

AMPHIBIAN SKIN SECRETIONS: A POTENTIAL SOURCE OF PROTEOLYTIC ENZYMES

I. Nikolaieva¹

Yu. Dudkina¹

D. Oliinyk¹

O. Oskyrko¹

O. Marushchak²

T. Halenova¹

O. Savchuk¹

¹Taras Shevchenko National University of Kyiv, Ukraine

²Schmalhausen Institute of Zoology
of the National Academy of Sciences of Ukraine, Kyiv

E-mail: irisha.nikolaeva@gmail.com

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The aim of the work was to study the protein content and proteolytic activity of the skin glands secretions of 10 the most common types of amphibians on the territory of Ukraine such as *B. bombina*, *B. variegata*, *B. bufo*, *B. viridis*, *R. temporaria*, *P. ridibundus*, *P. esculentus*, *P. fuscus*, *S. salamandra*, as well as the hybrid of *B. bombina* and *B. variegata* species. It was shown that the skin secretions of the studied amphibians contained a wide range of proteins with a molecular weight in the range from 8 to 150 kDa. By enzyme electrophoresis using gelatin, fibrinogen and collagen as substrates, it was found that they contained proteinases that differ in substrate specificity. It was revealed that the skin glands secretions of *B. bombina*, *S. salamander* species, as well as the hybrid of *B. bombina* and *B. variegata* species were characterized by the increased protein content with gelatinase and collagen activity.

Key words: amphibians, skin gland secretions, proteolytic activity.

Amphibians are recognized as a rich source of bioactive compounds with valuable biotechnology potential and according to their availability and accessibility they are supposed to be a superior raw material. The molecules derived from amphibian skin secretions have various activities that determine their profound applications in chemical and medical industries. In this regard, one of the main objectives of modern biotechnology is the search for new potential biologically active compounds of natural origin. Since their effectiveness and safety cannot be compared with chemically synthesized compounds, which characterized by a great number of side effects and unpredictable actions, they might have beneficial application in the science and industry.

The promising raw materials that contain different bioactive molecules are various plants, marine invertebrates and reptiles. In the last two decades scientists drew their attention to amphibians as potential objects for biochemical studies and industrial purposes [1]. The great number of researches

indicate that the amphibian glandular secretion is a rich source of various molecules with cardiotoxic [2, 3,], antidiabetic [4], immunomodulatory [5], antimicrobial [6, 7] and antiviral [8, 9] activities. It has also been established that they have sedative [10] and analgesic effects [11]. Moreover, considering the availability and accessibility of some species of amphibians the further study of the effects of the compounds from their skin secretions is very relevant.

In most of the cases the wide range of the effects of the components of skin secretions are associated with proteolytic activities. Proteolytic enzymes are capable of hydrolyzing peptide bonds in proteins and have great medical and pharmaceutical importance due to their key role in biological processes, such as: in digestion of food proteins, protein turnover, cell division, blood-clotting cascade, signal transduction, processing of polypeptide hormones, apoptosis and the life-cycle of several disease-causing organisms including the replication of retroviruses [12]. Alongside proteases are extensively applied enzymes in

several sectors of industry and biotechnology and numerous research applications require the use of them [1].

Although the territory of Ukraine is dwelled by numerous amphibians, there are a few studies concerning the nature and properties of the biologically active compounds of their skin secretion. So, the purpose of this work is to examine the presence of proteolytic enzymes in the skin secretions of the most common Ukrainian species of amphibians and to evaluate the gelatinolytic, fibrinogenolytic and collagenolytic activities to create a background for further investigations of amphibian secretions and for studying of their pharmaceutical potency.

