Exp Oncol 2004
26, 4, 278–281

Experimental ONCOLOGY

# SOME PHYSICOCHEMICAL PROPERTIES OF CATHEPSIN H FROM HUMAN MENINGIOMA

*Valentina Chornaya, Olga Lyannaya**
*Department of Experimental Physics, Dniepropetrovsk National University, Dniepropetrovsk 49050, Ukraine*

***Aim*: To compare some physicochemical properties of cathepsin H from human brain tumors and normal brain tissues. *Methods*: The enzyme was isolated from normal human brain tissue and fibrous meningioma and purified by methods of homogenization, saturation by $(NH_4)_2SO_4$ (30–80% saturation), followed by Concanavalin A and Azocazein-Sepharose chromatographies and gel filtration on Sephadex G-100 (75). *Results*: Cathepsin H was purified by 1795-fold from normal human brain tissue and by 2457-fold — from meningioma tissue. $V_{max}$ for cathepsin H from meningioma was found to be 3.5 times higher than that of normal brain tissue, whilst $K_m$ of the enzyme from tumor tissue is 1.3 times lower than that from normal human brain. The molecular weights of cathepsin H from normal human brain tissue and meningioma were 28 kDa and 25 kDa respectively. *Conclusion*: Cathepsin H isolated from human fibrous meningioma with microconcentric structures possesses higher activity levels and $V_{max}$ value and lower molecular weight than the enzyme from normal human neocortex tissue, without alteration in its pH optimum and $K_m$ value.**
***Key Words*: brain, meningioma, cathepsin H, kinetic parameters.**

Lysosomal cysteine cathepsins are the family of 11 proteases including cathepsins B, C, L, H, S and others [1]. During the past ten years, overview on cysteine cathepsins' role has been changed. It was established that these enzymes participate in remodeling and degradation of extracellular matrix proteins [2–5], control of immune response [6, 7], tumor metastasis and invasion [8, 9] and aging alterations in cell [10, 11]. Moreover, their ubiquity alone makes them potential targets for anticancer drugs [12], and their levels in tumor tissue extracts and extracellular fluids may serve as new prognostic factors for different tumor types [9, 13, 14].

Cysteine peptidehydrolases (EC 3.4.22) represent proteolytic enzymes with SH–group of cysteine in catalytic group of active site that are active in the presence of thyol containing compounds. Cysteine cathepsins are localized in lysosomes [15–17]. Salama et al. [18] revealed that about 65–80% of soluble lysosomal peptidehydrolases belong to cysteine family.

Cathepsin H (EC 3.4.22.19) is a peptidehydrolase that was firstly isolated from rat liver by Kirschke et al. [19], and then identified in liver, kidney, spleen and brain of different mammals. This enzyme has both aminopeptidase and endopeptidase activities. The increase of this enzyme activity and expression were found in blood sera of patients with melanoma, colorectal, lung and head and neck cancer [13].

The increase of expression and secretion of cysteine proteases in tumor tissues may be due to the changes in translation/transcription, posttranslational processing, and/or intracellular transport of those enzymes upon malignant transformation. The role of cathepsin H in human neoplasia is poorly studied yet. Immunohistochemical research has demonstrated that expression of cathepsin H in melanoma metastases is higher than that in primary tumor, and that cathepsin H–like enzyme may participate in destruction of extracellular matrix components of melanoma cells thus influencing proliferation and metastasis [20, 21].

The present study was aimed on comparative analysis of physicochemical properties of cathepsin H isolated and purified from human normal neocortex and brain tumor tissue.

Received: May 06, 2004.
*Correspondence: E–mail: olga_lyannaya@list.ru, olga_kakadij@ukr.net

*Abbreviations used*: BANA — Nα–benzoyl–DL–arginine–β–naphthylamide; EDTA — ethylenediaminetetraacetate; Leu–NA — L–leucine– naphthylamide; NA — β–naphthylamine; mRNA — matrix ribonucleic acid; RNAse — ribonuclease.

## MATERIALS AND METHODS

In the work, the samples of tissues of human brain neocortex and human brain tumor (biopsy material) were studied. Autopsy human brain neocortex samples were taken from the victims of accidents without CNS injuries in the Mechnikoff Regional Hospital (Dniepropetrovsk, Ukraine) not later than 12 h after the death. Biopsy brain tumor sample was received during the surgery in Neurosurgery Department of the same hospital, and classified by malignant grade and histological type according to [22].

