J. Microbiol. Biotechnol. (2004), **14**(4), 868–871

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Lysophosphatidylcholine Suppresses the Expression of Phr1p and Pra1p, Surface Proteins Involved in the Morphogenesis of *Candida albicans*

SHIN, DUCK-HYANG, WON-YOUNG CHOI, YUNG-JOON YOO[1], MIN-KYOUNG KIM, AND WONJA CHOI*

Department of Life Science, College of Natural Sciences, Ewha Women University, Seoul 120-750, Korea
[1]Department of Life Science, Kwangju Institute of Science and Technology (K-JIST), Kwangju 500-712, Korea

Received: September 5, 2003
Accepted: November 10, 2003

Abstract *Candida albicans* has become the most important human pathogen in immunocompromised patients. One important feature of the pathogenicity in *C. albicans* is the morphological transition from yeast to hyphae. Previously, we reported that lysophosphatidylcholine (Lyso-PC) suppressed the hyphal transition through the MAP kinase pathway (Min *et al.*, 2001). Therefore, it should be useful to examine the unknown genes involved in the MAP kinase pathway. As a way to identify target genes of Lyso-PC in hyphal suppression, this present study exploited two-dimensional electrophoresis. It was revealed that Lyso-PC suppressed expression of Phr1p and Pra1p, surface proteins involved in the morphogenesis.

Key words: *Candida albicans*, two-dimensional electrophoresis, morphological transition, hyphal suppression, lysophosphatidylcholine, *PHR1*, *PRA1*

Candida albicans is an opportunistic human pathogen, causing common superficial infections as well as life-threatening disseminated and organ infections, especially in immunocompromised patients with AIDS or diabetes and under cancer therapy or organ transplantation [8].

Although candidiasis has increased remarkably during the past decades, effective drugs are still not available. An azol compound, amphotericin, that has been used for the treatment of systemic candida infection is not applicable in the late stage of AIDS due to its toxicity. Even worse, candida strains resistant to azol have become widespread. Therefore, the development of a new drug is urgent and necessary for the treatment of candidiasis.

The morphogenic transition from yeast to hyphae that invade human tissues is induced by various stresses related to growth such as temperature, pH, nitrogen or carbon starvation, etc. [12, 13]. These exogenous factors transmit a signal to the mitogen-activated protein (MAP) kinase pathway, Ras-cyclic adenosine monophosphate (Ras-cAMP) pathway, or unknown pathways [3, 11, 16, 19]. Several transcriptional regulators, including *TUP1* [1], *CPH1*, and *EFG1* genes, have been identified as key elements in controlling filamentous growth. Each gene represents a separate signal transduction pathway [2].

Previously, we reported that 2-lysophosphatidylcholine (Lyso-PC) prepared from deer antler extract suppressed the hyphal transition from yeast to hyphae in *C. albicans* [21, 22]. Lyso-PC specifically inhibits the transition from yeast to hyphae, but does not affect the growth of either yeast or hyphae. It inhibits the hyphal transition through the MAP kinase pathway, rather than the Ras-cAMP pathway [15].

Since *C. albicans* is a diploid and has no known sexual cycle, identification of most genes involved in the transition pathway has been dependent on the information from *Saccharomyces cerevisiae*. To overcome this limitation and to identify the effects of Lyso-PC, in this study, we performed the proteomic analysis by using two-dimensional polyacrylamide gel electrophoresis (2-D PAGE). We compared protein profiles between Lyso-PC-treated and nontreated hyphal extract and identified the genes influenced by Lyso-PC. Here, we report that the expression of those proteins was considerably reduced by Lyso-PC, suggesting that Lyco-PC could be a good candidate for the development of a new drug for candidiasis.