Materials and Methods

Collection of frog skin secretions

There are a great number of ways of collection of the crude skin secretions that are shown in the studies, but most of them are inhumane. They usually include lethal release of the venoms, when the skin of animals that previously were subjected to decapitation, is removed, dried and ground to powder consistency for further use. Another lethal variant is to place a frog in a flask with anhydrous ether, which stimulates the secretion of the skin poison that is washed from the surface of the animal with deionized water. There are also some modern non-lethal methods, which include the usage of an electric current that causes synchronous release of toxic secretions from the glands or the stimulation of poison release by chemical injection [13]. In our research, we have used safe methodological approach, which allows us to use amphibians the unlimited number of times.

Adult pubescent specimens (both sexes) of *Bombina bombina* ($n = 20$), *Bombina variegata* ($n = 20$), *Bufo bufo* ($n = 15$), *Bufo viridis* ($n = 10$), *Rana temporaria* ($n = 10$), *Pelophylax ridibundus* ($n = 8$), *Pelophylax esculentus* ($n = 5$), *Pelobates fuscus* ($n = 7$), *Salamandra salamandra* ($n = 2$) and hybrid of *Bombina bombina* and *Bombina variegata* ($n = 5$) were collected outdoors in Kyiv region of Ukraine. The crude skin secretions were obtained by washing the skin with ultrapure water beyond mechanical stimulation of skin glands. Water solutions of skin secretions of all species were centrifuged at 3000 rpm for 15 min to remove debris. The supernatants were lyophilized (TestarLyoQuest) and kept at 4 °C till use.

Samples preparation

The samples of lyophilized skin secretions were resuspended in Tris-buffered saline (TBS), pH 7.4 (30 mg of dried material/ml) and centrifuged at 7000 g for 15 min. Protein concentration in supernatant was assayed by Bradford method [14], using bovine serum albumin as a standard. Samples for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and for zymography assay were mixed in equal volumes with the standard SDS-PAGE sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 5% sucrose, and 0.002% bromophenol blue) without heating.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis

SDS-PAGE of crude skin secretions was carried out using 4% (w/v) stacking gel and 12% (w/v) separating gel [15]. SDS-PAGE was performed using Mini-Protean Tetra System (Bio Rad, USA) at 19 mA for stacking and 36 mA for separating gels. The volume of sample applied per line was 15 μ l. The gels were stained with 2.5% coomassie brilliant blue R-250 in 10% (v/v) ethanol, 10% (v/v) acetic acid, 15% (v/v) isopropanol and the background of the gel was destained with 7% (v/v) acetic acid for 30 min. Apparent molecular weights of proteins were estimated using protein calibration mixture (Bio Rad, USA) containing myosin, β -galactosidase, phosphorylase b, serum albumin, ovalbumin (Mr 97; 66; 45; 31; 21; 14 kDa).

Zymography

Zymography was done according to the method Ostapchenko et al [16]. Separating gel (12% w/v) was polymerized in the presence of gelatin (1 mg/ml), fibrinogen (1 mg/ml) or collagen (1 mg/ml). The volume of samples applied to the gel was 15 μ l per line. After electrophoresis, the gels were incubated for 30 min at room temperature on a rotary shaker in 2.5% Triton X-100. Then the gels were washed with distilled water to remove Triton X-100 and incubated in 50 mM Tris-HCl (pH 7.4) at room temperature for 12 hours. The gels were stained with 2.5% coomassie brilliant blue R-250 in 10% (v/v) ethanol, 10% (v/v) acetic acid, 15% (v/v) isopropanol for 30 min and then destained. The digested bands were visualized as the nonstained regions of the zymogram gel.

Calculation of the results

TotalLab 2.04 program was used to analyze the resultant electrophorograms. The

represented electrophorograms and zymograms are typical for the series of the repeated experiments (at least three in each series).

Results and Discussion

Amphibians' glandular skin secretions are a rich source of potent biologically active compounds, many with high potential for therapeutic drug development [17]. While voluminous researches concerning the chemical structure and properties of crude skin secretions have been made all over the world [18], relatively little is known about the protein composition and biological activities of glandular secretion of different families of amphibians that represent the batrahofauna of Ukraine.

