine in the ceramide moieties instead of the sphingosine used mainly by mammals and also have longer fatty acids (C24-C26 instead of C16-C18) (9). Fungi then preferentially add inositol to the ceramide group, leading to the family of inositol phosphoceramides composed of inositol phosphoceramide (IPC), mannose inositol phosphoceramide (MIPC), and mannose diinositol phosphoceramide (M(IP)2C) instead of phosphocholine in mammalian cells (9). These differences between mammalian cells and fungi represent a target for antifungal drugs such as aurobasidin A (10) and khafrefungin (11), which inhibit IPC synthase. Along with sphingomyelin of mammals and IPCs of fungi and plants, glycosylceramides are another important class of sphingolipids described in fungi and mammals in which the sugar moiety is directly linked to the ceramide (6). The biosynthetic pathway of fungal sphingolipids has only been studied in the yeast *Saccharomyces cerevisiae*. Numerous steps in the pathway are still unknown (12), and the enzymes involved have not yet been purified or characterized. This lack of knowledge contrasts with the key role of these molecules in cell growth (13) and viability (10), resistance to various environmental stresses (14), remodeling of glycosyl phosphtidylinositol anchors (15), endocytosis (16), and calcium homeostasis (17).

Although *Candida albicans* is the prominent opportunistic fungal pathogen causing mucocutaneous and systemic infections (18, 19), almost nothing is known about its sphingolipids with the exception that a glucosyleramidase has been described.

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¶ To whom correspondence should be addressed. Tel.: 33-3-20-62-34-20; Fax: 33-3-20-62-34-16; E-mail: dan_poulain@compuserve.com.
previously (20) and that IPCs, MIPC, and M(IP)2C have recently been characterized in its hyphal form (21). In a series of studies, some immunochemical properties of a *C. albicans* glycolipid containing long chains of β-1,2-linked mannose residues have been demonstrated and characterized previously (22). *C. albicans* β-1,2-oligomannosides, which act as adhesins (23, 24), induce cytokine production, and generate protective antibodies (25, 26), are considered to be among the virulence factors of this species. Using a model of *C. albicans* macrophage interaction, it was shown that phospholipomannan (PLM) shed by *C. albicans* acted as a vector for β-1,2-oligomannosides in inducing strong cellular stimulation (27).

The aim of this study was to investigate the complete structure of this pathophysiologically relevant molecule of *C. albicans*. A purification procedure was developed to obtain large quantities of soluble PLM, and phase gas chromatography (GC) coupled to mass spectrometry (MS) analysis of methanolysis products, extensive NMR, and MS analyses were carried out. A combination of the results of these analyses allowed the complete structure of PLM to be determined and demonstrated that PLM is a new type of mannose inositol phosphoceramide.

**EXPERIMENTAL PROCEDURES**

**Strain and Growth Conditions—** *C. albicans* serotype A, strain VW32, was used throughout this study. For large scale cell production, cells were inoculated on Sabouraud’s agar for 18 h at 28 or 37 °C and then inoculated at a concentration of 106 cells/ml in 1-liter Erlenmeyer flasks containing 500 ml of Sabouraud’s broth. Growth was performed to stationary phase at 28 or 37 °C on an orbital shaker (180 rpm). Cells were finally collected by centrifugation.

**Purification of PLM—** Cells were broken with a French press (Amino) at 20,000 p.s.i., dialyzed, and lyophilized. PLM was then extracted and purified as described previously (22) with the exception that care was taken to avoid micelle formation to improve PLM solubility for NMR studies. Methodologies to the modification were mainly the lyophilization of most water phases or fractions instead of centrifuge concentration. Modifications to the method were mainly the lyophilization of most water phases or fractions instead of centrifuge concentration. NMR studies. Some immunochemical properties of a *C. albicans* strain were investigated (20) and that IPCs, MIPC, and M(IP)2C have revealed for PLMs synthesized by *C. albicans*.

