# Streszczenie i słowa kluczowe w języku angielskim

Human lysozyme, classified as type c lysozyme, is one of the key elements of innate immunity in humans. It is present in most body fluids, including saliva, tears, blood and lymph. It protects the body against the attack of microorganisms from both the external and internal environment. *Candida albicans* is an example of an opportunistic pathogen, because on the one hand, it ca be present in the human body as a commensal, and on the other hand, under favorable conditions, it can become the cause of superficial or systemic infections. Favorable conditions for the development of such an infection are conditions leading to a decrease in the body's immunity, including, but not limited to, long-term antibiotic therapy, chemotherapy and radiotherapy, and surgery.The neonatal period, pregnancy and breastfeeding can also contribute to the development of candidiasis.

The antimicrobial activity of type c lysozymes, the most extensively studied representative of which is hen egg white lysozyme (EWL), is well understood in a bacterial context. It involves the hydrolysis of glycosidic bonds between N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) molecules in peptidoglycan, which results in relaxation and loss of stability of the cell wall structure leading to the death of the bacterial cell [Ragland and Criss, 2017; Sahoo et al., 2012]. In addition to its antibacterial activity, EWL has antifungal activity against yeasts and filamentous fungi, including those isolated from patients, incl. *Candida albicans*, *Candida parapsilosis*, *Aspergillus fumigatus*, *Penicillum sp.*, *Acremonium sp.* [Samaranayake et al., 1997; Woods et al., 2011]. In the case of *C. albicans*, enzymatic hydrolysis of N-glycosidic bonds linking polysaccharides and cell wall structural proteins, activation of mannan synthetase and chitin synthetase, which occurs in response to cellular damage by lysozyme [Samaranayake et al., 1997], as well as the influence of EWL on the metabolism of Sap aspartyl proteases [Wu et al., 1999] were described. In studies that used $(GlcNAC)_5$ as a substrate, it was shown that human lysozyme, like EWL, can hydrolyze glycosidic bonds and catalyze transglycosylation reactions in chitooligosaccharides. Despite this, the antifungal activity of human lysozyme has not been fully elucidated.

The aim of the research presented in this dissertation was to analyze the effect of human lysozyme, which is a representative of the c-type lysozyme family, on *C. albicans* cells. The studies used the wild-type strain of *C. albicans*, which is a clinical isolate from the human oral cavity, and two mutants in the cell wall: *C. albicans* NGY 357 and *C. albicans* Myco 3.

In the initial phase of the study, the survival rate of *C. albicans*cells treated with human lysozyme was determined. Using the colony counting method on solid agar plates, it was found that human lysozyme at the lowest concentration used (0.5 µM) reduced the viability of *C. albicans* cells to 85.2% compared to the control. In the case of the mutant *C. albicans* NGY 357, the survival rate was 67.39%, and in the case of *C. albicans* Myco 3 - 104.96%. The killing activity of lysozyme against Myco 3 was only observed at a concentration of 1 µM. The survival of the Myco 3 mutant at this concentration of lysozyme was 83.24%. The survival test in the case of protoplasts devoid of the cell wall showed no sensitivity to lysozyme, even at the highest concentration used - 4 µM. On this basis, it was suggested that the cell wall plays an important role in the interactions between *C. albicans*cells and human lysozyme.

Subsequently, a metabolic activity study was performed using a LIVE/DEAD Yeast Viability Kit. It was shown that lysozyme caused a decrease in metabolic activity in all the strains used. Thus, in the case of the wild-type strain, the activity decreased from 79.66% in the control to 55.55% after treatment of the cells with human lysozyme. The *C. albicans* NGY 357 mutant showed a decrease in metabolic activity from 88.76% to 63.66%, while the *C. albicans* Myco 3 mutant from 82.88% to 48.33%.