Therefore, on the first stage of our work we wanted to get information about the protein composition of glandular skin secretions of the most common Ukrainian species of amphibians. To achieve this the SDS-PAGE analysis was performed. The typical electrophorograms of crude skin secretions are shown on the Fig. 1. The results of electrophoretic protein separation revealed the presence of proteins with molecular weights ranging from 6 to 149 kDa. It indicates that the crude skin secretions of studied amphibian species are characterized by a wide range of proteins with different molecular weights and alongside confirm a diverse protein composition of all studied secretions.

To define the exact molecular weights of identified protein fractions the electrophorograms were analyzed using the TotalLab 2.04 program (Table). It was shown

that different representatives of one type of species had similar protein composition. Thus, five common proteins were observed on the electrophoretic profile of crude skin secretion of amphibian species of Bombina family. The differences between these two studied secretions were only in two proteins (31 and 102 kDa), which were present in crude skin secretion of *B. variegata*. Noteworthy is that the protein composition of the hybrid of *B. bombina* and *B. variegata*, was almost identical to *B. variegata*. The protein composition of the representatives of the Bufo family had more pronounced differences. Even though all proteins that were discovered within this family had low molecular weight, their amount varied. Therefore, in *B. bufo* crude skin secretion five proteins ranging from 29 to 72 kDa was identified and nine proteins ranging from 8 to 68 kDa was observed in *B. viridis* skin venom. The protein composition of two representatives of Pelophylax family was also similar, except two proteins with molecular weights 31 kDa and 149 kDa, which were present in *P. ridibundus*. The largest number of proteins that were observed among the studied amphibian species was identified in *R. temporaria*. It was found eleven low molecular proteins ranging from 11 to 64 kDa in its glandular skin secretion. Only three proteins 18, 31 and 46 kDa were observed in *P. fuscus*. It was also identified six proteins in glandular skin secretions of *S. salamander* ranging from 10 to 60 kDa.

The variety of proteins with different molecular weights that are present in the crude skin secretions of studied amphibian species indicates the presence of different molecules

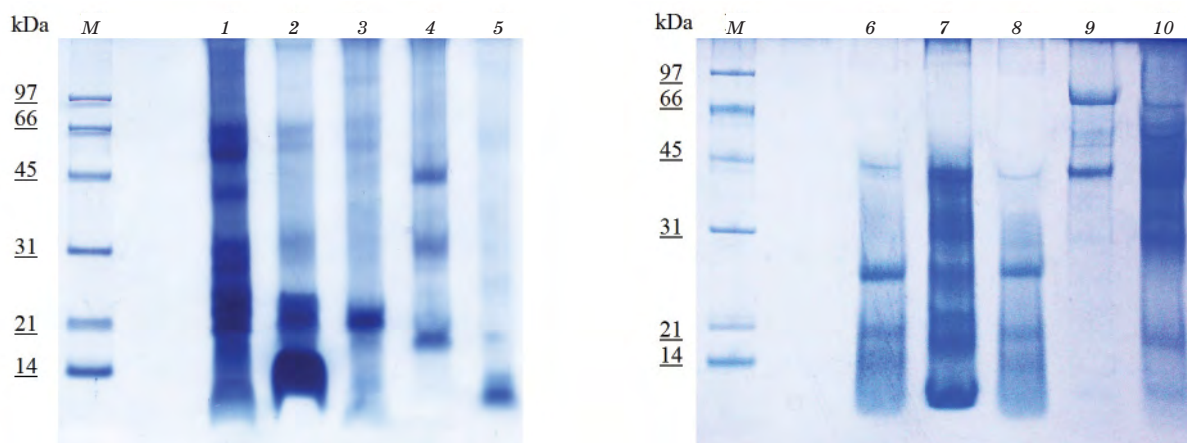


Fig. 1. Typical electrophoregram of crude skin secretions of studied amphibian species:
 1 — *B. bombina*; 2 — *B. variegata*; 3 — hybrid of *B. bombina* and *B. variegata*; 4 — *B. bufo*; 5 — *B. viridis*;
 6 — *R. temporaria*; 7 — *P. ridibundus*; 8 — *P. esculentus*; 9 — *P. fuscus*; 10 — *S. salamander*;
 M — markers of molecular weight