**RESULTS**

Analysis of Molar Composition of PLM Revealed That the β-Oligomannosside Chain Is Anchored to a Ceramide Moiety—Our GC/MS analysis of the methanolysis products of PLM allowed us to establish in a single experiment the identification and relative ratio of each PLM component (Table I). It revealed for PLMs synthesized by *C. albicans* strain VW32 at 28°C (PLM-28) or 37°C (PLM-37) an average composition of 12 or 13 mannose residues for one inositol, one long chain base and one fatty acid. The major long chain bases were identified by their electron impact spectra (Fig. 1) as the 1,3,4-trihydroxy-2-amino-15-methyl-heptadecane (ramified C18 phytosphingosine or rC18 PHS) (Fig. 1c) and 1,3,4-trihydroxy-2-amino-15-cyclohexyl-nonadecane (rC18 PHS) (Fig. id). Minor components were identified as 1,3,4-trihydroxy-2-amino-octadecane and 1,3,4-trihydroxy-2-amino-eicosane (Fig. 1i), i.e., the corresponding non-ramified PHSs. The major fatty acid (FA) was 2-hydroxy-21-methyl-tricosanoate, a ramified hydroxylated fatty acid with 24 carbon atoms (OH rC24 FA) (Fig. 1b). Other fatty acids were the homologues with 25 and 26 carbon atoms all

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hydroxylated on carbon 2 and ramified in the \( n-3 \) position (OH rC25 FA and OH rC26 FA, respectively). Linear fatty acids (hexadecanoate and octadecanoate) were also present at a lower level. No trace of monosaccharides other than mannose was detected. In contrast, a small peak with a typical fragmentation pattern revealed the presence of mannose-6-phosphate.

The molar composition of PLM-28 and PLM-37 appeared to be similar as did their OH rC24 FA ratio (Table I). The main modifications concerned an inversion of the relative ratios of OH rC25 FA and OH rC26 FA and a marked inversion of rC18 PHS and rC20 PHS, the latter becoming the most abundant in PLM-37.

**Table I**

Comparison of the composition of phospholipomannans synthesized by *C. albicans* VW32 at 28 and 37 °C

| Component         | No. residues | Component     | Total FA  | Component     | Total LCB |
|-------------------|--------------|---------------|-----------|---------------|-----------|
| Mannose           | 12.07        | OH rC24      | 71.16     | rC14 PHS      | 52.81     |
| Inositol          | 0.987        | OH rC25      | 4.75      | C14 PHS       | 2.68      |
| Fatty acid\(^a\)  | 0.992        | OH rC26      | 6.22      | rC26 PHS      | 41.8      |
| LCB\(^b\)         | 1.00         | C16:0        | 7.63      | C20 PHS       | 2.69      |
|                   |              | C18:0        | 7.96      |               |           |
|                   |              | C18:1        | 2.28      |               |           |
| Mannose           | 13.21        | OH rC24      | 70.21     | rC14 PHS      | 29.13     |
| Inositol          | 0.891        | OH rC25      | 9.02      | C14 PHS       | 2.19      |
| Fatty acid\(^a\)  | 0.975        | OH rC26      | 4.54      | rC26 PHS      | 64.54     |
| LCB\(^b\)         | 1.00         | C16:0        | 9.68      | C20 PHS       | 4.12      |
|                   |              | C18:0        |           |               |           |

\( ^a \) See the details of the fatty acids composition in the column Fatty Acids.

\( ^b \) See the details of the long chain base composition in the column Long Chain Base.

\( ^c \) r refers to a ramification of the chain in the position of (n-3).

**MS Analysis of PLM Revealed Crossed Heterogeneity Arising from Its Carbohydrate and Lipid Moieties**—Electrospray mass spectra of PLM-37 (Fig. 2a) and PLM-28 (Fig. 2b) demonstrated a series of groups of five peaks that covered the entire spectra. Among these groups, those spaced by an average \( m/z \) ratio of 81 were observed. These corresponded to (M + 2H)\(^+\) molecular-related ions and consequently to the varying degrees of polymerization of the glycan chain of PLM seen in a previous study (22). Conversely, the five peaks within each group arose from heterogeneity of the lipid part of PLM, because they displayed spaced \( m/z \) ratios of 7, which therefore corresponded to a mass change of 14 (one \( \text{CH}_2 \) group). According to the results of

The molar composition of PLM-28 and PLM-37 appeared to be similar as did their OH rC24 FA ratio (Table I). The main modifications concerned an inversion of the relative ratios of OH rC25 FA and OH rC26 FA and a marked inversion of rC18 PHS and rC20 PHS, the latter becoming the most abundant in PLM-37.
GC/MS analysis of methanolysis products and to the relative ratios of their lipid components, these peaks can be attributed to the different compositions of the ceramide moiety as shown in Table II for PLM-37. This interpretation of the results was confirmed when the PLM-28 and PLM-37 spectra were compared (Figs. 2, a and b), because at 28 °C, for each degree of

FIG. 2. Electrospray mass spectra of phospholipomannan synthesized by C. albicans strain VW32 at 37 (a) and 28 °C (b). Two complex families of molecular-related ions, (M - 2H)/2 (for example, m/z 1650.49) and (M - 2H + Cl)/3 (for example, m/z 1274.49), that partially overlapped were produced from the original molecules. For each family, groups of peaks corresponded to the various degrees of polymerization of the glycan part, and peaks within each group arose from the variability of the lipid part. The shift observed for the main peak of each group between PLM-37 and PLM-28 corresponds to a change of the number of carbons of the phytosphingosine unit. The m/z values observed on these spectra differed slightly from the values reported in Table II because of smoothing of the spectra.

