ABSTRACT

The subject of the research described in this paper are yeast-like fungi of the genus *Candida*, including *C. albicans*, *C. tropicalis* and *C. parapsilosis*, as well as the species *Nakaseomyces glabrata* previously included in the genus *Candida*. They inhabit the oral cavity, digestive tract and genitourinary system, usually without causing infection symptoms. However, an imbalance, which may be caused by a decrease in the host's immunity, leads to difficult to treat fungal infections, called candidiasis. Fungal infections are a significant medical problem due to the increasing resistance of yeasts to treatment.

One of the most important components of the yeast cell is the cell wall, which is the first to come into contact with the host, providing both defense and attack. In a large part it consists of proteins divided into typical, anchored with a glycosylphosphatidylinositol (GPI) link, and atypical, more loosely associated with the cell wall. In addition to these constant proteinaceous components of the cell wall, additional adsorbed proteins are also observed, referred to as "moonlighting proteins". Among this group of proteins, one can find enzymes involved in intracellular metabolic processes, such as the glycolysis pathway, the pentose phosphate pathway or the Krebs cycle, and even proteins involved in translation and some chaperones. The origin of moonlighting proteins on the surface of yeast cells is not fully understood. It is assumed that their presence is related to the release of cytoplasm contents into the extracellular space by dead yeast cells or as a result of transport by extracellular vesicles, followed by their adsorption to the surface of living cells via typical adhesion proteins, such as, in *C. albicans*, adhesins with agglutinin-like sequences (Als), hyphal wall proteins (Hwp) or proteins with increased adhesion to polystyrene (Eap).

One of the "moonlighting" proteins is phosphoglycerate mutase (Gpm1), a cofactor-dependent isomerase involved in the glycolysis pathway, where it is responsible for the conversion of 3-phosphoglycerate to 2-phosphoglycerate in the presence of the cofactor 2,3-bisphosphoglycerate. So far, several extracellular functions of Gpm1 on the surface of yeast cells have been described, including the interactions with human proteins: prekallikrein, high molecular weight kininogen (HMWK), factor XII and plasminogen, as well as factors H and FHL-1 (factor H-like protein 1) of the complement system, and the binding of extracellular matrix proteins (ECM) such as fibronectin (Fn), vitronectin (Vtr) or laminin.

The subject of this doctoral thesis is Gpm1 of the yeast from *Candida* and *Nakaseomyces* genera, its localization on the surface of these yeast-like fungi and the interaction of this enzyme with human ECM proteins (Vtr, Fn) and HMWK – a key component of two host

hemo(homeo)static systems: the contact activation of blood coagulation (CAS) and the kinin-kallikrein system (KKS). The ECM is the outermost zone surrounding human cells and is one of the first places where pathogen-host contact occurs. This contact depends on the ability of yeast cell wall proteins to interact with ECM components. The role of HMWK in infections is different. HMWK, present in blood serum, is attracted to non-physiological negatively charged surfaces, such as cell surfaces of pathogenic microorganisms. Many studies have indicated that bacteria are able to use HMWK to spread infection by releasing proinflammatory peptides – kinins that increase vascular permeability, allowing migration and escape from blood clots and providing nutrients.

In this study, the presence of Gpm1 was demonstrated on the cell surface of the tested yeasts by microscopic observations after visualization with antibodies against *S. cerevisiae* Gpm1 (anti-Gpm1) and in cell wall extracts using the Western blotting technique. In addition, a comparison of the enzymatic activity of recombinant Gpm1 preparations with enzymes isolated and purified from yeast cell wall showed that recombinant Gpm1 exhibited significantly higher activity than that obtained from yeast surface.

The interaction of Gpm1 with selected human proteins (Fn, Vtr and HMWK) was confirmed by microplate binding assays. In addition, by blocking the surface of *Candida* species or *Nakaseomyces glabrata* with anti-Gpm1 antibodies, the contribution of Gpm1 to the total binding of human proteins by whole yeast cells was estimated to be about 25% relatively to all components of the cell wall. The interaction sites of Gpm1 – human proteins were mapped using chemical cross-linking and mass spectrometry, with confirmation using microplate competition tests using synthetic peptides, corresponding to the indicated segments of protein sequences – both in Gpm1 and in human proteins. One Gpm1 peptide was indicated for Vtr binding: aa138-158, for Fn: aa138-158 and aa158-175, and for HMWK and low molecular weight kininogen: aa61-80, aa116-136 and aa158-175. For the mapping of Gpm1 binding sites on human proteins, aa354-367 was indicated for Vtr and aa904-922 and aa1117-1129 for Fn. Mapping of HMWK interaction sites required the use of microplate displacement assays with peptides corresponding to HMWK segments, which identified domains 3 and 6 as the main areas of interaction with Gpm1.

The ability of Gpm1 to interact with the cell surface of the tested yeasts was demonstrated by the addition of Gpm1 labeled with biotin to the monolayer of *Candida* spp. and *N. glabrata* yeast cells. In yeast cell wall extracts, proteins that potentially interact with Gpm1 on the yeast surface were identified using chemical cross-linking. These included both other moonlighting proteins and common proteinaceous components of the cell wall, including Als3. In order to

test the hypothesis of typical adhesins as potential "docking platforms" for moonlighting proteins, *S. cerevisiae* yeast mutants displaying *C. albicans* adhesins: Als3, Eap1 and Hwp1 on the cell surface were used to check the interaction ability with Gpm1. These analyzes indicated that Als3 is the main adhesin responsible for this interaction.

Als3 was isolated and purified from the *C. albicans* cell wall and it was shown that Als3 interacted with Gpm1 through similar sites as in the case of binding human proteins, namely: aa61-80, aa116-136, aa138-158 and aa158-175. Mapping of the interaction sites on Als3 required the use of yeast *S. cerevisiae* mutants that displayed Als3 on their surface with deletions of various fragments. These studies showed that only the Als3 region responsible for the formation of the amyloid is not involved in the interaction with Gpm1.

The role of both yeast proteins – Gpm1 and Als3 – in the interaction with HMWK was checked, with the use of the above-mentioned mutants of *S. cerevisiae* and the simultaneous addition of Gpm1 and HMWK to the cells. It was found that Gpm1 more readily interacted with HMWK.

To summarize, Gpm1 is a cytoplasmic enzyme which, probably on the basis of readsorption after prior release into the extracellular space (from extracellular vesicles or yeast cells dying in the same inflammatory focus), interacts with the cell wall of the tested yeasts. Its contribution to the binding of the studied human proteins (Fn, Vtr, HMWK) suggests a significant role in the pathogen-host interaction. Due to the indicated sites of interaction between Gpm1 and human proteins, it is likely that Gpm1 on the cell surface may participate in the inhibition of fibrillogenesis (Fn binding), interfere with blood coagulation (interaction with HMWK), and inhibit the activation of the complement system (Vtr binding).

It is assumed that the main proteins to dock moonlighting proteins on the cell wall are typical adhesins, including the main adhesin from the Als family – Als3, which is abundant in the hyphal form of *C. albicans*. However, studies have shown that the role of Als3 as a platform for Gpm1 ends with the appearance of a human ligand – in this case HMWK. This result suggests that Als3 may have a displaying role for Gpm1 on the yeast surface in anticipation of a better ligand such as HMWK.