Preparative SDS Gel Electrophoresis of Hydrophobic Cell Wall Proteins from *Candida albicans*

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Introduction

Candida albicans is an opportunistic fungal pathogen of humans. The mechanism by which *Candida* changes from a harmless inhabitant of the gastrointestinal tract to a pathogen infecting multiple tissue sites has been the subject of much investigation.[1, 2]

Fundamental to *Candida* pathogenesis is the organism's ability to adhere to host surfaces and interact with host cell proteins. A key adhesion factor is cell surface hydrophobicity (CSH).[3] *Candida* cells which are more hydrophobic interact more strongly with host cells and prosthetic devices.[4, 5] Our laboratory is interested in the biochemistry and regulation of *Candida albicans* hydrophobic/hydrophilic conversion.

Previous work using hydrophobic interaction chromatography (HIC) revealed a subset of cell surface proteins which are hydrophobic.[6] We believe that these hydrophobic proteins increase CSH and are integral to the *Candida albicans* adhesion mechanism. We were thus interested in isolating and purifying these proteins for further characterization. Due to their low abundance and their tendency to bind to inanimate surfaces, isolation of the hydrophobic proteins in quantity by available means was problematic. We report here the use of the Mini Prep Cell (Bio-Rad) for the isolation of one of the hydrophobic proteins by continuous elution electrophoresis.

Methods

SAMPLE PREPARATION

Candida cells were washed three times in ice cold, sterile water, and suspended in digest buffer (0.01 M $NaPO_4$, pH 7.4, containing protease inhibitors EDTA, PMSF, leupeptin, and pepstatin). Enzymatic release of cell wall proteins by a ß1-3 glucanase was monitored and stopped when the concentration of released protein reached 500 µg/ml. Cessation of the digest at this point produced a cell wall protein preparation essentially free from cytoplasmic contamination. Intact cells were removed by centrifugation, and the concentration of released protein in the final supernatant fluid was re-assessed. This preparation will be referred to as rapid crude digest (RCD).

Because we are specifically interested in the hydrophobic wall proteins, antibodies were prepared to track the hydrophobic proteins during isolation procedures. These polyclonal antibodies were generated by immunizing mice with hydrophobic proteins prepared from RCD by HIC-HPLC (phenyl-5-PW column, Bio-Rad).

PREPARATIVE ISOELECTRIC FOCUSING

Initial fractionation of the cell wall protein digest was carried out using the Mini Rotofor® preparative electrofocusing cell. Ampholytes (1% each of Bio-Lyte® 3–5 and Bio-Lyte 5–7 ampholyte) were added to an RCD sample containing 12 mg total protein in 18 ml. Proteins were focused in the Rotofor cell at 12 W constant power for 3 hr at approximately 14 ˚C. Initial voltage was 550 V and reached a plateau of 1,100 V after 2 hr. Twenty fractions were collected and analyzed by SDS-PAGE and western blotting.

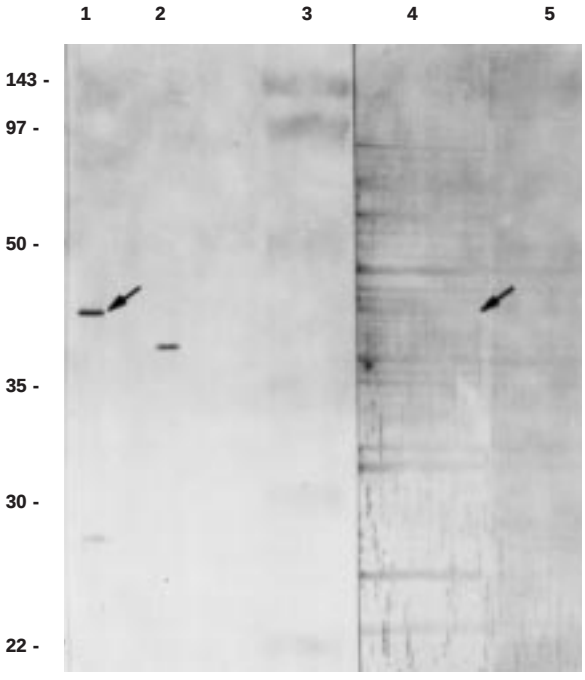

Fig. 1. Blot of Candida albicans *proteins. Lanes 1, 2, and 4 contain blotted proteins from pooled Rotofor fractions 12 and 13. Lanes 1 and 2 were probed with different polyclonal antisera that detect* Candida *hydrophobic cell wall proteins. Lane 3 contains prestained low MW markers (Bio-Rad), molecular weights are listed at left. Lane 5 contains blotted whole RCD from* Candida albicans*. Lanes 4 and 5 were stained with colloidal gold (Bio-Rad).*

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PREPARATIVE ELECTROPHORESIS WITH THE MINI PREP CELL

As a final purification step, the discontinuous buffer system of Laemmli was used for preparative SDS electrophoresis.[7] An 8 cm, 10% (30.0:0.8 acrylamide, bis-acrylamide, w/w) resolving gel was poured and allowed to polymerize overnight. A stacking gel, 500 µl of a 4% acrylamide solution, was layered on the resolving gel and allowed to polymerize.