The *C. albicans* strain used in this study was SC5314 (wild-type). Cells were routinely grown in liquid Sabouraud-dextrose medium (Difco, Detroit, MI, U.S.A.) at 30°C overnight. For hyphal induction, yeast cells grown to the stationary phase at 30°C were transferred into RPMI-1640 medium (Gibco-BRL, Gaithersburg, MD, U.S.A.) and further grown at 37°C for 24 h. For the repression of hyphal transition, cells were treated with 200 μM of Lyso-PC (Sigma, St. Louis, MO, U.S.A.). Then cell extracts

**Corresponding author*
Phone: 82-2-3277-2892; Fax: 82-2-3277-2385;
E-mail: wjchoi@ewha.ac.kr

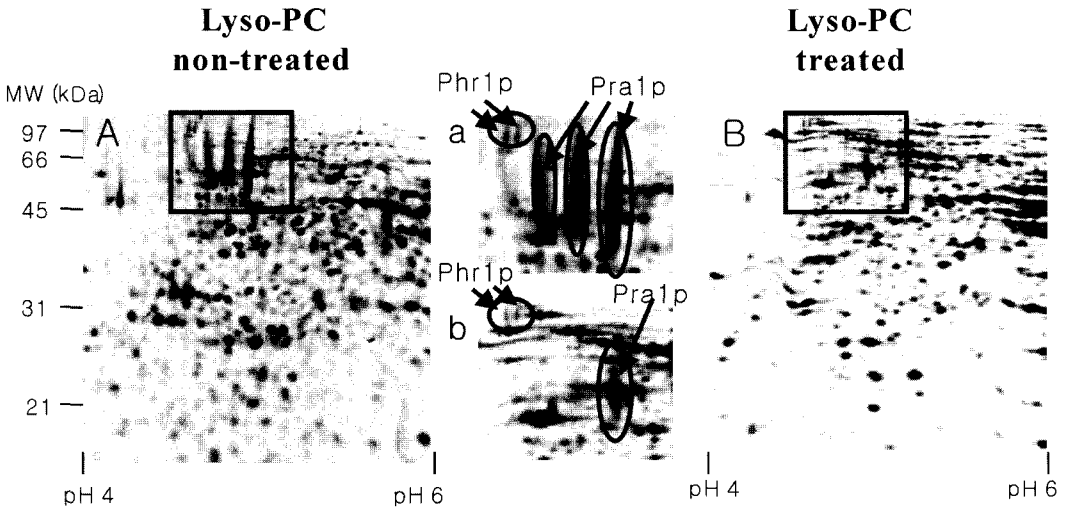

Fig. 1. Protein profile comparison between Lyso-PC-treated and nontreated cells of *C. albicans*.
2D-PAGE analyses of control cells (hyphal cells) (A) and yeast cells suppressed by Lyso-PC-treatment (B) in the hyphae-induced conditions. Higher magnification of regions in boxes of A and B shows the expression pattern of Pra1p and Phr1p in Lyso-PC-nontreated (a) and Lyso-PC-treated (b) cells. Pra1p and Phr1p appear as streaks with long and short tails, as expected from previous reports, revealing that they are glycosylated [22, 23]. As glycosylation causes a shift in molecular mass and pH, each molecule is positioned at more than two sites. Lyso-PC treatment reduces the extent of glycosylation and the level of expression, especially in the case of Pra1p.

were prepared from the Lyso-PC-treated and nontreated cultures as described previously [23].

2D-PAGE was performed using an IPG (Immobilized pH gradient) system [10, 28]. Samples were applied to an IPG strip that was in-gel rehydrated using IPGphor (Pharmacia, Picastaway, NJ, U.S.A.) under constant 10 V for 12 h. After isoelectrofocusing with stepwise increase of voltage up to a total of 15,000 Vh, the strip gel was run on 13% SDS-PAGE gels (T=30%, C=2.8%). The gel was stained with ammonia silver solution (47 mM silver nitrate, 3.3 mM ammonia, 20 mM sodium hydroxide). Images were obtained with a GS-710 densitometer (Bio-Rad, Hercules, CA, U.S.A.) and analyzed with PDQUEST program (V. 6.1). Molecular weight was determined with a standard size marker (Bio-Rad).

Proteins were identified by peptide mass fingerprinting (PMF) as described previously with minor modifications [4]. The mass of the peptides was determined using a Voyager-DE STR MALDI-TOF (matrix-assisted laser desorption/ionization-time-of-flight) mass spectrometer (PE Biosystem, Framingham, MA, U.S.A.). Calibration was performed using the internal mass of trypsin. The database was searched by MS-Fit (http://prospector.ucsf.edu/ucsfhtml4.0/msfit.htm). Protein sequences were determined by tandem mass spectrometry and used to search for homologous protein from databases with NCBIs Basic Local Alignment Search Tool (BLAST).

