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Exploring an antioxidant and hemostasis activity of peptides from Antarctic krill *Euphausia superba*

Abstract. The goal of the study was to obtain the fractions of endogenous and hydrolytic peptides from the hydrobiont *Euphausia superba* and evaluate their antioxidant potential and possible activity against certain hemostasis factors. The fraction of endogenous peptides was isolated by stepwise precipitation of proteins with perchloric acid and ethanol. Peptides with a molecular weight up to 5 kDa were isolated by by ultrafiltration. Hydrolysis with trypsin was used to obtain hydrolytic peptides. The purity of peptide fractions was confirmed by SDS-polyacrylamide gel electrophoresis. Antioxidant activity was assessed by analyzing the peptides' reducing power, 2,2-diphenyl-1-picrylhydrazyl, and nitric oxide radical scavenging activity. To assess the effect of peptides on the amidolytic activity of thrombin, active thrombin was pre-incubated with peptide fractions, and further thrombin activity was determined using the chromogenic substrate S2238. The ability of the peptides to influence ADP-induced platelet aggregation was tested in platelet-rich plasma. The results showed that endogenous and hydrolytic peptides exhibit moderate antioxidant activity; however, endogenous peptides were more potent antioxidants than peptides produced by trypsin hydrolysis. The influence of *E. superba* peptides on some hemostasis factors has been established. Inhibition of ADP-induced platelet aggregation by hydrolytic peptides (by 1.76 times) was found, while endogenous peptides possess the opposite effect. The differences in the activity and effectiveness of the peptides indicate that the fractions contain molecules that differ in amino acid composition. Considering the data, *E. superba* can be a source for peptides with moderate antioxidant activity and peptides that can affect the activity of key hemostasis factors.

Keywords: Antarctic hydrobiont, endogenous peptides, hydrolytic peptides

1 Introduction

In recent years, the role and importance of peptides as physiologically active and therapeutically beneficial molecules have been increasingly recognized. Being involved in the regulation of various biochemical processes, peptides are gaining attention as possible candidates in drug development. Due to the broad spectrum of activities, namely antioxidant, antihypertensive, antiviral, antiproliferative, anticoagulant, metal-binding, anti-obesity, and anti-diabetic, peptide-based therapeutics are used to treat a wide range of pathologies such as neurological problems, diabetes, cancer, obesity, cardiovascular disorders, and other diseases (Newman & Cragg, 2020; Cappello & Nieri, 2021).

It was also reported that peptides provide muscle endurance, pain relief, injury recovery (Corrochano et al., 2021; Khatri et al., 2021). The advantages of peptides as therapeutic agents include high biocompatibility, selectivity, efficiency, and fewer side effects, in particular, the lack of immunogenicity typical for protein-based drugs. To date, pharmaceutically attractive peptides have been isolated from various natural sources such as plants, animals, bacteria, and fungi, which has encouraged researchers to search for new sources of bioactive peptides (Blunt et al., 2017; Romano et al., 2022).

The marine environment is by far the major storage of the planet's biodiversity and represents a promising source of untapped chemical richness (Carson & Clarke, 2018). To adapt to unique living conditions (variations in pressure, salinity, lighting, and temperature), marine organisms have developed many compounds with more pronounced or even unique properties than their terrestrial counterparts. Moreover, the structural features of proteins of marine organisms in terms of amino acid composition, the presence of branched, cyclic amino acids, as well as the presence of both D and L amino acids allow considering them as possible raw materials for the production of peptides for pharmaceutical use (Jo et al., 2016; Ahmed et al., 2022).