The molecular weights (MW) of proteins that are present in crude skin secretions of studied amphibian species

Amphibian species	MW, kDa
<i>Bombina bombina</i>	7; 14; 20; 27; 42.
<i>Bombina variegata</i>	7; 17; 22; 26; 31; 40; 102.
Hybrid of <i>B. bombina</i> and <i>B. variegata</i>	6; 13; 19; 27; 29; 41; 99.
<i>Bufo bufo</i>	29; 41; 48; 52; 72.
<i>Bufo viridis</i>	8; 18; 29; 32; 35; 40; 50; 56; 68.
<i>Rana temporaria</i>	11; 17; 20; 22; 24; 28; 31; 35; 42; 51; 64.
<i>Pelophylax ridibundus</i>	14; 22; 24; 31; 54; 66; 109; 149.
<i>Pelophylax esculentus</i>	11; 18; 22; 54; 69; 115.
<i>Pelobates fuscus</i>	18; 31; 46.
<i>Salamander salamander</i>	10; 19; 26; 30; 38; 60.

that could have biological significance and might be a source of different types of enzymatic activities. The presence of proteins with molecular weight that is lower than 100 kDa especially binds attention, since it is known that most of the proteolytic enzymes have molecular weight up to 100 kDa.

One of the simplest and the most sensitive visual methods of the detection of active proteases in the biological material is zymography method [19]. In this methodological approach the gels polymerize in the presence of the corresponding substrate proteins. And using different polymerized substrates we can identify the presence or absence of proteolytic enzymes.

In this research gelatin, fibrinogen and collagen were used as substrates to evaluate the proteolytic potential of crude skin secretions of studied amphibian species. Our aim was to test the substrate specificity of proteolytic enzymes and to identify the presence of gelatinolytic, fibrinogenolytic and collagenolytic activities.

The typical zymograms of the detection of gelatinolytic, collagenolytic, and fibrinogenolytic activities are shown on Fig. 2. The appearance of the light digested zones of hydrolysis was due to the manifestation of enzymatic activity and indicated the presence of active proteolytic enzymes with the substrate specificity in the studied amphibian crude skin secretions. The active proteins trypsin (24 kDa) and plasmin (84 kDa) were used to identify the approximate molecular weights of active compounds.

Gelatin is considered as a common substrate which helps to study the overall proteolytic activity and usually used to pre-evaluate the presence of active forms of enzymes. According to the results the components of the crude

skin secretions of *B. bombina*, *B. variegata*, the hybrid of *B. Bombina* and *B. variegata*, *B. viridis*, *P. esculentus* and *S. Salamander* had pronounced gelatinolytic activity. Most of the light digested areas corresponds to fractions of proteins with molecular weight up to 70 kDa. Whereas it was not observed expressed gelatinolytic activity in the study of the components of crude skin secretions of other amphibian species.

Collagen and fibrinogen are substrates with high specificity and the small amount of enzymes are capable to hydrolyse them. On the other hand, using these substrates can help us to identify and detail the proteolytic enzymes action.

While studying the zymograms of crude skin secretions with fibrinogen as a substrate, the total fibrinogenolytic activity was insignificant and the hydrolysis zone was identified only in the regions that corresponds to the fractions of proteins with high molecular weight. The light digested zones correspond to the pronounced fibrinogenolytic activity and were revealed on the zymograms of the crude skin secretions of *B. bombina*, *S. salamander* and the hybrid of *B. bombina* and *B. variegata*.

Generally, the true fibrinogenolytic enzymes have a molecular weight in the range from 20 to 60 kDa, but the enzymes with both lower and higher molecular weights are also known [20]. The presence of high molecular weight proteolytic enzymes with specificity to fibrinogen might be due to the inclusion of the enzymes in the complexes.

