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Michaelis's Constant of Cathepsin *H* From Nontransformed And Tumors Tissues Of Women's Breast.

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ABSTRACT

Cathepsin *H* is lysosomal cysteine protease (EC 3.4.22.16), which is involved in the regulation of intracellular metabolism, post-translational modification of proteins, in the processes of cell differentiation, in the processes of growth and aging, in the activation and inactivation of peptide hormones and neuropeptides, in the immune response, in the destruction of components of the extracellular matrix and basal membrane, thereby promoting the proliferation and metastasis of tumor cells. The aim of the study was to research the Michaelis's constant of cathepsin *H* from women's breast with lobule-infiltrating form of tumor and from tissues which was adjacent of breast cancer. Cathepsin *H* from untransformed breast tissue degraded oxytocin with $K_m = 0,008$ mM and $V_{max} = 0,007$ mM per minute (at Lineweaver - Burk coordinates) was established. Cathepsin *H* from moderately differentiated form tissue with lobule-infiltrating forms of breast cancer hydrolyzed oxytocin with $K_m = 0,009$ mM and $V_{max} = 0,014$ mM per minute (at Lineweaver - Burk coordinates) was established. Cathepsin *H*, which was isolated from untransformed breast tissue had an insignificant affinity to substrate oxytocin compare with cathepsin *H* from moderately differentiated lobule-infiltrating form of breast cancer.

Keywords: cathepsin *H*, proteolysis, tumor, Michaelis's constant, mammary gland.

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INTRODUCTION

The increase of proteolytic enzymes activity in tumor tissue is shown for many types of human cancers, such as breast cancer [1, 2], lung [1, 3], gastrointestinal tract [1, 4], melanoma [1, 5], meningioma [6]. Cysteine proteinase relate to proteolytic enzymes and participate in many processes, which associated with cancerous progression: hyper proliferation of tumourous cells, apoptosis, tumourous induced angiogenesis, invasion and metastasis [7, 8]. One of the lysosomal cysteine protease is cathepsin *H* (EC 3.4.22.16), which is involved in the regulation of intracellular metabolism, post-translational modification of proteins, in the processes of cell differentiation, in the processes of growth and aging, in the activation and inactivation of peptide hormones and neuropeptides, in the immune response [1], in the destruction of components of the extracellular matrix and basal membrane, thereby promoting the proliferation and metastasis of tumor cells [6]. In contrast to other proteinases cathepsin *H* has both activities: proteinase and endopeptidase [6]. High activity of cathepsin *H* is discovered in tumors: glioblastoma, anaplastic astrocytoma, colorectal carcinoma [6], head and neck tumors [9, 10].

The aim of the study was to research the Michaelis's constant of cathepsin *H* from women's breast with lobule-infiltrating form of tumor and from tissues which was adjacent of breast cancer.

MATERIALS AND METHODS

The samples of mammary gland with no tumor (tumor adjacent tissue) and samples with lobule-infiltrating forms of breast cancer were investigated. The lobule-infiltrating forms of breast cancer, according to the histological classification, are a nodal formation with no clear boundaries, dense consistency, grey-yellow or white colour and have a different degree of tissue and cellular atypia [11]. Pathomorphological and histological diagnosis verification according to the international classification of the WHO was performed by specialists of the certified pathological laboratory of Odessa Regional Oncology Center who defined the morphological state of the tumour tissue [12]. Taking the anatomical materials for research, compliance of ethical and legal standards in accordance with: the Declaration of Helsinki (1964), the Convention on human rights and biomedicine (1996), the law of Ukraine "About transplantation of organs and other anatomic human materials" (1999) was provided by the medical institution. The patients were informed and gave their written consent for using anatomical material for biochemical studies. The tissue samples were frozen at -18°C immediately after collection during surgery, homogenated with 0,9% NaCl solution (in ratio 1 : 10), centrifuged at 9000g per hour (at $+4^{\circ}\text{C}$) throughout 30 min. The protein content in the supernatant was determined by biuret method [13]. The activity of cathepsin *H* was determined by hydrolysis of 0,04 mM oxytocin. The content of leucine was detected by modified ninhydrin method [14] expressed in micromoles of leucine per mg of protein in min (at 37°C). The hydrolysis of substrate (0,01 mM, 0,02 mM, 0,04 mM, 0,08 mM and 0,16 mM oxytocin solution) in phosphate buffer (pH 6,5) at constant of content enzyme (0,003 g per g tissue) was detected. The investigation of V_{\max} and K_m was analysed (at Lineweaver - Burk coordinates) [15, 16]. Statistical analysis was carried out by the Mann-Whitney method [17].