The success of using hydrobionts, namely invertebrates as sustainable sources of novel compounds, may be limited by several factors, such as the difficulty of their cultivation on a large scale. This problem can be partially addressed by utilizing commercial marine organisms to search for and produce molecules with target activities. Among commercial hydrobionts, Antarctic krill *Euphausia superba* represents a consumer resource with great exploitation potential. Given that Antarctic krill is one of the most abundant species in the ocean, as well as the amount and rate of krill catches, this hydrobiont may represent an inexhaustible source of protein for processing into biologically active peptides. A review of the literature shows that *E. superba* proteins are well-balanced in amino acids (Li et al., 2020), and their biological value is higher than milk and meat proteins, which fully comply with the dietary requirements of the Food and Agriculture Organization (FAO) / World Health Organization (WHO) for humans (Chen et al., 2009; Li et al., 2022). Even though peptides are being intensively studied, there are no publications devoted to comparing the activities of endogenous peptides and peptides produced by enzymatic hydrolysis of tissues of the same organism. Other than very broad generalizations, there are no results to suggest which peptides – hydrolytic or endogenous – will have greater activity or be more effective.

The purpose of the study is to produce from the Antarctic krill *E. superba* the peptide fractions (endogenous and obtained by enzymatic hydrolysis) with a molecular weight of up to 5 kDa, evaluate their antioxidant potential, and test for activity against certain hemostasis factors.

2 Materials and methods

2.1 Reagents

Tris(hydroxymethyl)aminomethane, Triton X-100, sucrose, CBZ-glycil-glycine dipeptide, 2,2-diphenyl-1-picrylhydrazyl, Griess reagent, potassium ferricyanide, adenosine diphosphate (ADP), ascorbic acid, trypsin, sodium dodecyl sulfate, Coomassie Blue R-250, acrylamide, N,N'-methylenebisacrylamide were purchased from Sigma-Aldrich (USA). The chromogenic substrate S2238 was purchased from Chromogenix (Sweden); polypeptide SDS-PAGE molecular weight standards were purchased from Bio-Rad Laboratories, Inc. (USA). All other chemicals such as $HClO_4$, KOH, ethanol, and trichloroacetic acid were of analytical grade quality and were purchased from KHIMLABOR-REAKTYV, LLC (Ukraine).

2.2 Preparation of tissue extract of Antarctic krill

Antarctic krill (*Euphausia superba*) was used to obtain the fractions of bioactive peptides. The specimens of hydrobiont were collected near Galindez Island of the Argentine Islands during the

Ukrainian Antarctic Expedition of 2013–2014. After being collected, the samples (total weight 75 g) were immediately frozen in liquid nitrogen to prevent enzymatic degradation of proteins. The samples were delivered to the Department of Biochemistry of Taras Shevchenko National University of Kyiv in a frozen state. The frozen mass of the hydrobionts was weighed and ground in liquid nitrogen. The powder was then suspended in extraction buffer (Tris-buffered saline containing 0.25% sucrose and 0.5% Triton X-100) at the ratio of 1:2 (w/v) and left to stir continuously at 4 °C for 30 min on an orbital shaker PSU-10i (BioSan, Latvia). After this, the sample was centrifuged (Allegra 64 R Centrifuge, Beckman Coulter, USA) at 10 000 × g for 30 min at 4 °C. The supernatant was collected and lyophilized with laboratory freeze-dryer LyoQuest-55 (Telstar, USA).

2.3 Preparation of the fractions of peptides with a molecular weight of up to 5 kDa

The fraction of endogenous peptides was extracted according to the method of Nikolajchik et al. (1991) with a slight modification. First, 1 gram of lyophilized material was dissolved in 5 mL of Tris-buffered saline and then mixed with 1.2 M $HClO_4$ at 1:1 (v/v) ratio to precipitate the proteins. After centrifugation at 10 000 × g for 20 min at 4 °C, the supernatant was adjusted by 5 M KOH to pH 7.0 and the sample was centrifuged again. After adding ethanol to the supernatant to a final concentration of 80%, the sample was kept at 4 °C for 30 min and centrifuged.