By analyzing the reproductive potential and examining the effect of human lysozyme on cell lifespan, it was found that human lysozyme leads to a decrease in the reproductive potential in the case of the wild strain, the NGY 357 and Myco 3 mutant 3.8-, 1.7- and 1,8- times, respectively. Cells treated with human lysozyme did not start budding, in contrast to the control where all the cells start proliferation. It was found that in the case of the wild-type strain, 38.8% of cells started budding, while in the case of NGY 357 and Myco 3 mutants it was 61.3% and 57.5%, respectively. Cells that started the process of proliferation were characterized by a shorter reproduction time. A reduction in this time was observed for wild-type *C. albicans* cells, as well as NGY 357 and Myco 3 mutants, 3.1-, 1.6- and 1.8-fold, respectively. There was also a reduction in the mean post-reproductive life time after treatment with lysozyme. For *C. albicans* cells and NGY 357 and Myco mutants the post-reproductive life time decreased 2.5-, 2.2- and 4.7-fold, respectively. Thus, the mean lifetime of *C. albicans*, *C. albicans* NGY 357 and Myco 3 cells was significantly shortened by the action of human lysozyme - 2.3-, 1.2-, and 1.7-fold, respectively.

Using the CaspACE ™ FITC-VAD-FMK In Situ Marker caspase activity assay kit, *C. albicans* cells with active metacaspase were found in both wild-type *C. albicans* and *C.*

*albicans* NGY 357 strains. In *C. albicans* control 23.83% cells exhibited active metacaspase, while after treatment with human lysozyme the number of cells increased to 58.5%. The NGY 357 mutant had a metacaspase activity in 19.8% of control cells, while the value increased to 49% after lysozyme treatment.

Analysis of the surface topography and nanomechanical properties of *C. albicans* cells was performed using atomic force microscopy (AFM). Human lysozyme led to an increase in stiffness and a decrease in the adhesion forces of the cell surface of the wild-type *C. albicans* strain, and cells of the mutant *C. albicans* Myco 3 showed an increase in surface roughness. For all three strains used, the loss of the oval shape of the cells was observed under the action of human lysozyme.

Selected components of the *C. albicans* cell wall were also stained. Chitin was stained with Calcofluor White, while Congo Red was used to image β-glucan, and imaging was performed with a confocal laser scanning microscope. The cells of all three strains after treatment with human lysozyme were characterized by changes in staining the cell wall and loss of the oval shape.

Using the colorimetric method, the lack of β-1,3-glucanase and chitinolytic activity of human lysozyme *in vitro* was found. On the other hand, the study of the interaction of human lysozyme with laminarin by biolayer interferometry showed that lysozyme bound specifically to laminarin, which has β-1,3-glycosidic bonds in its structure.

Summarizing, it was demonstrated that human lysozyme effectively reduces the survival of *C. albicans* and *C. albicans* NGY 357 and Myco 3 cells. Based on the insensitivity of protoplasts to the action of human lysozyme, it was found that the cell wall is important for the interaction between the lysozyme and the *C. albicans* cells. The metabolic activity of the studied strains decreased under the treatment of lysozyme. Human lysozyme also shortened the lifetime of wild-type strain cells and mutants, both in the reproductive and post-reproductive phases. The action of human lysozyme resulted in a decrease in the number of cells that undertook the reproductive process and a decrease in the number of cell doublings of all three *C. albicans* strains used. The study of metacapase activity showed its more than twofold increase under the influence of human lysozyme, which, together with other results, may suggest cell cycle disorders caused by lysozyme. Cell swelling observed during microscopic examinations - staining of components of the cell wall and AFM imaging suggests osmotic imbalance. On the other hand, the possibility of binding human lysozyme to β-1,3-glucan of the cell wall in the absence of β-1,3-

glucanase and chitinolytic activity *in vitro* suggests that a mechanism other than enzymatic may also be involved in the action of human lysozyme against *C. albicans*.

Key words: human lysozyme, *Candida albicans*, atomic force microscopy, cell wall, metacaspase, reproductive potential