According to the results of zymography of crude skin secretions using collagen as a substrate, it was revealed the presence active hydrolytic enzymes that are capable to cleave collagen. Moreover, the clearly defined zones of hydrolysis were not detected, since the collagenolytic activity

with different degree of severity was noted throughout the length of the tracks.

Enzymes with collagenolytic activity are very interesting as they have a lot of practical application in medicine, biotechnology and food industry. Based on collagenolytic enzymes several medicines have already been developed to treat wounds, burns and other skin lesions [21]. The new types of collagenolytic

enzymes are a promising material for the development of more specialized drugs [22].

Thus, considering the obtained results, we can state that the crude skin secretions of studied amphibian species have a pronounced protease activity with specificity to different substrates. The components of crude skin secretions of *B. bufo* and *R. temporaria* had the least evident gelatinolytic, fibrinogenolytic

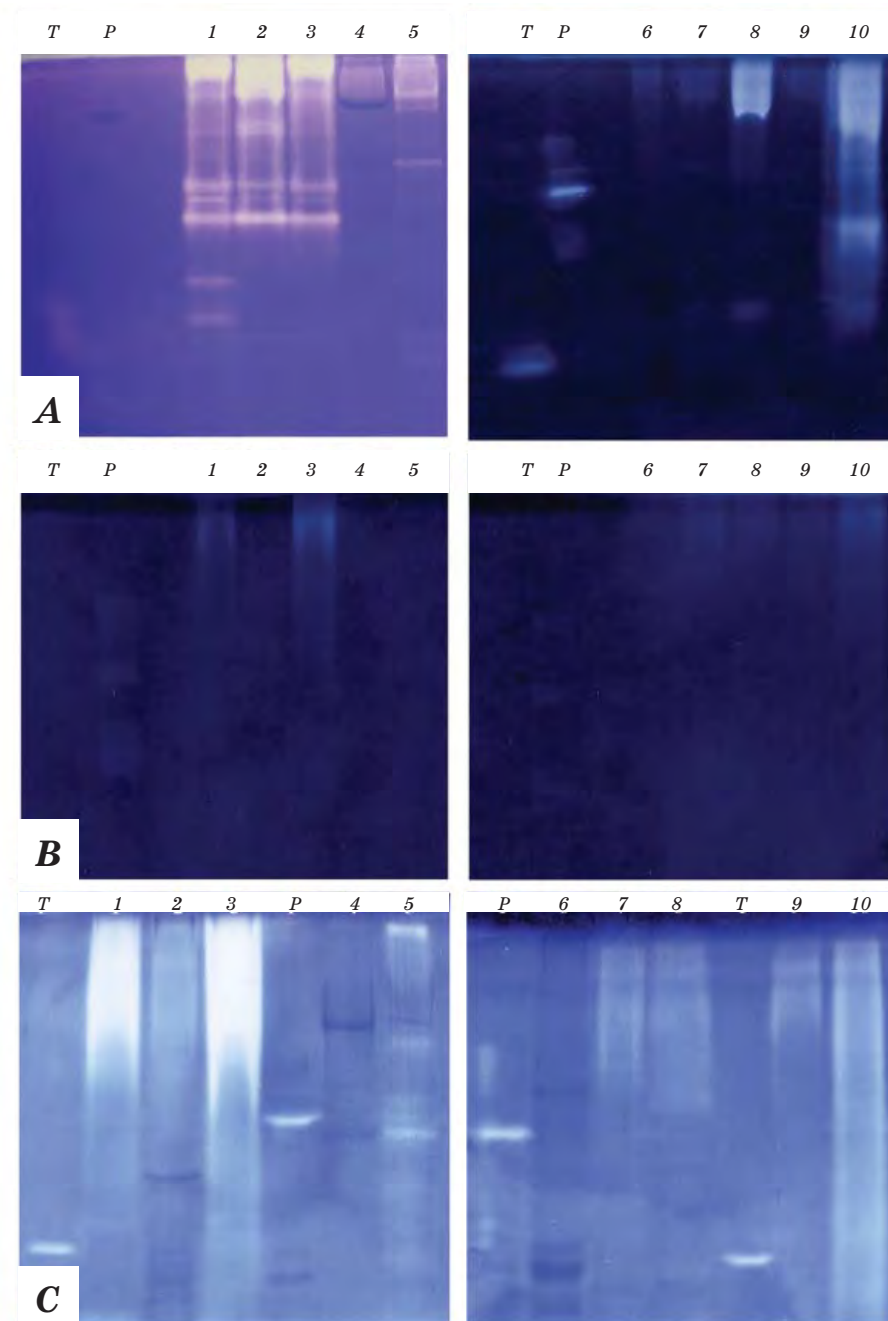


Fig. 2. Typical zymograms of crude skin secretions using gelatin (A), fibrinogen (B) and collagen (C) as substrates:

- 1 — *B. bombina*; 2 — *B. variegata*; 3 — hybrid of *B. bombina* and *B. variegata*; 4 — *B. bufo*; 5 — *B. viridis*;
 6 — *R. temporaria*; 7 — *P. ridibundus*; 8 — *P. esculentus*; 9 — *P. fuscus*; 10 — *S. salamander*;
 T — trypsin (24 kDa) and P — plasmin (84 kDa)

and collagenolytic activities, whereas the crude skin secretions of *B. bombina*, *S. Salamander* and the hybrid of *B. Bombina* and *B. variegata* characterized by the most expressive activities and, what is worth noting, some zones of hydrolysis on the zymograms with gelatin, fibrinogen and collagen used as substrates

coincided. This can be the evidence of the presence of enzymes that simultaneously have two or three kinds of activities. Further investigations concerning the identification of other biologically active compounds in the skin secretions of amphibians and their characterization are required.