To obtain the fraction of hydrolytic peptides, 1 gram of lyophilized material was dissolved in 5 mL of 50 mM Tris-HCl (pH 8.0) and digested with trypsin (3000 U/g protein) at 37 °C. In 24 hours, the hydrolysate was heated at 95 °C for 10 min and centrifuged at 10 000 × g for 20 min. In both methods, the fraction of peptides with a molecular weight of up to 6.5 kDa was obtained by ultrafiltration of the supernatant on a 5 kDa molecular weight cut-off (MWCO) membrane. The optical density of samples was measured at 210 nm, and the concentrations of peptides were calculated using a conversion factor. CBZ-glycyl-glycine dipeptide was used as the peptide to make a calibration curve. The conversion factor was 14.5.

The molecular weight of peptides was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 18% PAGE. SDS-PAGE was performed using the Mini-Protean Tetra System (Bio-Rad Laboratories, Inc., USA) at 19 mA for stacking and 36 mA for separating gel. Fifteen microliters of each peptide fraction were added per lane. Gels were stained with 2.5% Coomassie brilliant blue G-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. The molecular weight of peptides was estimated using polypeptide SDS-PAGE molecular weight standards (triosephosphate isomerase 26.6 kDa; myoglobin 16.6 kDa; lactalbumin 14.4 kDa; aprotinin 6.5 kDa; insulin, b chain, oxidized 3.4 kDa; bacitracin 1.4 kDa) (Bio-Rad Laboratories, Inc., USA). The electrophoregrams were analyzed using the TotalLab 2.01 program.

2.4 Estimation of the antioxidant potential of peptides

The 2,2-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide radical scavenging activity, and the reducing ability of peptides were analyzed according to the methods described by Moon and Shibamoto (2009) and Royer et al. (2011), respectively. Lyophilized peptide samples were dissolved in 20% ethanol at a concentration of 2 mg per mL.

To determine the DPPH radical scavenging activity, peptide fractions (100 μg) were mixed with DPPH (0.15 mM) previously dissolved in 96% ethanol and kept in the dark at room temperature for 30 min. The optical density of the samples was measured at 517 nm. To determine the nitric oxide radical scavenging activity, peptide fractions (100 μg) were added to the reaction mixture containing sodium nitroprusside (5 мМ)

in 0.1 M phosphate-buffered saline, pH 7.4. After incubation at room temperature for 120 min, Griess reagent (5%) was added, and the optical density of the samples was measured at 546 nm. The control samples contained 20% ethanol instead of peptide fraction.

The percentage of DPPH and nitric oxide radical scavenging activity was calculated as follows:

$$\frac{K - S}{K} \times 100\% \quad (1)$$

K – absorbance of the control sample; S – absorbance of the peptide sample.

To determine the reducing power of the peptides, the fractions (100 μg) were added to the reaction mixture containing potassium ferricyanide (0.5%) and 0.2 M sodium phosphate buffer, pH 6.6 (Fu et al., 2010). The mixture was incubated at 50 °C for 30 min, and the reaction was terminated adding trichloroacetic acid (5%). Centrifugation (5,000 g, 10 min) was followed by mixing of the supernatant with 0.1% $FeCl_3$ (1:1, v:v). The optical density was measured at 700 nm. The control samples contained 20% ethanol instead of peptide fraction.

The percentage of reducing power was calculated as follows:

$$\frac{S - K}{S} \times 100\% \quad (2)$$

For all tests, ascorbic acid (1 mM) was used as a positive control.

2.5 Platelet aggregation assay

Blood was taken from healthy rabbits. All experiments carried out with blood were approved by the Bioethical Committee of the Taras Shevchenko National University of Kyiv, Ukraine. Blood was collected from the rabbit ear artery into polyethylene tubes with 3.8% sodium citrate (9:1). Platelet-rich plasma (PRP) was obtained by centrifugation at 150 × g for 10 min at room temperature. Platelet-poor plasma (PPP) was prepared by further centrifugation of the remaining blood at 2 500 × g for 20 min at room temperature. To prevent spontaneous platelet activation, PRP was placed in a water bath at 37 °C for 30 min prior to aggregation experiments, which were undertaken within the first three hours after blood sampling.