RESULTS AND DISCUSSION

In own previous studies of substrate specificity we found that cathepsin *H* from women's breast tumour and from adjacent tissues of breast cancer particularly hydrolyzed low-molecular substrates, some native proteins and preferably oxytocin (0,04 mM). We established that the cathepsin *H* from malignant tumor had slightly greater activity to oxytocin compare with enzyme from untransformed tissues of breast cancer [18].

We found out for enzyme from untransformed breast tissue the maximum speed of oxytocin hydrolysis was detected at the 0,02 mM of oxytocin and then remained constant (Fig. 1).

Cathepsin *H* from untransformed breast tissue degraded oxytocin with $K_m = 0,008$ mM and $V_{\max} = 0,007$ mM per minute (at Lineweaver - Burk coordinates) it was established. Michaelis's constant of cathepsin *H* from untransformed breast tissue is different from K_m of cathepsin *H* from other biological objects (Fig. 2).

Michaelis's constant from untransformed breast tissue to the natural oxytocin substrate was 160 times higher than K_m of cathepsin *H*, which isolated from the neocortex of human brain ($K_m = 5,0 \cdot 10^{-5}$ M) [6]

and 80 times higher than K_m of cathepsin H from human brain (relative to the substrate $N\alpha$ -benzoyl-DL-arginine- β -naphthylamide (BANA) ($K_m = 7,0 \cdot 10^{-4} M$) [19].

However the K_m of cathepsin H from untransformed breast tissue was 7,5 – 362,5 times lower (to the natural substrate of oxytocin) in comparison with K_m of cathepsin H, which isolated from rabbit lung (relative to synthetic substrates that had amino acid – pNA or Z-amino acid – 4MeO β NA), respectively [20].

Michaelis’s constant of cathepsin H from untransformed breast tissue to oxytocin was 1275-fold lower than K_m of cathepsin H, which isolated from rat liver (which detected to the synthetic substrate Suc-Ala-Ala-pNA) [21].

We found out that the maximum speed of oxytocin hydrolysis of enzyme from untransformed breast tissue was detected at the 0,04 mM of oxytocin (Fig. 1).

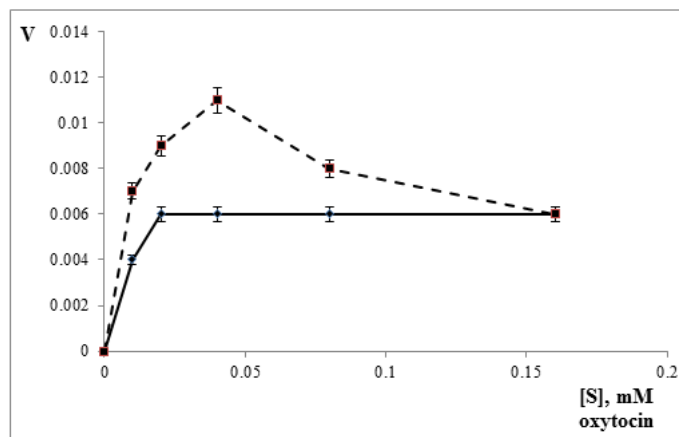


Figure 1. Effect of substrate concentration on the speed of enzymatic reaction which was catalyzed by cathepsin H from untransformed tissue and from moderately differentiated lobule-infiltrating forms of breast cancer (n = 3).

Note: A – untransformed breast tissue; B – moderately differentiated lobule-infiltrating forms of breast cancer.