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were spaced by an average 1274.35 (M/H11002 1657.80 (M − 2H)/2 3317.61 14 1 1 rC20 1O H rC26 2 mass value 1731.59 (M − 2H)/2 3465.19 (Major)b 15 1 1 rC18 1O H rC26 2 1210.62 (M − 2H + Cl)/3 3598.42 16 1 1 rC18 1O H rC26 2 1220.38 (M − 2H + Cl)/3 3614.23 16 1 1 rC18 1O H rC26 2 1224.89 (M − 2H + Cl)/3 3641.23 16 1 1 rC18 1O H rC26 2 1274.35 (M − 2H + Cl)/3 3789.61 (Major)b 17 1 1 rC18 1O H rC26 2 of the glycan part of PLM (Table II). Peaks within each group were spaced by an average m/z ratio of 4.66, which corresponded to a mass variation of 14 arising from the lipid part (Table II). As confirmed from their respective spacing, groups of peaks from the two families regularly overlapped, leading to common groups of peaks, i.e. groups with a main peak at m/z 1166.19 or 1327.51 on Fig. 2a. This finding was also confirmed by a slight shift of the m/z values (data not shown) and from a modification of the relative ratios of the peaks within these groups explained by the heterogeneous overlapping of the families, because they vary respectively according to M/3 and M/2.

Therefore, the two families of groups of peaks observed in the two spectra appeared to originate from the same native molecules. According to these results, PLM may contain 3 − 20 Man residues/molecule with PLM containing 14 Man residues being the most abundant. In both spectra, the two main peaks at m/z ~678 and ~1357 appeared unrelated to the PLM families and seemed to correspond to (M − 2H)/2 and (M − H) molecular-related ions from the same molecule, which according to its mass and the results of NMR analysis (see below) is probably M(IP)3C. As observed for PLM, M(IP)3C is also heterogeneous and seems to express the same variability in its ceramide moiety.

NMR Analysis of PLM Revealed That a Specific Spacer Is Used to Anchor the β-1,2-Man Polymer to the PHS of the Ceramide Moiety—Low solubility of glycolipids leads to micelle formation, which affects the relaxation time of molecules in NMR experiments. Therefore, previous NMR analysis of PLM has been restricted to water-soluble glycolipids released by alkaline treatment (22). A new PLM preparation procedure, avoiding micelle formation, and the use of Me3SO-d6, known to improve the solubility of glycolipids, have allowed NMR studies of the native molecule. In complementation to previous analysis of the glycan part performed in D2O, this study mainly focused on the structure of the spacer that links glycans (β-1,2-oligomannosides) to the lipid moiety and resolves previous ambiguity regarding the position of the second phosphate group of PLM.

NMR analyses have consisted of: (i) a series of COSY experiments to define the 1H chemical shifts in the sugar, inositol, fatty acid, and phytosphingosine units; (ii) 1H-13C and 1H-31P HMBC correlations to define the 13C chemical shifts and the nature of the phosphodiester bridges; and (iii) a ROESY experiment that has established the linkage anomer of the sugar units, their sequence, and their linkage with inositol and phytosphingosine.

The 1H NMR spectra of PLM-28 and PLM-37 of C. albicans were almost identical, and the anomeric signals of the sugar units were designated A to G according to the decreasing chemical shift of their resonances (Fig. 3a and b). IN, SP, and FA were also used to indicate myo-inositol, phytosphingosine, and fatty acid signals, respectively, to avoid excessive spectra overload. Because the coupling constants of the anomeric signals could not be measured accurately, the anomeric configuration of Man units A to G was established on the basis of their intraresidues connectivities, H-1/2H and H-1/H-3, H-5 for β-Man by ROESY experiments (see below). The anomeric configuration of α-Man units A and B was also revealed by their characteristic downfield-shifted 1H-5 resonance as well as by their upfield-shifted 13C-5 resonance (see Table III). In this way, sugar units A and B on the one hand and C to G on the other hand were identified as α- and β-Man units, respectively. Moreover, the relatively high coupling (J9 = 7.2 Hz), which splits the H-1 resonance of α-Man unit A, is most probably attributed to the presence of a phosphate group as confirmed by heteronuclear 1H-31P NMR spectroscopy.

