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Novel mechanisms of surfactants against *Candida albicans* growth and morphogenesis

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ABSTRACT

Candida albicans is a common opportunistic fungal pathogen, causing not only superficial mucosal infections but also life-threatening systemic candidiasis in immune-compromised individuals. Surfactants are a kind of amphiphilic compounds implemented in a wide range of applications. Although their antimicrobial activity has been characterized, their effect on *C. albicans* physiology remains to be elucidated. In this study, we investigated the inhibitory effect of two representative surfactants, cetyltrimethylammonium bromide (CTAB) and sodium dodecyl sulfate (SDS), on *C. albicans* growth and morphogenesis. Both surfactants exhibited inhibitory effect on *C. albicans* growth. This effect was not attributed to plasma membrane (PM) damage, but was associated with mitochondrial dysfunction. Excitingly, the surfactants, especially CTAB, showed strong inhibitory effect on hyphal development (IC_{50} = 0.183 ppm for CTAB and 6.312 ppm for SDS) and biofilms (0.888 ppm for CTAB and 76.092 ppm for SDS). Actin staining and Hwp1-GFP localization further revealed that this inhibition is related to abnormal organization of actin skeleton and subsequent defect in polarized transport of hyphae-related factors. This study sheds a novel light on the antimicrobial mechanisms of surfactants, and suggests these agents as potential drugs against *C. albicans* hyphae-related infections in clinical practice.

1. Introduction

Candida albicans is a common opportunistic fungal pathogen, causing not only superficial mucosal infections but also life-threatening systemic candidiasis [1,2]. One of the most important properties of this pathogen is its ability of hyphal development under certain environmental stimuli, such as amino acids, low nitrogen source, serum and hypoxia [3]. Hyphal development is governed by a complicated signaling network that elaborately senses the stimuli and activates expression of hyphae-related genes, such as *HWP1* [4,5]. Their products are then transported from the endoplasmic reticulum to the hyphal tip and provide materials for hyphal elongation [6]. Since the significance of hyphal development during invading host tissues, the system that mediates this process is a potential target against *C. albicans* infections [7,8].

Surfactants, also named surface active agents, are a kind of compounds containing both hydrophobic groups and hydrophilic groups. Due to the amphiphilic property, they are implemented in a wide range of applications [9–11]. Especially, these compounds are widely used as broad-spectrum antimicrobial agents [12,13]. Moreover, evidence suggests that these agents, in spite of their potential toxicity to mammalian cells, have potential use as anticancer agents [14]. Therefore, the surfactants may be developed as novel drugs in clinical practice.

Cetyltrimethylammonium bromide (CTAB) and sodium dodecyl sulfate (SDS), two representative surfactants, are widely used in biological researches. In this study, we investigated their effect on *C. albicans* growth and morphogenesis, and found that both agents had much stronger inhibitory effect on hyphal development than on growth. Furthermore, this inhibition is related to abnormal organization of actin skeleton and subsequent defect in transport of hyphae-related factors. Our findings suggested that the surfactants may be potential agents against *C. albicans* infections.

2. Materials and methods

2.1. Agents, strains and growth conditions

The surfactants used in this study were purchased from Sigma, USA. The stock solutions of the agents were prepared in liquid RPMI-1640 medium (RPMI-1640 powder (Sigma, USA) 1%,

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3-(N-morpholino) propanesulfonic acid (Sigma, USA) 0.418%, uridine 80 ppm, pH 7.4) at the initial concentration of 1000 ppm, and diluted with the same medium to certain concentrations.

Normally, the wild-type *C. albicans* strain BWP17 was used for growth inhibition tests, hyphal induction and biofilm assays. To investigate the effect of the surfactants on polarized transport of Hwp1, the strain NKF152 containing the Hwp1-GFP fusion gene (based on the plasmid pMG2082) was used [15]. The strains were overnight cultured in liquid YPD medium (yeast extract 1%, peptone 2%, glucose 2%) with shaking at 30 °C, and then used for further experiments.

2.2. Growth inhibition assays

To investigate the inhibitory effect of the surfactants on *C. albicans* growth, overnight cultured cells were washed twice with PBS, and suspended in RPMI-1640 medium to an initial optical density at 600 nm (OD_{600}) of 0.2. 50 μL of the suspension were added into 96-well polystyrene microplate wells, and then 50 μL of surfactant solutions were mixed with the cell suspensions, obtaining the mixture containing the yeast cells with the OD_{600} of 0.1 and the surfactants with the following concentrations, 0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16 ppm for CTAB, and 0, 1.25, 2.5, 5, 10, 20, 40, 80, 160 ppm for SDS. The plates were covered with their lids, sealed with parafilm and incubated at 37 °C for 24 h. The OD_{600} of each well was then determined using a microplate reader (Bio-Rad, USA). The percent of growth for each treatment was calculated as the OD_{600} of each surfactant-treated group divided by the control (without the treatment of surfactants). The growth inhibition tests were also performed in liquid YPD medium (yeast extract 1%, peptone 2%, glucose 2%) and SC medium (yeast nitrogen base 0.67%, glucose 2%, amino acid mixture 0.2%) to evaluate the effect of media in CTAB activity.