The effect of peptide fraction on *in vitro* aggregation of rabbit platelets was assessed using a photo-optical aggregometer AT-02 (Medtech, Belarus) according to Halenova et al. (2020). The peptide fractions were used at the concentrations of 0.4 μg/mL and 40 μg/mL. Before the assessment, the platelet count in PRP was adjusted with PPP to about 230×10^3–250×10^3 cells per μL. The peptide fraction was added to PRP, and after 3 min of incubation, ADP was added to the samples at a final concentration of 5×10^{-6} M to induce platelet aggregation. The control sample contained an equal volume of pure water instead of the peptide fraction. The aggregation process was monitored for 10 min. Assays were performed in triplicates using plasma from three different rabbits.

2.6 Chromogenic substrate spectrophotometric assays

To assess the effect of peptides on thrombin activity, the thrombin at a final activity of 0.1 U was pre-incubated in Tris-buffered saline (pH 7.4) in the presence of peptide fraction (100 μg) dissolved in pure water. After 15 min of incubation at room temperature, the chromogenic substrate S2238 (Chromogenix, Sweden) (0.3 mM) was added to the reaction mixture. The control sample contained an equal volume of pure water instead of the peptide fraction. The optical density of the samples was recorded at equal intervals for an hour at 405 nm wavelength using the kinetic microplate spectrophotometer μQuant (BioTek Instruments Inc., USA).

2.7 Data analysis

All values were expressed as means ± standard deviations (SD). Statistical treatment was performed using Statistica 8.0 software. The Kolmogorov-Smirnov test was used to verify the normal distribution of results. After normality test,

the data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's test. The value $p < 0.05$ was considered statistically significant. All experiments were repeated at a minimum of three times each.

3 Results

3.1 Characterization of peptide fractions by molecular weight

Electrophoresis in 18% polyacrylamide gel with SDS was used to check the purity of the peptide fractions and estimate the molecular weight of the peptides. As can be seen from Figure 1, the molecular weight of both endogenous peptides and peptides produced by enzymatic hydrolysis of *E. superba* tissue was below 6.5 kDa, which fully satisfies the task of work – to produce the peptides of a certain molecular weight. This characterization of peptides is critical because, according to numerous studies, most bioactive peptides consist of 3–20 amino acid residues and have a molecular weight of 0.4–5 kDa (Akbarian et al., 2022).

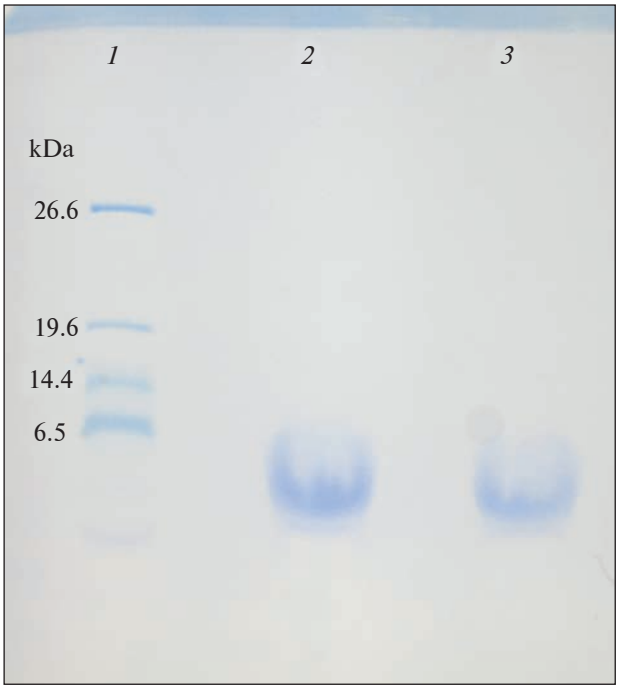

Figure 1. Electrophoregram of the peptide fractions from *Euphausia superba*: lane *1* – molecular weight markers; lane *2* – the fraction of hydrolytic peptides; *3* – the fraction of endogenous peptides

3.2 Antioxidant potential of hydrolytic and endogenous peptides from *Euphausia superba*

Due to the complexity of oxidative processes in living organisms and different mechanisms of antioxidant activity, the use of a single method for assessing antioxidant activity may not be informative. To overcome this limitation, three types of radical scavenging assays were applied to evaluate the possible antioxidant abilities of *E. superba* peptides.