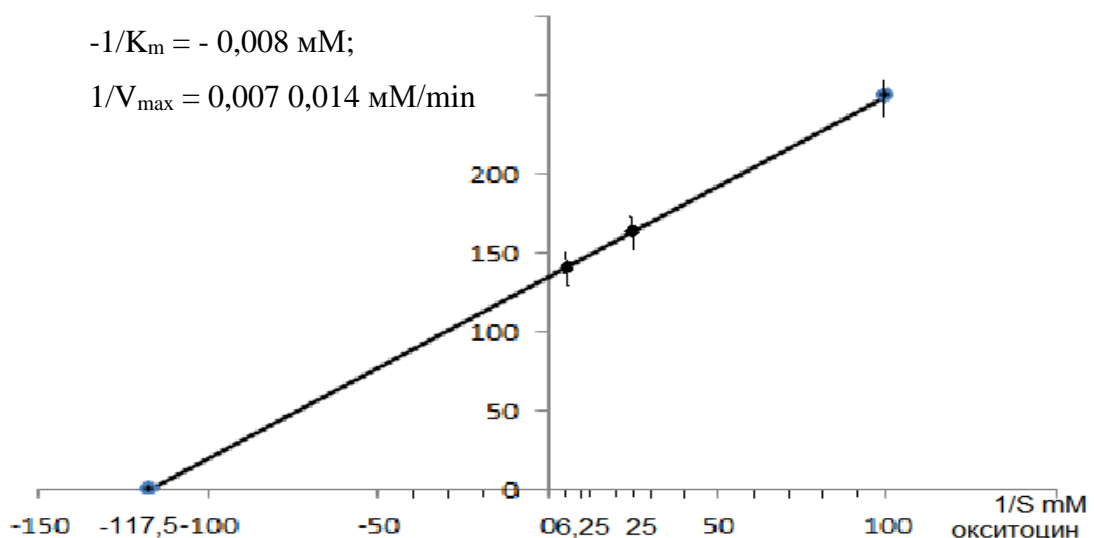


Figure 2. Effect of the substrate concentration on the speed of hydrolysis oxytocin in the presence of cathepsin H from untransformed tissue and from moderately differentiated lobule-infiltrating forms of breast cancer (at Lineweaver - Burk coordinates, n = 3).

Note: A - untransformed breast tissue; B - moderately differentiated lobule-infiltrating forms of breast cancer.

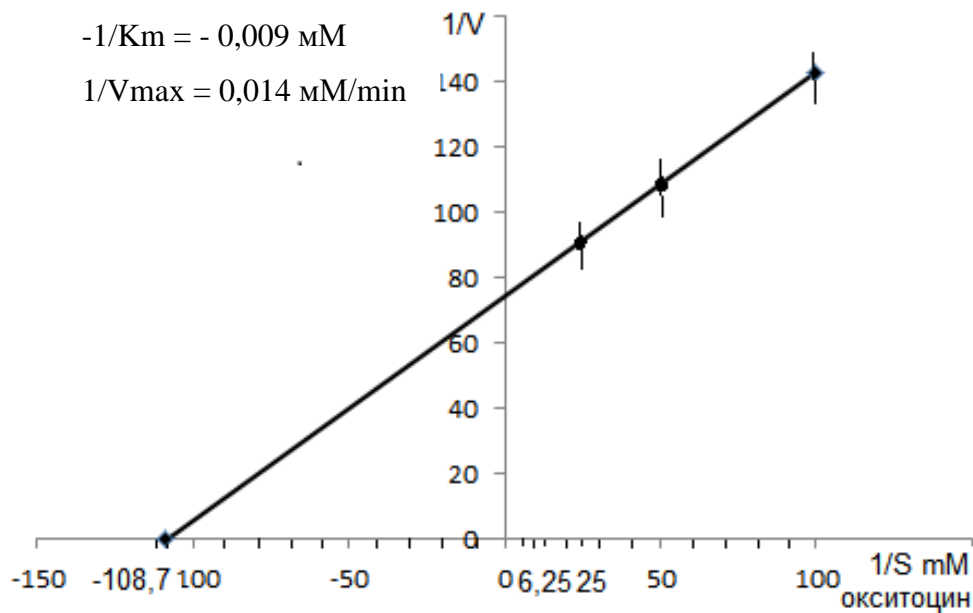


Figure 3. Effect of the substrate concentration on the speed of hydrolysis oxytocin in the presence of cathepsin H in untransformed tissue and moderately differentiated lobule-infiltrating forms of breast cancer (at Lineweaver - Burk coordinates, n = 3).

Note: A - untransformed breast tissue; B - moderately differentiated lobule-infiltrating forms of breast cancer.

Cathepsin H from moderately differentiated form with lobule-infiltrating forms of breast cancer hydrolyzed oxytocin with $K_m = 0,009 \text{ mM}$ and $V_{\max} = 0,014 \text{ mM per min}$ (at Lineweaver - Burk coordinates), and its K_m is different as K_m of cathepsin H from other biological objects (Fig. 3). Michaelis's constant of cathepsin H from moderately differentiated form with lobule-infiltrating forms of breast cancer to the oxytocin substrate was 230,769 times greater than the Michaelis's constant of cathepsin H, which was isolated from tumors of human brain, which was detected by hydrolysis of $N\alpha$ -benzoyl-DL-arginine- β -naphthylamide (BANA) substrate ($K_m = 5 \cdot 10^{-5} \text{ mM}$) [6].

Cathepsin H from moderately differentiated lobule-infiltrating forms of breast cancer hydrolyzed oxytocin with $K_m = 0,009 \text{ mM}$ and $V_{\max} = 0,014 \text{ mM per min}$ (at Lineweaver - Burk coordinates) was established (Fig. 3).

CONCLUSION

According to the results of research Michaelis's constant of cathepsin H from untransformed breast tissue ($K_m = 0,008 \text{ mM}$) was 1,123 times lower than the Michaelis constant of cathepsin H from moderately differentiated form with lobule-infiltrating forms of breast cancer ($K_m = 0,009 \text{ mM}$).

Cathepsin H, which was isolated from untransformed breast tissue had an insignificant affinity to substrate oxytocin compare with cathepsin H from moderately differentiated lobule-infiltrating form of breast cancer.

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