Because two-dimensional COSY, ROESY, and 1H-13C or 1H-31P HMBC correlation spectra of both PLMs were essentially identical (data not shown), the assignment of 1H and 13C chemical shifts was performed using two-dimensional NMR spectra of PLM-28 or PLM-37.

The starting point for interpretation of the COSY (Fig. 4) and one-step or two-step delayed COSY spectra (data not shown) was the H-1 signal of sugar residues A to G and the H-5 signal of IN and (CH2) of FA and SP. A combination of these NMR analyses allowed the assignment of the 1H chemical shifts of H-1, H-2, H-3, H-4, H-5, and H-6 of Man units A to G and also
the assignment of GA2, SP, and IN1–6 resonances (Table III). A split of the IN2, IN3, and IN6 signals (IN2*, IN3*, and IN6*, respectively) was also observed for PLM-28 (Fig. 4) and was attributed to heterogeneity of the material as described below.

The two-dimensional ¹H-¹³C HMQC spectrum of PLM-28 (Fig. 5a) allowed the assignment of most of the ¹³C resonances (Table III) with the exception of those belonging to α-Man unit B, which were deduced from a comparison of the ¹H-¹H COSY, ¹H-¹³C, and ¹H-³¹P HMQC spectra (data not shown). The complete assignment of the ¹³C resonances was achieved by interpretation of the ¹H-¹³C HMQC-HOHAHA spectrum (Fig. 5b). This experiment provided individual C-2 to C-6 resonances of sugar units G, C, D, A, F, and E, starting from their respective H-2 atom resonance as explained in Fig. 5. The complete series of ¹H and ¹³C resonances of the inositol unit was also observed, and the IN C-4 and C-6 resonances in particular were clearly
assigned although they were masked on the $^1$H-$^{13}$C HMQC spectrum by C-3 resonances of sugar units C, D, F, and G. Moreover, the complete series of $^1$H resonances belonging to sugar unit B was also observed.

The $^1$H-$^{31}$P HMQC spectrum of PLM-28 (data not shown) revealed the presence of two $^{31}$P resonances connected to the four protons indicated by arrows in Fig. 6. Two of these protons were unambiguously assigned to A1 and IN1 by comparison.
FIG. 5. $^1$H-$^{13}$C HMQC (a) and HMQC-HOHAHA (b) spectra of PLM-28 allowed the assignments of $^{13}$C resonances precised in Table III. Labels A to G, IN, FA, and SP refer to signals from Man units inositol, fatty acid, and phytosphingosine, respectively. On the HMQC spectrum (a), the $x$ axis displayed the $^1$H shifts of protons as previously determined by COSY experiments. Their projection on the $y$ axis allowed us to define the corresponding $^{13}$C chemical shift. On the HMQC-HOHAHA spectrum (b), the $^1$H-$^{13}$C correlations were read vertically, i.e. from $^1$H-2 E (E2) at $\delta = 3.781$, the $^{13}$C-6, $^{13}$C-4, $^{13}$C-2, $^{13}$C-3, and $^{13}$C-5 signal resonances (noted E6, E4, E2, E3, and E5) were assigned, respectively. $^{13}$C-$^1$H correlations were read horizontally, i.e. from $^{13}$C-1 signal at $\delta = 76.85$ of inositol IN1, the $^1$H signal resonances IN5, IN3, IN4, IN6, and IN2 were assigned.
with the COSY spectrum. The assignment of SP1a and SP1b 1H resonances was also inferred from this comparison and was in agreement with previous NMR data (7).

The 1H,31P HMQC-HOHAHA spectrum exhibited a complex panel of correlations for the two 31P resonances (Fig. 6). By comparison with the COSY spectra, the A2, A3, and B2 signals could easily be connected with the 31P signal at δ = 0.9. This observation proved the presence of a phosphodiester bridge between the anomeric carbon of sugar unit A and one of the carbons of sugar unit B. From the 1H,13C HMQC spectrum (Fig. 5a) and comparison with the 1H,31P HMQC spectrum, the 1H resonance observed at δ = 3.88, which correlated with a 13C signal at δ = 65.85, could therefore be defined as the H6a-H6b resonance of sugar unit B. This assertion was confirmed by the observation of B2, B3, B4, B5, and B6 horizontal alignment at δ = 71.85 on the 1H,13C HMQC-HOHAHA spectrum (Fig. 5b). This observation was consistent with the establishment of the sequence A-P-B, namely Manα-P-6-Mano. The second line of 1H-31P correlations at δ = 3.22 exhibited the complete IN proton spin system as well as the H-1a, H-1b, H-2, and H-3 atom resonances of the phytosphingosine unit (SP1a, SP1b, SP2, and SP3). From this observation, the linking structure IN-1-P-SP was established.