REFERENCES

- Gomes A., Giri B., Saha A., Mishra R., Dasgupta S., Debnath A., Gomes A. Bioactive molecules from amphibian skin: Their biological activities with reference to therapeutic potentials for possible drug development. *Ind. J. Exp. Biol.* 2007, 45, 579–593.
- Clarke B. T. The natural history of amphibian skin secretions, their normal functioning and potential medicinal applications. *Biol. Rev.* 1997, 72 (3), 365–379. <https://doi.org/10.1111/j.1469-185X.1997.tb00018.x>
- Morishita S., Shoji M., Oguni Y., Ito C., Noguchi K., Sakanashi M. Congestive heart failure model in rabbits: effects of digoxin and a drug containing toad venom. *Jpn. J. Pharmacol.* 1991, 56 (4), 427–432. <https://doi.org/10.1254/jjp.56.427>
- Marenah L., Flatt P. R., Orr D. F., McClean S., Shaw C., Abdel-Wahab Y. H. Skin secretion of the toad *Bombina variegata* contains multiple insulin-releasing peptides including bombesin and entirely novel insulinotropic structures. *Biol. Chem.* 2004, 385 (3–4), 315–321. <https://doi.org/10.1515/BC.2004.027>
- Shimizu Y., Inoue E., Ito C. Effect of the water-soluble and non-dialyzable fraction isolated from Senso (Chan Su) on lymphocyte proliferation and natural killer activity in C3H mice. *Biol. Pharm. Bull.* 2004, 27 (2), 256–260. <https://doi.org/10.1248/bpb.27.256>
- Barberio C., Delfino G., Mastromei G. A low molecular weight protein with antimicrobial activity in the cutaneous ‘venom’ of the yellow-bellied toad (*Bombina variegata pachypus*). *Toxicon.* 1987, 25 (8), 899–909.
- Soravia E., Martini G., Zasloff M. Antimicrobial properties of peptides from *Xenopus* granular gland secretions. *FEBS Lett.* 1988, 228, 337–342. [https://doi.org/10.1016/0014-5793\(88\)80027-9](https://doi.org/10.1016/0014-5793(88)80027-9)
- Yasin B., Pang M., Turner J. S., Cho Y., Dinh N. N., Waring A. J., Lehrer R. I., Wagar E. A. Evaluation of the inactivation of infectious Herpes simplex virus by host-defense peptides, *Eur. J. Clin. Microbiol. Infect. Dis.* 2000, 19 (3), 187–194.
- Chinchar V. G., Wang J., Murti G., Carey C., Rollins-Smith L. Inactivation of frog virus 3 and channel catfish virus by esculentin-2P and ranatuerin-2P, two antimicrobial peptides isolated from frog skin. *Virology.* 2001, 288 (2), 351–357. <https://doi.org/10.1006/viro.2001.1080>.
- Montecucchi P. C., Gozzini L., Erspamer V., Melchiorri P. The primary structure of tryptophan containing peptides from skin extracts of *Phyllomedusa rhodei* (tryptophyllins). *Int. J. Pept. Protein Res.* 1984, 24 (4) 276–281. <https://doi.org/10.1111/j.1399-3011.1984.tb02720.x>