All manipulations with brain tissue samples were carried out at +4 °C. Cathepsin H activity was determined by hydrolysis of L–leucine β–naphthylamide (Leu–NA) (Koch–Light Laboratories, England) with some modifications using Nα–benzoyl–DL–arginine–β–naphthylamide (BANA) (Reanal, Hungary) [23]. The reaction of hydrolysis was carried out in 1.0 ml of incubation mixture after 15 min preincubation of the enzyme with 2 mM 2–mercapthoethanol (2–ME) and 2 mM $Na_2EDTA$. The activity toward BANA and Leu–NA was expressed in μM of naphthylamine (NA) per 1 mg protein per min.

Tissues were homogenized in 0.02 M Tris–HCl buffer (pH 7.2) at the ratio 1 : 1.2 and centrifuged at 10 000 g

for 20 min. Then the extracts were fractionated using ammonium sulfate in 30–80% saturation range [24], dialyzed against 0.05 M citrate (or 0.02 M phosphate) buffer containing 0.8 M NaCl, 0.002 M 2–ME and 0.001 M EDTA; otherwise, gel–filtration on Sephadex G–25 was used. Then affine chromatography on Con–canavaline–A (ConA) Sepharose and Azocasein–Sepharose was carried out. The sorbent was synthe–sized using CNBr–activated Sepharose 4B (Pharma–cia, Sweden). The column was washed with buffer solution (0.1 M acetate buffer, pH 6.0 with 0.5 M NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$, 1 mM $MgCl_2$) to remove proteins that did not bind with Con–A Sepharose. The elution of proteins that bind with Con–A Sepharose was held using the buffer with 10% methylglucoside, and of those, bind with azocasein — by buffer with 1 M NaCl, 0.001 M 2–ME (pH 9.0). For further purification of cathe–psin and for determination of its molecular weight, the gel–filtration of proteins that did not bind with Azo–casein–Sepharose was carried out using Sephadex G–100 (75) (Pharmacia, Sweden) both. The column with Sephadex was balanced with 0.01 M phosphate buffer, pH 7.5 with 2 mM EDTA and calibrated by solu–tions of bovine serum albumin (Sigma,USA), hemo–globin, trypsin, soya trypsin inhibitor, RNAse. The evaluation of protein concentration in the samples the methods of Bradford [25], Lowry [26] and spectropho–tometry [24] were applied.

## RESULTS AND DISCUSSION

Purification of cathepsin H from tissue samples of neocortex and brain fibrous meningioma with micro–concentric structures was carried out as follows (see Materials and Methods): homogenization, $(NH_4)_2SO_4$ precipitation, affine chromatography on ConA–Sepharose, chromatography on Azocasein–Sepharose for fractions that bound with Con–A, and gel–chroma–tography on Sephadex G–100, G–75.

The application of affine chromatography on ConA–Sepharose resulted in 410– and 1420–folds purifica–tion of cathepsin H glycoprotein from neocortex tissue and brain tumor tissue in comparison with homoge–nate, respectively (Fig. 1, 3). The peak of cathepsin H activity in both cases coincided with protein peak in frac–tions that bound to ConA–Sepharose. That fraction underwent further purification by chromatography on Azocasein–Sepharose (Fig. 2, 4). Kirschke et al. [19] to obtain high–purity preparation of cathepsin H, used the affine chromatography on Hg–Sepharose or ConA–Sepharose. From different sources it is known that molecular weight of the enzyme is 28 kDa–30 kDa and pI is ranging from 6.5 to 7.5 [18, 19]. Besides, the cathe–psin H molecule isolated from rat liver is composed from 220 amino acid residues and shares high sequence homology with cathepsin B from the same source and papain [27]. The presence of mannose residues in oli–gosaccharide component of cathepsin H explains its high affinity to Concanavaline A and proves its lysoso–mal localization [19, 27].