The definitive sequence of PLM was finally achieved through ROESY experiments. The sequences G-F-A and B-IN were suggested from the interresidual correlations between G1-F2, F1-A1, and B1-IN2 (Fig. 7). Because the 1H,31P HMQC-HOHAHA spectra previously analyzed (Fig. 6) have shown that the two phosphate groups were part of the two sequences IN-P-SP and A-P-6-B, the sequence of the linking structure was fully established as α-α-Manp-1→P-(O→6)-α-α-Manp-(1→2)-IN-1-P-(O→1)-SP. Concerning the glycan part whose β-1,2-oligomannoside structure was established previously (22), a comparison of the proton and carbon chemical shift values (Table III) provided evidence for the non-reducing terminal position of the β-Man E unit. However, for Man units C and D involved in the repeated sequence of β-1,2-linked mannoses, no more information could be deduced regarding their exact position because of the polydispersity of the molecule.

These results allowed us to define the respective positions of units A to G, IN, SP, and the phosphate groups (Fig. 5a) and to propose a schematic structure of PLM, which specifies its heterogeneity (Fig. 8b).

The split of IN2, IN3, IN6, and B1 signals, previously referred to as IN2*, IN3*, IN6*, and B1* and observed mainly in PLM-28 (Figs. 4 and 7) appeared to arise from the same molecule, which may correspond to the (M - 2H)/2 molecular-related ion observed on MS spectra at m/z 677.94 (Fig. 2b). Its deduced mass closely fits the following sequences: Man-α-P-Man-α-Ins-P-Cer or Ins-P-Man-α-Ins-P-Cer (M/IP2C). We suggest that this structure is most probably M(IP)2C because: (i) examination of the COSY and HMQC spectra of PLM-28 revealed that β-Man unit E was the only one that occurred in the terminal position; (ii) NMR data revealed the absence of a terminal non-reducing α-Man unit; and (iii) the HMQC spectrum of PLM-28 clearly showed that the IN* unit was terminal according to its C-2 atom resonance observed at δ = 73.5 (Fig. 5).

DISCUSSION

The poor solubility of glycolipids leading to the formation of micelles is a major limitation to their physicochemical analysis. Therefore, a previous structural study of C. albicans PLM has been restricted to its highly soluble glycan part released by saponification (22). This current NMR and MS study has confirmed the two unusual properties of this molecule that were expected from previous immunchemical analyses, i.e., the β-anomery of the linkages and the high degrees of polymerization of the oligomannoside chains (22, 28). The presence of two phosphate groups in the molecule was also demonstrated, most probably the first connecting the glycan chain to the lipid moiety and the second presumed to be used to branch a short oligomannoside chain. The presence of this second phosphate group was confirmed by fluorhydric acid hydrolysis that led to a marked modification of the major polymer migration on TLC together with the liberation of a dimannoside or trimannoside (data not shown). The most appropriate method to address the question of the position of the phosphate groups is phosphor/ proton NMR analysis. This required: i) the purification of large quantities of PLM (>5 mg), ii) the analysis of the whole molecule since saponification was shown to affect the environment of at least one phosphate group and to induce an additive heterogeneity of the mass spectra (22), and iii) high solubility of the whole molecule in a solvent system. These problems were overcome by improving the step-by-step purification procedure and by using as the solvent for NMR studies dimethyl sulfoxide, which allowed a better solubility of PLM than H2O.
Methanolysis of PLM revealed the presence of rC18 or rC20 PHS and rC24, rC25, or rC26 hydroxylated fatty acids in addition to mannoses and inositol. These results showed that PLM is related to the family of IPC, MIPC, and M(IP)2C previously observed in C. albicans (21). Nevertheless, the electron impact spectra of methanolysis products newly evidenced predominant branched aliphatic chains in both the long chain bases and fatty acids of the ceramide moiety.