When the protein profiles were compared between Lyso-PC treated and nontreated cell extracts by 2-D PAGE, several spots were reproducibly noticed (Fig. 1A). Three protein spots identified by tandem mass as *PRA1* (pH regulated antigen) [20] were expressed at a severely reduced level in the Lyso-PC-treated extract (Fig 1B). The extent of glycosylation characterized by vertical streaks at different pH was also severely reduced. Previously, Pra1p was reported as a putative secreted glycoprotein expressed in neutral pH which functions indirectly in hyphae formation [25]. Two spots representing *PHR1* (pH responsive protein), which also had vertical streaks at different pHs (Fig. 1B), were reduced when hyphal transition was suppressed by Lyso-PC. Phr1p is a cell surface protein and null mutations in *PHR1* result in pH-conditional morphological defects. At alkaline pH, the Phr1p-deficient mutant is unable to achieve the apical growth [24] that is a characteristic of hyphae. Reduced expression of Phr1p and Pra1p by Lyso-PC also confirms that these two genes are involved in the morphogenesis.

To examine the regulation of these two genes at the transcriptional level, Northern analyses were performed (Fig. 2). Total RNAs were prepared from Lyso-PC-treated and nontreated culture as described previously [5]. To prepare probes, the DNA fragments of *PHR1, PRA1,* and *EFG1* were amplified by PCR using primers (sense, 5'-GTGTACACAAGTTGATTTCAAATTGG-3', and antisense, 5'-AATTCATCGACGCCTCATTCATACTAA-3' for PHR1; sense, 5'-CAGTTTCTGTATAAACCTTAGTC-3', and antisense, 5'-TTACTCTTTTGTCTACAAGCG-3' for PRA1; sense, 5'-GTCAACGTATTCTATACCC-3', and antisense, 5'-CTTCTTTGGCAACAGTGC-3' for EFG1). *ACT1* encoding actin was used as an internal control. The expression of *PHR1* was suppressed when hyphal transition was suppressed by Lyso-PC, while the expression of *PRA1* was

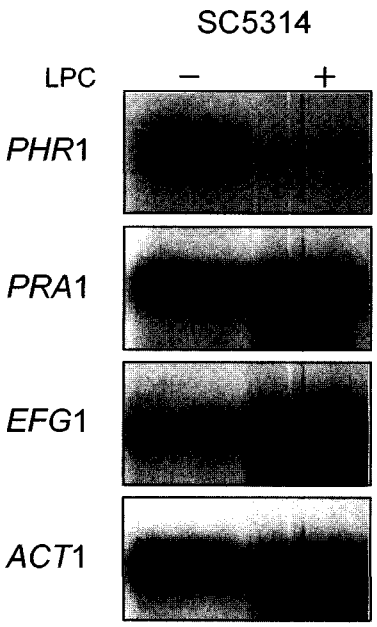

Fig. 2. Northern blot analysis. Total RNAs were prepared from Lyso-PC-treated and nontreated cells of *C. albicans* and hybridized with *PHR1*, *PRA1*, *EFG1*, and *ACT1*.

not (Fig. 2). To investigate the relationship of these two genes with either the MAP kinase pathway or the cyclic AMP pathway, the transcriptional levels *CPH1* and *EFG1* that represent each pathway were examined in Lyso-PC-treated and nontreated cultures. The expression of *EFG1*, which is known to be the transcriptional regulator of the cyclic AMP pathway, was surprisingly increased when cells were treated with Lyso-PC. Meanwhile, transcripts of *CPH1*, the transcriptional regulator of the MAP kinase pathway, were not detected (data not shown).

Efg1p protein has been proposed as a regulator of the morphogenetic processes in *C. albicans*, since it influences yeast-hyphae interconversion [14, 27] and regulates phenotypic switching and chlamydospore formation [26]. Efg1p is an activator and also a repressor in the process of morphogenesis [6]. It is likely that increase of Efg1p by Lyso-PC might inhibit the expression of *PHR1* at the transcriptional level, resulting in a decreased protein level, and that decreased Phr1p might affect the degree of glycosylation of Pra1p. Our finding that the two surface proteins involved in the morphogenesis were suppressed by Lyso-PC revealed the downstream effect of Lyso-PC.