Next, the fraction that did not bind with azocasein was studied by gel–filtration on Sephadex G–100 to

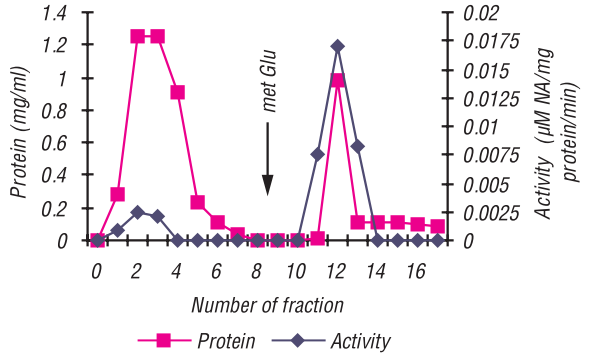


**Fig. 1.** Affine chromatography on concanavaline A sepharose. The elution profile of cathepsin H from normal neocortex frac–tions that were eluted by 10% methylglucoside (met–Glu)

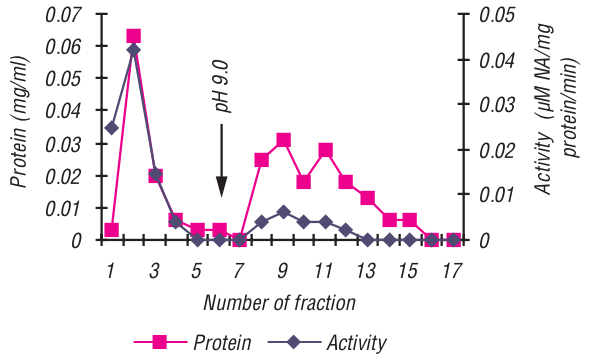


**Fig. 2.** The elution profile of normal neocortex cathepsin H frac–tions, bound with concanavaline A sepharose, after chromatog–raphy on azocasein–sepharose

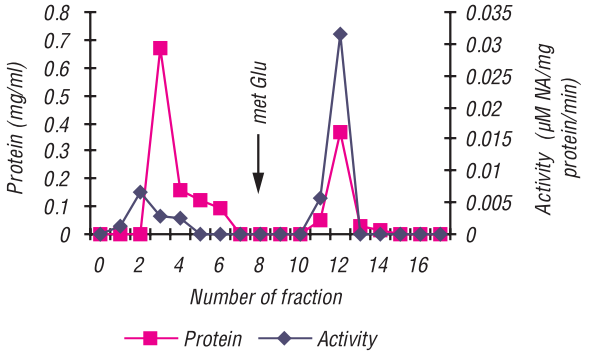


**Fig. 3.** The elution profile of brain meningioma cathepsin H frac–tions after affine chromatography on concanavaline A sepharose (the fractions that had affinely bound with the carrier were eluted by 10% methylglucoside (met–Glu))

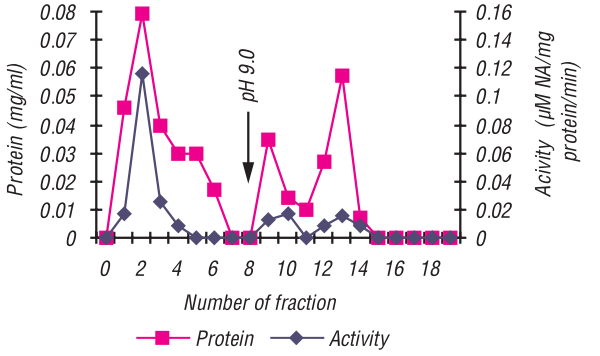


**Fig. 4.** The elution profile of brain meningioma cathepsin H frac–tions, bound with concanavaline A sepharose, after chromatog–raphy on azocasein–sepharose

determine the molecular weight of the enzyme. Established molecular weights of cathepsin H from neocortex tissue and brain tumor tissue were 28 kDa and 25 kDa, respectively. Our data are in agreement with the data of Sivaparvathi et al. [28] that had showed that cathepsin H molecular weight was 27 kDa and 25 kDa in normal brain and tumor samples (gliomas) correspondingly. The applied 5–stage scheme of purification resulted in 1795 fold–and 2457–fold purification of cathepsin H from human neocortex tissue and human brain tumor in comparison with homogenate respectively. During both the research of cathepsin H activity dependence on substrate concentration and determination of $V_{max}$ and $K_m$ kinetic values, the graphical analysis methods of Michaelis–Menten and Lineweaver–Burk were used [29].