2.3. PM damage assays

To evaluate the effect of CTAB on PM integrity, overnight cultured fungal cells were suspended in fresh YPD medium containing CTAB with the following concentrations, 0, 0.25, 0.5, 1, 2, 4, 8 ppm. The cells were cultured with shaking at 30 °C for 24 h and stained with PI (final concentration 5 ppm, Sigma, USA) for observation. At least 30 fields were observed. PI-positive and total cells in each group were quantified. The percent of PI-positive cells for each treatment was then calculated as the number of PI-positive cells divided by that of total cells. PM integrity was also evaluated by lactate dehydrogenase (LDH) assays. The CTAB-treated cultures were centrifuged at 12,000 rpm for 10 min, and the supernatant was used for determining LDH activity by an LDH Assay Kit (KEYGEN BIOTECH, China).

2.4. Measurement of mitochondrial membrane potential (MMP)

CTAB-treated cells were washed and suspended with PBS and stained with JC-1 (final concentration 1 ppm, Sigma, USA) for 30 min. Fluorescence intensity of JC-1 aggregates (red, FL-1) and monomers (green, FL-2) were recorded by a flow cytometer (FACSCalibur, BD, USA). The cells exhibiting decreased red fluorescence density than the control (untreated) cells were considered as the cells with decreased MMP, and the percent of cells with decreased MMP was recorded.

2.5. Hyphal induction

C. albicans hyphae were induced as described previously [15]. Briefly, overnight cultured cells were washed with PBS and suspended in RPMI-1640 medium containing the surfactants with the indicated concentrations. The suspensions were then incubated with shaking at 37 °C for 4 h. Cells were harvested, fixed with 4% formaldehyde, and observed by a light microscope (Olympus, Japan). The number of hyphal cells and total cells was counted in each field, and the percent of hyphal cells were calculated as the number of hyphal cells divided by the number of total cells. At least 30 fields were determined.

2.6. Biofilm assays

C. albicans biofilms were cultured in polystyrene microtiter plates (Corning Inc., USA). Overnight cultured cells were washed with PBS and suspended in RPMI-1640 medium containing the surfactants at different concentrations, with the OD_{600} of 0.1. 100 μL of the suspensions were then added into 96-well microtiter plates. The plates were covered with lids and incubated at 37 °C. After 24 h of incubation, the plates were washed 3 times with PBS, and the biofilm activity in each well was detected by XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) reduction assays [16]. To observe biofilm structures, the cultured biofilms were washed with PBS and fixed with 3% glutaraldehyde. The samples were then sectioned, dehydrated with ethanol, dried in vacuum desiccators, coated with gold and observed by a scanning electron microscope (SEM, QUANTA 200, Czech).

To evaluate the effect of surfactants on biofilm maintenance, the biofilms were pre-formed as described above. The wells were then washed with PBS, and 100 μL RPMI-1640 medium containing the surfactants with different concentrations were added into the wells. The plates were covered with their lids and incubated at 37 °C for further 24 h. The metabolic activity of biofilms was also measured by the XTT reduction assay.

2.7. Actin staining

To observe actin skeleton, the *C. albicans* cells were washed twice with PBS, fixed with 4% formaldehyde for 10 min, permeabilized with 0.5% Triton 100 for 30 min and washed again with PBS. 5 μL of rhodamine phalloidin (final concentration 1 ppm, Cytoskeleton, USA) were then added into 100 μL of the cells. The cells were incubated at the room temperature for 30 min, and washed 3 times with PBS for observation.

2.8. Fluorescence microscopy

The PI-stained and rhodamine phalloidin-stained cells were observed by a fluorescence microscope (Olympus, USA) using the RFP filter set. To detect Hwp1 localization, the strain NKF152 was cultured in RPMI-1640 containing the surfactants as described above, and Hwp1-GFP was observed by the fluorescence microscope using the GFP filter set.

2.9. Statistical analysis

The experiments were performed with five replicates, and the values represent the means ± standard deviations (SD). Significant differences between the treatments were determined using one-way ANOVA ($P < 0.05$). Statistical analysis and IC_{50} calculation were performed by SPSS (Version 20, IBM, USA).

3. Results

3.1. The surfactants exhibit a distinct inhibitory effect on C. albicans growth

Surfactants are well-known antimicrobial agents. To investigate whether the agents have antifungal activity, we first tested the