- Montecucchi P. C., de Castiglione R., Piani S., Gozzini L., Erspamer V. Amino acid composition and sequence of dermorphin, a novel opiate-like peptide from the skin of *Phyllomedusa sauvagei*. *Int. J. Pept. Protein Res.* 1981, 17 (3), 275–283.
- Mecikoglu M., Saygi B., Yildirim Y., Karadag-Saygi E., Ramadan S., Esemeli T. The effect of proteolytic enzyme serratiopeptidase in the treatment of experimental implant-related infection. *J. Bone Joint Surg. Am.* 2006, 88 (6), 1208–1214. <https://doi.org/10.2106/JBJS.E.00007>
- Jianwu Z., McClean S., Thompson A., Yang Z., Shaw C., Rao P., Bjourson A. J. Purification and characterization of novel antimicrobial peptides from the skin secretion of *Hylarana guentheri*. *Peptides.* 2006, 27, 3077–3084. <https://doi.org/10.1016/j.peptides.2006.08.007>
- Bradford M. M. A rapid and sensitive method for quantities of utilizing the principle of protein binding. *Anal. Biochem.* 1976, 7 (72), 248–254.
- Laemmli K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* 1970, 227 (5259), 680–685.
- Ostapchenko L., Savchuk O., Burlova-Vasilieva N. Enzyme electrophoresis method in analysis of active components of haemostasis system. *Adv. Biosci. Biotechnol.* 2011, 2, 20–26. <https://doi.org/10.4236/abb.2011.21004>.
- Abhishek D., Hippargi R. V., Amit N. Gandhare Toad skin-secretions: Potent source of pharmacologically and therapeutically significant compounds. *Int. J. Pharmacol.* 2008, 5 (2), 17–23. <https://doi.org/10.5580/18b6>
- van Zoggel H., Hama-Kourbali Y., Galanth C., Ladram A., Nicolas P., Courty J., Amiche M., Delbé J. Antitumor and

- angiostatic peptides from frog skin secretions. *Amino Acids*. 2012, 42 (1), 385–395. <https://doi.org/10.1007/s00726-010-0815-9>
19. Wilkesman J., Kurz L. Protease analysis by zymography: a review on techniques and patents. *Recent Pat. Biotechnol.* 2009, 3 (3), 175–184.
20. Joung-Yoon K., Seung-Bae L., Ki Rok K., Suk-Ho C. Isolation and characterization of a 32-kDa fibrinolytic enzyme (FE-32kDa) from *Gloydius blomhoffii siniticus* venom. *J. Pharmacopunct.* 2013, 17 (1), 44–50. <https://doi.org/10.3831/KPI.2014.17.006>
21. Shekhter A.B., Balakireva A.V., Kuznetsova N.V., Vukolova M.N., Litvitsky P.F., Zamyatnin A.A. Collagenolytic enzymes and their applications in biomedicine. *Curr. Med. Chem.* 2017, 24, 1–19. <https://doi.org/10.2174/0929867324666171006124236>
22. Alipour H., Raz A., Zakeri S., Dinparast Djadid N. Therapeutic applications of collagenase (metalloproteases): A review. *Asian Pacific J. Trop. Biomed.* 2016, 6 (11), 975–981. <https://doi.org/10.1016/j.apjtb.2016.07.017>