We have shown that $K_m$ value for cathepsin H from human brain neocortex is $5 \cdot 10^{-5}$ M, and $V_{max}$ is 0.12 μM NA/mg protein/min. These values are close to the data obtained by Azaryan et al. [30] that have shown that $K_m$ value for enzyme from human brain toward arginine–N–naphthyleamide and benzoyl–arginine–N–naphthylamide were $1.0 \cdot 10^{-4}$ and $7.0 \cdot 10^{-4}$ M respectively.

It is known that the cathepsin H activity levels in human astrocytomas, gliomas and glioblastomas are noticeably higher than those in normal brain and gliomas with low grade of malignancy [28]. Guinec et al. [31] demonstrated that the highest level of cathepsin H activity in tumor tissues is observed in the pH range of 5.0–7.0. Besides, they showed that cathepsin H participates in protein degradation of basal membrane and thus promoting tumor progression.

It was found that $V_{max}$ for the enzyme in brain tumor was 0.41 μM 2NA /mg protein/min, that was in 3.5 folds higher than $V_{max}$ for normal brain cathepsin H, and $K_m$ was $3.9 \cdot 10^{-5}$ M.

The increase in brain tumor lysosomal cysteine cathepsin H activity level may be probably caused by increased enzyme expression on protein and mRNA levels [28, 32], as well as alterations in processing [33], subcellular localization and endogenous inhibitor regulation [34].

The influence of pH value on the rate of hydrolysis of synthetic substrates BANA and Leu–NA by cathepsin H isolated from human neocortex is presented on Fig. 5, *a*. One may see that the dependence of Leu–NA hydrolysis on pH value reaches the maximum at pH 6.0 that points to aminopeptidase activity of the cathepsin. At the same time, even insignificant shifts of pH from the optimal value led to abrupt reduction of cathepsin H activity (for example, at pH 5.0 the enzyme activity yields only 7.6% from the maximal level, at pH 6.5 — 35%). The maximal activity toward BANA was also reached at pH 6.0, pointing for the presence of endopeptidase activity of cathepsin H. Those data are in agreement with the data of other authors on substrate specificity of cathepsin from human brain, bovine spleen, muscles [28, 30] demonstrating that combination of thioldependent endopeptidase and aminopeptidase activities is the characteristic feature of cathepsin H. The dependence of cysteine cathepsin H aminopeptidase activity from brain fibrous meningioma with microconcentric structures on pH milieu (Fig. 5, *b*) indicated that maximum activity value responds to pH 6.0 too. It was shown, that there was no shift in pH optimum for cathepsin H activity from tumor brain tissue in comparison with that in normal brain.

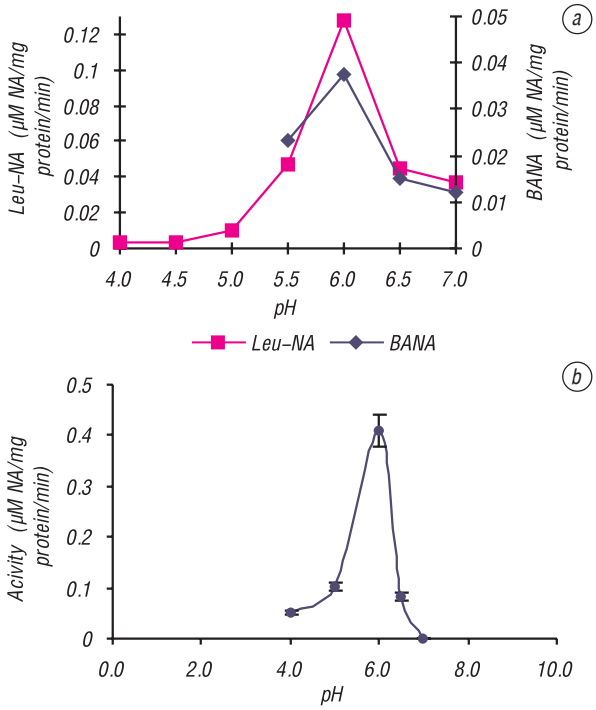


**Fig. 5.** Influence of pH on the activity of cathepsin H from neocortex (*a*) and brain fibrous meningioma with microconcentric structures (*b*)

In conclusion, we have shown that cathepsin H isolated from human fibrous meningioma with microconcentric structures possesses higher activity levels and $V_{max}$ value and lower molecular weight than the enzyme from normal human neocortex tissue, without alteration in its pH optimum and $K_m$ value.