As shown previously, the glycan chain was the major source of heterogeneity of the molecule because electrospray mass spectrometry revealed 3–20 mannose units/molecule, the majority of PLM molecules presenting >11 mannose units. Such heterogeneity could be constitutive or may result from the different steps of biosynthesis. The first hypothesis is more coherent with Western blot analysis of cell wall extracts of C. albicans that are supposed to contain mature PLM, which nevertheless revealed heterogeneity of the molecule. However, mass spectrometry together with analysis of methanolysis products also demonstrated the heterogeneity of the ceramide moiety. This was based mainly on the presence of rC18 or rC20 PHS-OH rC24 FA for PLM at 28 °C or rC20 PHS-OH rC24 FA for PLM at 37 °C.

**FIG. 7.** ROESY spectrum of PLM-37. α-Man units A and B were characterized by H-1/H-2 intraresidual NOE contacts, whereas β-Man units C to G showed intense H-1/H-3, and H-5 intraresidual connectivities. Interresidual correlations between A to F and B-IN are also observed.

**FIG. 8.** Schematic structure of PLM of C. albicans strain VW32, serotype A. a, detailed structure showing the position in the molecule of the components that displayed nuclear magnetic resonance signals A to G, IN, SP, and FA. b, general structure of PLM pointing out the heterogeneity of its glycan moiety. The ceramide moiety (Cer) also heterogeneous is mainly composed of rC18 PHS-OH rC24 FA for PLM at 28 °C or rC20 PHS-OH rC24 FA for PLM at 37 °C.

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P.-A. Trinel, E. Maes, J.-P. Zanetta, F. Delplace, B. Coddeville, T. Jouault, G. Strecker, and D. Poulain, unpublished data.
the $C_{20}/C_{18}$ PHS ratio has also been reported for S. cerevisiae during heat shock. It was transient for free PHS (15 min) but more stable for PHS found in phytoceramides (at least 1 h) (32) and was proposed to act as a key signaling event in the heat stress response including the induction of trehalose accumulation (32), a cellular thermoprotectant (33). Therefore, it seems that C. albicans and S. cerevisiae respond similarly to temperature by increasing $C_{20}$ PHS. However, this increase is continuous, and $r_{C_{20}}/r_{C_{18}}$ PHS ratios are more important in C. albicans for which the consequences of this shift on both yeast cell metabolism and host-parasite relationships remain to be determined.

Similar modifications linked to growth temperature were also observed in a molecule associated with our PLM preparations with the characteristics of $M(IP)_2C$, the main sphingolipid of plasma membranes (3). This was deduced from MS analysis, which displayed the expected masses related to variability of the ceramide moiety similar to that of PLM, whereas NMR studies revealed additional $IN_2^a$, $IN_3^a$, and $IN_6^a$ signals arising from unsubstituted inositol. The presence of this structurally related molecule was not surprising because our purification procedure was designed to address to the whole PLM family, which encompasses a number of molecules that diverge according to their amphipathic character. Among these, poorly glycosylated PLMs have physicochemical properties closely related to $M(IP)_2C$. We may also expect in the peaks allocated to $M(IP)_2C$ a minor interference of Man-P-Man-IPC that display the same masses and correspond to the first biosynthesis step specific for PLM.

However, because of the selectivity of the NMR signals, the presence of $M(IP)_2C$ did not change the interpretation of our results and allowed us to determine the general structure of PLM, particularly the position of the two phosphate groups. As suggested previously, the first phosphate group links the inositol to the PHS of the lipid moiety. The second group, formerly thought to be associated with a long lateral oligomannose chain, appears to be a component of the spacer that links the $\beta$-1,2-oligomannose chain to the lipid anchor. The structure of this spacer (Man-P-Man-Ins-P-), although closely related to the polar group of $M(IP)_2C$, -Ins-P-Man-Ins-P-, has never been described for a glycosphingolipid and appears to be more complex than those of other fungal glycoinositol sphingolipids (8) described for a glycosphingolipid and appears to be more complex than those of other fungal glycoinositol sphingolipids (8). Nevertheless, the $\beta$-1,2-oligomannosidase is regulated by transcription pathways will be an interesting question to address.

In conclusion, this study shows that the prominent opportunistic fungal pathogen C. albicans uses the biosynthetic pathway of membrane sphingolipids to express an unusual linear mannose polymer of $\beta$-1,2-linked mannose on its surface. As $\beta$-1,2-oligomannosides are known to have an important role as adhesins, induce cytokine production, and generate protective antibodies, it can be anticipated that this alternative mode of presentation to host cells may have some significance in terms of pathogenesis.

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