Previously, we reported that Lyso-PC suppresses hyphal transition [15]. In this study, we showed reduced expression of Phr1p and Pra1p when treated with Lyso-PC, using 2D electrophoresis. Neither protein was recognized as a single spot, but rather as a streak with a long tail, as expected from the previous reports that they are glycosylated proteins [24, 25]. The extent of glycosylation was also reduced by treatment with Lyso-PC. *PHR1* is apparently associated with dimorphism, since its null mutant exhibits aberrant cell morphology and reduced virulence in the mouse systemic model [7, 9, 18, 21]. Phr1p is both N- and O-glycosylated, accounting for the difference in its native molecular mass [17, 24]. *PRA1* is differentially expressed by pH and media conditions used to induce hyphal formation [25]. Pra1p is also found to be a highly N and O glycosylated protein [25].

The morphological transition of *C. albicans* is related to its survival capability as a pathogen. Therefore, a chemical, if any, which inhibits this morphological plasticity may be used to control candidiasis. According to our 2D electrophoresis data, Lyso-PC inhibited the expression of two genes that have roles in morphogenesis and virulence. In this regard, Lyso-PC is possibly a novel candidate as a chemical for the treatment of candidiasis.

Acknowledgments

We thank Dr. W. Fonzi for the gift of SC5314. This work was supported by KOSEF (No. R04-2000-000-00022-0) for Wonja Choi and by BK21 project and K-JIST Internal Grant for Yung Joon Yoo.

References

1. Braun, B. R. and A. D. Johnson. 1997. Control of filament formation in *Candida albicans* by the transcriptional repressor *TUP1*. *Science* **277:** 105-109.
2. Braun, B. R. and A. D. Johnson. 2000. *TUP1*, *CPH1* and *EFG1* make independent contributions to filamentation in *Candida albicans*. *Genetics* **155:** 57-67.
3. Brown, A. J. P. and N. A. R. Gow. 1999. Regulatory networks controlling *Candida albicans* morphogenesis. *Trends Microbiol.* **7:** 333-338.
4. Chang, J. W., H. B. Jeon, J. H. Lee, J. S. Yoo, J. S. Chun, J. H. Kim, and Y. J. Yoo. 2001. Augmented expression of peroxiredoxin I in lung cancer. *Biochem. Biophys. Res. Commun.* **289:** 507-512.
5. Domdey, H., B. Apostol, R. J. Lin, A. Newman, E. Brody, and J. Abelson. 1984. Lariat structures are *in vivo* intermediates in yeast pre-mRNA splicing. *Cell* **39:** 611-621.
6. Ernst, J. F. 2000. Transcription factors in *Candida albicans* - enviromental control of morphogenesis. *Microbiology* **146:** 1763-1774.
7. Fonzi, W. 1999. *PHR1* and *PHR2* of *Candida albicans* encode putative glycosidases required for proper cross-linking of beta-1,3- and beta-1,6-glucans. *J. Bacteriol.* **181(22):** 7070-7079.
8. Fridkin, S. and W. Jarvis. 1996. Epidemiology of nosocomial fungal infections. *Clin. Microbiol. Rev.* **9(4):** 499-511.
9. Ghannoum, M. A., B. Spellberg, S. M. Saporito-Irwine, and W. A. Fonzi. 1995. Reduced virulence of *Candida albicans* *PHR1* mutants. *Infect. Immun.* **63(11):** 4528-4530.