## REFERENCES

1. **Wolters PJ, Chapman HA.** Importance of lysosomal cysteine proteases in lung disease. Resp Res 2000; **1**: 170–7.

2. **Riese RJ, Chapman HA.** Cathepsins and compartmentalization in antigen presentation. Curr Opinion Immunol 2000; **12**: 107–13.

3. **Buck MR, Karustis DG, Day NA, Honn KV, Sloane BF.** Degradation of extracellular-matrix proteins by human cathepsin B from normal and tumor tissues. Biochem J 1992; **28**: 273–8.

4. **Sires UI, Schmid TM, Fliszar CJ, Wang ZQ, Gluck SL, Welgus HG.** Complete degradation of type X collagen requires the combined action of interstitial collagenase and osteoclast-derived cathepsin B. J Clin Invest 1995; **95**: 2089–95.

5. **Lah TT, Buck KM, Honn KV.** Degradation of laminin by human tumor cathepsin B. Clin Exp Metastasis 1989; **7**: 461–8.

6. **Antoniou AN, Blackwood SL, Mazzeo D, Watts C.** Control of antigen presentation by a single protease cleavage site. Immunity 2000; **12**: 391–8.

7. **Riese RJ, Mitchell RN, Villadangos JA, Shi GP, Palmer JT, Karp ER, De Sanctis GT, Ploegh HL, Chap-**

**man HA.** Cathepsin S activity regulates antigen presentation and immunity. J Clin Invest 1998; **101**: 2351–63.

8. **Coulibaly S, Schwihla H, Abrahamson M, Albini A, Cerni C, Clark JL, Ng KM, Katunuma N, Schlappack O, Glossl J, Mach L.** Modulation of invasive properties of murine squamous carcinoma cells by heterologous expression of cathepsin B and cystatin C. Int J Cancer 1999; **83**: 526–31.

9. **Lah TT, Kos J.** Cysteine proteinases in cancer progression and their clinical relevance for prognosis. Biol Chem 1998; **379**: 125–30.

10. **Cuervo AM, Dice JF.** When lysosomes get old. Exp Gerontol 2000; **35**: 119–31.

11. **Juhg H, Lee EY, Lee SI.** Age-related changes in ultrastructural features at cathepsin B- and D-containing neurons in rat cerebral cortex. Brain Res 1999; **844**: 43–54.

12. **Turk D, Guncar G.** Lysosomal cysteine proteases (cathepsins): promising drug targets. Acta Cryst 2003; **D59**: 203–13.

13. **Kos J, Schweiger A.** Cathepsins and cystatins in extracellular fluids — useful biological markers in cancer. Radiol Oncol 2002; **36**: 176–9.

14. **Lah TT, Kos J, Blejec A, FrKovic-Georgio S, Golouh R, Vrhovec I, Turk V.** The expression of lysosomal proteinases and their inhibitors in breast cancer: possible relationship to prognosis of the disease. Pathol Oncol Res 1997; **3**: 89–98.

15. **Barrett AJ, McDonald J.** Mammalian proteases: a glossary and bibliography. V.1.: The endopeptidases. London: Academic press, 1980; 213 pp.

16. **Tang J, Wong R.** Evolution in the structure and function of aspartic proteases. J Cell Biochem 1987; **33**: 53–63.

17. **Pokrovskiy AA, Tutelyan VA.** Lysosomes. Moskow: Nauka, 1976; 382 pp (In Russian).

18. **Salama ZB, Bohley P.** Studies on some effectors of lysosomal proteinases from rat liver. Acta Biol Med Germ 1977; **36**: 1939–44.

19. **Kirschke H, Langner J, Riemann S, Wiederanders B, Ansorge S, Bohley P.** Lysosomal cysteine proteinases: protein degradation in health and disease. Elsevier, North Holland, 1980; 15–35.