СЕКРЕТИ ШКІРНИХ ЗАЛОЗ АМФІБІЙ — ПОТЕНЦІЙНЕ ДЖЕРЕЛО ПРОТЕОЛІТИЧНИХ ЕНЗИМІВ

І. Ніколаєва¹, Ю. Дудкіна¹,
Д. Олійник¹, О. Марущак²,
О. Осирко¹, Т. Галенова¹,
О. Савчук¹

¹Київський національний університет
імені Тараса Шевченка, Україна

²Інститут зоології ім. І. І. Шмальгаузена
НАН України, Київ

E-mail: irisha.nikolaeva@gmail.com

Метою роботи було вивчити вміст протеїнів та протеолітичну активність секретів шкірних залоз 10 найпоширеніших на території України видів амфібій: *B. bombina*, *B. variegata*, *B. bufo*, *B. viridis*, *R. temporaria*, *P. ridibundus*, *P. esculentus*, *P. fuscus*, *S. salamandra*, а також гібриду видів *B. bombina* та *B. variegata*. Показано, що секрети шкірних залоз досліджуваних видів містять широкий спектр протеїнів з молекулярною масою від 8 до 150 кДа. Методом ензимелектрофорезу з використанням желатину, фібриногену та колагену як субстратів виявлено, що вони містять протеїнази, які відрізняються за субстратною специфічністю. Встановлено, що секрети шкірних залоз видів *B. bombina*, *S. salamander*, а також гібриду видів *B. bombina* та *B. variegata* характеризуються підвищеним вмістом протеїназ із желатиназою та колагеназою активністю.

Ключові слова: амфібії, секрети шкірних залоз, протеолітична активність.

СЕКРЕТЫ КОЖНЫХ ЖЕЛЕЗ АМФИБИЙ — ПОТЕНЦИАЛЬНЫЙ ИСТОЧНИК ПРОТЕОЛИТИЧЕСКИХ ЭНЗИМОВ

И. Николаева¹, Ю. Дудкина¹,
Д. Олейник¹, О. Марущак²,
О. Осирко¹, Т. Галенова¹,
О. Савчук¹

¹Киевский национальный университет
имени Тараса Шевченко, Украина

²Институт зоологии им. И. И. Шмальгаузена
НАН Украины, Киев

E-mail: irisha.nikolaeva@gmail.com

Целью работы было изучить содержание протеинов и протеолитическую активность секретов кожных желез 10 самых распространенных на территории Украины видов амфибий: *B. bombina*, *B. variegata*, *B. bufo*, *B. viridis*, *R. temporaria*, *P. ridibundus*, *P. esculentus*, *P. fuscus*, *S. salamandra*, а также гибрида видов *B. bombina* и *B. variegata*. Показано, что секреты кожных желез исследуемых видов содержат широкий спектр протеинов с молекулярной массой от 8 до 150 кДа. Методом энзимелектрофореза с использованием желатина, фибриногена и коллагена как субстратов обнаружено, что они содержат протеиназы, которые отличаются по субстратной специфичности. Установлено, что секреты кожных желез видов *B. bombina*, *S. salamander*, а также гибрида видов *B. bombina* и *B. variegata* характеризуются повышенным содержанием протеиназ с желатиназой и колагеназой активностью.

Ключевые слова: амфибии, секреты кожных желез, протеолитическая активность.