CELL SURFACE ENOLASE OF CANDIDA SPECIES IS INVOLVED IN THE INTERACTIONS WITH HUMAN HOST AND THE FORMATION OF POLYMICROBIAL BIOFILMS

M. Rapala-Kozik, J. Karkowska-Kuleta, D. Satala, M. Gogol, D. Bartnicka, M. Zawrotniak, A. Kozik
Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland

INTRODUCTION

Enolase, a known cytoplasmic enzyme involved in the glycolytic pathway and abundant moonlighting protein found at the cell surface of numerous types of prokaryotic and eukaryotic cells, plays an important role in fungal infections caused by *Candida* spp. yeasts. These opportunistic microorganisms take part in physiological microbiota of more than 50% of individuals in the human population but are also dangerous pathogens that cause life-threatening systemic infections, particularly in immunocompromised patients. Candidal enolases at the cell surface acquire completely new functions, often becoming receptors for a number of important host ligands and also may be a promising target for new antifungal therapies.

AIMS

- The analysis of the presence of enolase at the surface of *C. albicans* and *C. tropicalis* cells, growing under various conditions, including mixed-species biofilms.
- Quantitative characterization of the possible interactions of the surface-exposed enolase with host proteins – plasminogen and fibrinogen.

RESULTS

Fig. 1. The surface exposition of enolase on the *C. albicans* cells during their growth in YPD medium. The cell growth in rich complete YPD medium at 30°C was analyzed by OD measurements at 600 nm and the presence of enolase on the cell surface was detected using a specific antibody, recognizing this fungal protein.

Fig. 2. The correlation of the enolase presence at the surface of *C. albicans* cells with growth conditions. *C. albicans* cells were cultured for 48 hours in: (1) YPD medium at 30°C under aerobic conditions or in anoxia (mimicking large intestine conditions); (2) YAPD medium with the reduced amount of nitrogen; (3) minimal defined Lee medium and (4) fungal-selective Sabouraud medium at 37°C. The percentage of enolase at the cell surface was calculated after cell surface shaving with trypsin and LC-MS/MS analysis.

Fig. 6. The proposed model for the interaction between *C. albicans* enolase and plasminogen.

For enolase-plasminogen complexes, a crosslinking method was used, followed by trypsin digestion and LC-MS/MS analysis to determine the protein fragments, directly involved in this mutual protein-protein interaction. Two peptides localised to enolase were identified – $_{57}$ KWLKGKGVKAV $_{67}$ and $_{104}$ KSKLGANAILGVSLAA $_{120}$. The interaction model was proposed using the Hex Protein Docking software based on a full-length plasminogen structure (PDB: 4DUU). The *C. albicans* enolase structure was obtained by homology modelling using a Modeller software, based on PDB: 1EBH structure (*S. cerevisiae* enolase, 78% identity). The structure of enolase is shown in green, plasminogen in yellow and peptides identified by MS in red.

CONCLUSIONS

- The exposition of enolase at the cell surface of selected *Candida* species differs, depending on the growth conditions that mimics nutrient starvation or invasion of the host at various sites of infection.
- Location of enolase to the cell surface is activated after *C. albicans* has reached the stationary phase of growth.
- A significant enrichment with fungal enolase was detected on the surface of mixed biofilms formed with bacteria (e.g., *P. gingivalis*) at different oxygen availability.
- The fungal enolase present at the *Candida* cell surface during mono- or poly-microbial biofilm formation is involved in mutual interactions of the species and possibly contributes to the stabilizing forces of the biofilm structure.
- Studies on the interaction between plasminogen and purified native candidal enolase revealed the dissociation constants (KD) for complexes of human plasminogen with fungal enolases in a 10^{-7} M range, corresponding to the strength of plasminogen binding by human enolase that shares a high sequence similarity to this microbial protein.
- The identification of enolase-derived peptides directly involved in the interaction with human plasminogen allowed to present a structural model for mutual interactions of these two protein molecules.
- Enolase produced by *Candida* species during mono- or poly-microbial host infections could serve as a bridging molecule that facilitates the host colonization and the regulation of host responses.

Fig. 3. The participation of fungal enolase in the binding to the cell surface of *Porphyromonas gingivalis* cells. The binding of fluorescently (FITC)-labeled enolase to *P. gingivalis* cells was detected by flow cytometry.

<i>P. gingivalis</i> strain in mixed biofilm	Anaerobic conditions		Aerobic conditions	
	W83	ATCC 33277	W83	ATCC 33277
% increase of surface-exposed enolase	1124	970	296	728

Tab. 1. The percentage increase in the amount of enolase on the surface of the *C. albicans* cells during formation of mixed-species biofilm with *P. gingivalis*. *C. albicans* cells and *P. gingivalis* cells (1:10) of wild strains W83 and ATCC 33277 were co-cultured in RPMI 1640 medium at 37°C for 24 hours with gentle agitation (30 rpm) to form biofilm under aerobic or anaerobic conditions. The mono-species fungal biofilm served as a control. Surface-exposed proteins were identified using MS/MS analysis.

Fig. 4. The increase in the amount of enolase at the surface of *C. albicans* and *C. tropicalis* cells during fungal growth in the presence of human plasminogen. *C. albicans* or *C. tropicalis* cells were grown in YPD medium at 30°C or YAPD medium at 37°C, respectively, with or without addition of 120 nM human plasminogen (HPG). To identify surface-exposed enolases, the cell surface shaving with trypsin and LC-MS/MS analysis were performed.

Fig. 5. Surface plasmon resonance (SPR) sensograms representing interactions between human plasminogen and *C. albicans*, *C. tropicalis*, *Saccharomyces cerevisiae* or human enolases. SPR characteristics of the interaction between the HPG and a particular enolase immobilized onto a surface of the CM5 chip was performed with BIACORE 3000 system. The plasminogen solutions at a concentration of 100 nM were injected at a flow rate of 30 μ l/min for 120 seconds.

K_D (M)	Plasminogen	Fibrinogen
enolase		
<i>C. albicans</i>	2.57×10^{-7}	7.40×10^{-6}
<i>C. tropicalis</i>	2.53×10^{-7}	2.02×10^{-6}
<i>S. cerevisiae</i>	-	-
<i>H. sapiens</i>	1.58×10^{-7}	8.23×10^{-6}

Table 2. The binding parameters for the interactions of fungal enolase with human host proteins – plasminogen and fibrinogen.