20. **Kageshita T, Yoshii A, Kimura T, Maruo K, Ono T, Himeno M, Nishimura Y.** Biochemical and immunohistochemical analysis of cathepsins B, H, L and D in human melanocytic tumors. Arch Dermatol Res 1995; **287**: 266–72.

21. **Friedrich B, Yung K, Lein M, Turk I, Rudolph B, Hampel G, Schnorr D, Loening SA.** Cathepsins B, H, L and cysteine protease inhibitors in malignant prostate cell lines, primary cultured prostatic cells and prostatic tissue. Eur J Cancer 1999; **35**: 138–44.

22. Histologic classification of CNS tumors. WHO: Geneva, 1983.

23. **Schwartz WN, Barrett AJ.** Human cathepsin H. Biochem J 1980; **191**: 487–97.

24. **Kochetov GA.** Manual on enzymology. Moskow: Vyschaya shkola, 1980; 272 pp (In Russian).

25. **Bradford MM.** A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; **72**: 248–54.

26. **Lowry OH, Rosenbrough HI, Farr ZA, Randall RJ.** Protein measurement with the folin phenol reagent. J Biol Chem 1951; **193**: 265–75.

27. **Azaryan AV.** Peptide hydrolases of nervous system and their biological functions. Erevan: Ajastan, 1989; 207 pp (In Russian).

28. **Sivaparvathi M, Sawaya R, Gokaslan Z, Chintala SK, Rao JS, Chintala KS.** Expression and the role of cathepsin H in human glioma progression and invasion. Cancer Lett 1996; **104**: 121–6.

29. **Cornish-Bouden A.** Fundamental of enzyme kinetics. London: Portland Press Ltd. 2002; 343 pp.

30. **Azaryan A, Galoyan A.** Human and bovine brain cathepsin L and cathepsin H: purification, physico-chemical properties, and specificity. Neurochem Res 1987; **12**: 207–14.

31. **Guinec N, Dalet-Fumeron V, Pagano M.** Digestion *in vitro* of basal membrane, bovine lens capsule, by human liver lysosome cathepsins B, H and L and ascitic fluid tumor cathepsin B from ovarian adenocarcinoma. Pathol Biol (Paris) 1990; **38**: 988–1092.

32. **Koblinski JE, Ahram M, Sloane BF.** Unraveling the role of proteases in cancer. Clin Chim Acta 2000; **291**: 113–5.

33. **Kos J, Werle B, Lah T, Brunner N.** Cysteine proteinases and their inhibitors in extracellular fluids: markers for diagnosis and prognosis in cancer. Int J Biol Markers 2000; **15**: 84–9.

34. **Schwartz MK.** Tissue cathepsins as tumor markers. Clin Chim Acta 1995; **237**: 67–78.

# НЕКОТОРЫЕ ОСОБЕННОСТИ ФИЗИКО–ХИМИЧЕСКИХ СВОЙСТВ КАТЕПСИНА Н, ВЫДЕЛЕННОГО ИЗ МЕНИНГИОМЫ ЧЕЛОВЕКА

***Цель* данной работы — сравнительный анализ некоторых физико-химических свойств катепсина Н, выделенного из нормальной ткани мозга человека и менингиомы. *Методы:* фермент выделяли путем последовательной гомогенизации ткани, высаливания сульфатом аммония (30–80% насыщение), хроматографии на конканавалин А-сефарозе и азоказеин сефарозе, гель-фильтрации на сефадексе G-100 (75). *Результаты:* катепсин Н был очищен в 1795 раз из неокортекса головного мозга человека и в 2457 раз — из опухолевой ткани. Установлено, что значение $V_{max}$ для катепсина Н из менингиомы головного мозга в 3,5 раза превышало таковое для катепсина Н нормального мозга, а $K_m$ фермента менингиомы был в 1,3 раза ниже нормы. Молекулярные массы катепсина Н, выделенного из нормальной и опухолевой тканей мозга составили 28 кДа и 25 кДа соответственно. Выводы: катепсин Н, выделенный из ткани фиброзной менингиомы человека, обладает более высокими значениями активности и $V_{max}$ и более низкой молекулярной массой, чем фермент нормальной ткани мозга, но не отличается от такового по величине $K_m$ и оптимальному значению pH.**

***Ключевые слова*: головной мозг, менингиома, катепсин Н, кинетические параметры.**