Sample (100 µl each from Rotofor fractions 12 and 13), was mixed with sample buffer and heated at 95 °C for 5 min. Based on the Rotofor fraction protein concentrations, the sample applied to the Mini Prep Cell contained ~80 µg of total protein. The heated sample was briefly cooled on ice, then loaded onto the gel. Electrophoresis was carried out at 1 W constant power. Elution buffer was continuously pumped (Econo Pump) through the system at a flow rate of 0.1 ml/min. The dye front eluted after ~4.5 hours. When the dye front had cleared the elution line, fractions (2.5 min) were collected using the Model 2128 Fraction Collector. Seventy-two fractions were collected.

MINI PREP CELL FRACTION ANALYSIS

To locate the protein of interest, 20 µl of every third fraction was mixed with sample buffer, heated and analyzed by SDS-PAGE (12.5%). A second SDS-PAGE analysis (12.5%) was done using every fraction neighboring the fraction containing the protein of interest.

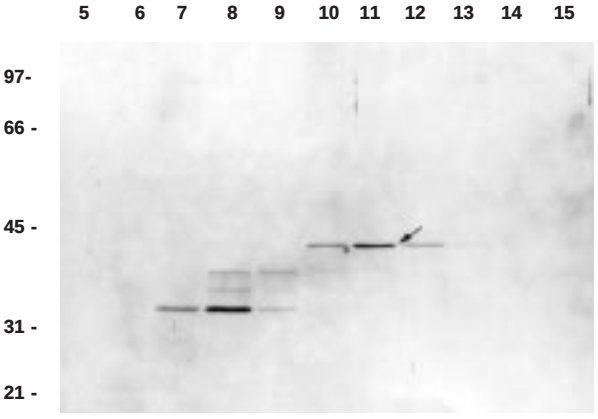

Fig. 2. SDS-PAGE of Mini Prep Cell fractions. Outside lanes are low MW standards (Bio-Rad). Labeled lanes contain 20 µl of the Mini Prep Cell fraction indicated. Proteins were visualized by silver staining.

Results

Initial SDS-PAGE analysis of the Rotofor fractions indicated that three hydrophobic proteins were isolated in fractions 12 and 13 (pH 5.2 and 5.49, respectively, data not shown). Aliquots of these fractions were pooled and analyzed by western blot (Figure 1). Total protein was visualized by colloidal gold staining (pooled Rotofor fractions 12 and 13 and whole RCD in lanes 4 and 5, respectively). The pooled Rotofor fractions were also tested with polyclonal antibodies directed against hydrophobic proteins (lanes 1 and 2). The protein of interest (approximately 42 kDa) for this separation is indicated by the arrows.

Aliquots of Rotofor fractions 12 and 13 were then pooled and loaded onto the preparative electrophoresis gel. Mini Prep Cell fractions were analyzed as described above. The first SDS-PAGE indicated that the protein of interest was located near fraction 11. The second gel was run using samples from fractions 5 through 15 (Figure 2). This figure demonstrates the high degree of purification that can be achieved by this method. Most of the 42 kDa hydrophobic protein was found in a single fraction,11. Some is also found in 10 and 12. However, all three fractions are virtually free of other proteins.

Summary

The hydrophobic proteins in the *Candida albicans* cell wall contribute to the ability of this organism to adhere to host surfaces. Characterization of these proteins will facilitate investigation of their specific role in adhesion mechanisms and aid in the design of better strategies for providing protection against candidiasis.

However, these fungal proteins are difficult to isolate due to their hydrophobic nature. In particular, these proteins stick to dialysis membranes, most chromatographic matrices, and glass or plastic surfaces. Thus any method which involves sample dilution and subsequent dialysis or concentration leads to substantial protein loss. This makes isolation of pure protein by current strategies such as chromatography or electro-elution of bands from SDS-PAGE gels problematic and inefficient.

Preparative PAGE allows protein separation without dilution. By combining this method with Rotofor isoelectric focusing, we established a two-dimensional separation of *Candida* hydrophobic proteins while maintaining protein solubility, providing rapid protein isolation, and achieving yields sufficient for use in protein sequencing.

References

1 *Odds, F. C.,* Candida and Candidosis*, 2nd Ed (Bailliere Tindall, London, 1988).*
2 *Cutler, J. E., Ann. Rev. Microbiol., 45, 187 (1991).*
3 *Hazen, K.C. in Microbial Cell Surface Hydrophobicity (Doyle, R.J. & Rosenberg, M., eds.), 249 (American Society for Microbiology, Washington DC, 1990).*
4 *Hazen, K. C., Infect. Immun., 57, 1894 (1989).*
5 *Hazen, K. C., Brawner, D. L., Riesselman, M. H., Cutler, J. E. and Jutila, M. A., Infect. Immun., 59, 907 (1991).*
6 *Hazen, K. C. and Hazen, B. W., Infect. Immun., 60, 1499 (1992).*
7 *Laemmli, U. K., Nature, 227, 680 (1970).*

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