10. Kim, T. W., S. H. Jung, J. Y. Lee, S. K. Choi, S. H. Park, J. S. Jo, and H. Y. Kim. 2003. Identification of lactic acid bacteria in Kimchi using SDS PAGE profiles of whole cell proteins. *J. Microbiol. Biotechnol.* **13(1):** 119-124.
11. Kobayashi, S. D. and J. E. Cutler. 1998. *Candida albicans* hyphal formation and virulence: Is there a clearly defined role? *Trends Microbiol.* **6:** 92-96.
12. Kron, S. J. and N. A. Gow. 1995. Budding yeast morphogenesis: Signaling, cytoskeleton and cell cycle. *Curr. Opin. Cell Biol.* **7:** 845-855.
13. Liu, H., J. Kohler, and G. R. Fink. 1994. Suppression of hyphal formation in *Candida albicans* by mutation of a *STE12* homolog. *Science* **266:** 1723-1726.
14. Lo, H. J., J. R. Köhler, B. DiDomenico, D. Loebenberg, A. Cacciapuoti, and G. R. Fink. 1997. Nonfilamentous *C. albicans* mutants are avirulent. *Cell* **90(5):** 939-949.
15. Min, J. Y., Y. J. Lee, Y. A. Kim, H. S. Park, S. Y. Han, G. J. Jhon, and W. J. Choi. 2001. Lysophosphatidylcholine derived from deer antler extract suppresses hyphal transition in *Candida albicans* through MAP kinase pathway. *Biochim. Biophys. Acta* **1531:** 77-89.
16. Mitchell, A. P. 1998. Dimorphism and virulence in *Candida albicans*. *Curr. Opin. Microbiol.* **1:** 687-92.
17. Mouyna, I., T. Fontaine, M. Vai, M. Monod, W. A. Fonzi, M. Diaquin, L. Popolo, R. P. Hartland, and J. P. Latge. 2000. Glycosylphosphatidylinositol-anchored glucanosyltransferases play an active role in the biosynthesis of the fungal cell wall. *J. Biol. Chem.* **275:** 14882-14889.
18. Muhlschlegel, F. A. and W. A. Fonzi. 1997. *PHR2* of *Candida albicans* encodes a functional homolog of the pH-regulated *PHR1* with an inverted pattern of pH-dependent expression. *Mol. Cell. Biol.* **17:** 5960-5967.
19. Odds, F. C. 1994. *Candida* species and virulence. *ASM NEWS* **60:** 313-318.
20. Pardo, M., M. Ward, A. Pitarch, M. Sànchez, C. Nombela, W. Blackstock, and C. Gil. 2000. Cross-species identification of novel *Candida albicans* immunogenic proteins by combination of two-dimensional polyacrylamide gel electrophoresis and mass spectrometry. *Electrophoresis* **21:** 2651-2659.
21. Park, H. S., G. J. Jhon, and W. J. Choi. 1998. Deer antler extract selectively suppresses hyphal growth in dimorphic fungus, *Candida albicans*. *J. Microbiol. Biotechnol.* **8(3):** 291-294.
22. Park, H. S., G. J. Jhon, and W. J. Choi. 1998 Hyphal growth inhibition by deer antler extract mimic the effect of chitin synthase deletion in *Candida albicans*. *J. Microbiol. Biotechnol.* **8(4):** 422-425.
23. Pitarch, A., M. Pardo, A. Jimenez, J. Pla, C. Gil, M. Sanchez, and C. Nombela. 1999. Two-dimensional gel electrophoresis as analytical tool for identifying *Candida albicans* immunogenic proteins. *Electrophoresis* **20(4-5):** 1001-1010.
24. Saporito-Irwin, S. M., C. E. Birse, P. S. Sypherd, and W. A. Fonzi. 1995. *PHR1*, a pH-regulated gene of *Candida albicans*, is required for morphogenesis. *Mol. Cell. Biol.* **15:** 601-613.
25. Sentandreu, M., M. V. Elorza, R. Sentandreu, and W. A. Fonzi. 1998. Cloning and characterization of *PRA1*, a gene encoding a novel pH-regulated antigen of *Candida albicans*. *J. Bacteriol.* **180:** 282-289.
26. Sonneborn, A., D. Bockmûhl, and J. F. Ernst. 1999. Chlamydospore formation in *Candida albicans* requires the Efg1p morphogenetic regulator. *Infect. Immun.* **67:** 5514-5517.
27. Stoldt, V. R., A. Sonneborn, C. Leuker, and J. F. Ernst. 1997. Efg1, an essential regulator of morphogenesis of the human pathogen *Candida albicans*, is a member of a conserved class of bHLH proteins regulating morphogenetic processes in fungi. *EMBO J.* **16:** 1982-1991.
28. Choi, W. Y., Y. J. Yoo, M. K. Kim, D. H. Shin, H. B. Jeon, and W. J. Choi. 2003 Identification of proteins expressed highly in the hyphae of *Candida albicans* by two-dimensional electrophoresis. *Yeast* **20:** 1053-1060.