As illustrated in Table, both peptide fractions from *E. superba* could scavenge DPPH and nitric oxide radicals and possess reducing power ability. However, in general, the antioxidant potential of endogenous peptides was higher than that of hydrolytic peptides. Thus, the 2,2-diphenyl-1-picrylhydrazyl scavenging activity of endogenous peptides was 36.0 ± 2.5% versus 11.0 ± 1.5% for

Table. Antioxidant potential of hydrolytic and endogenous peptides from *Euphausia superba*

Type of activity	Ascorbic acid (1 mM)	Fraction of hydrolytic peptides (100 μg)	Fraction of endogenous peptides (100 μg)
2,2-diphenyl-1-picrylhydrazyl scavenging activity, %	98.0 ± 5.0	11.0 ± 1.5	36.0 ± 2.5
Reducing power, %	90.0 ± 4.5	37.0 ± 3.5	64.0 ± 4.5
Nitric oxide radical scavenging activity, %	10.0 ± 2.0	28.0 ± 1.5	34.0 ± 3.5

Note: Data are expressed as mean ± standard deviation ($n = 6$)

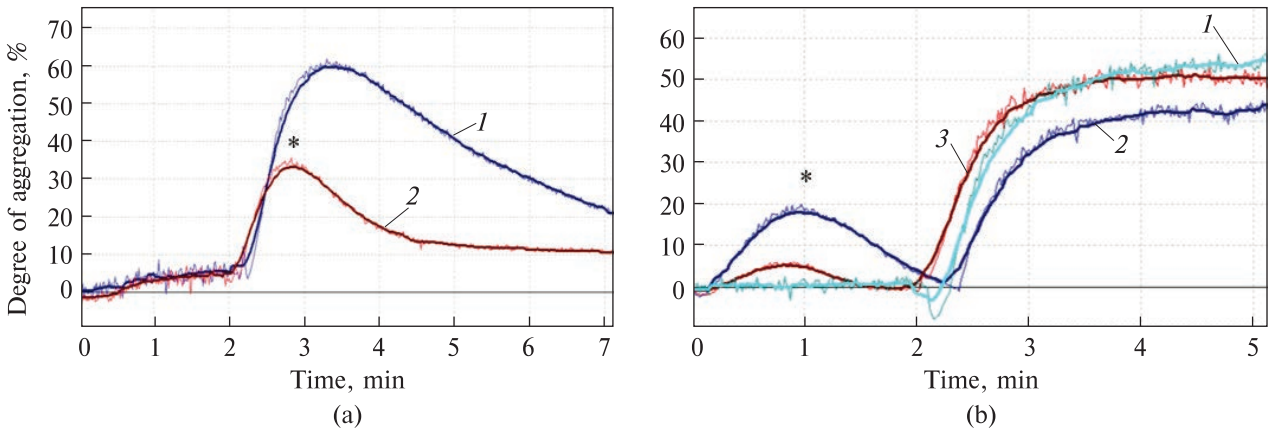

Figure 2. The effect of the hydrolytic (a) and endogenous (b) peptides from *Euphausia superba* on ADP-induced platelet aggregation: *1* – the control sample; *2* – the peptide fraction at the concentration of 40 μg per mL of PRP; *3* – the peptide fraction at the concentration of 0.4 μg per mL of PRP. * $p < 0.05$ – significantly different from the control sample

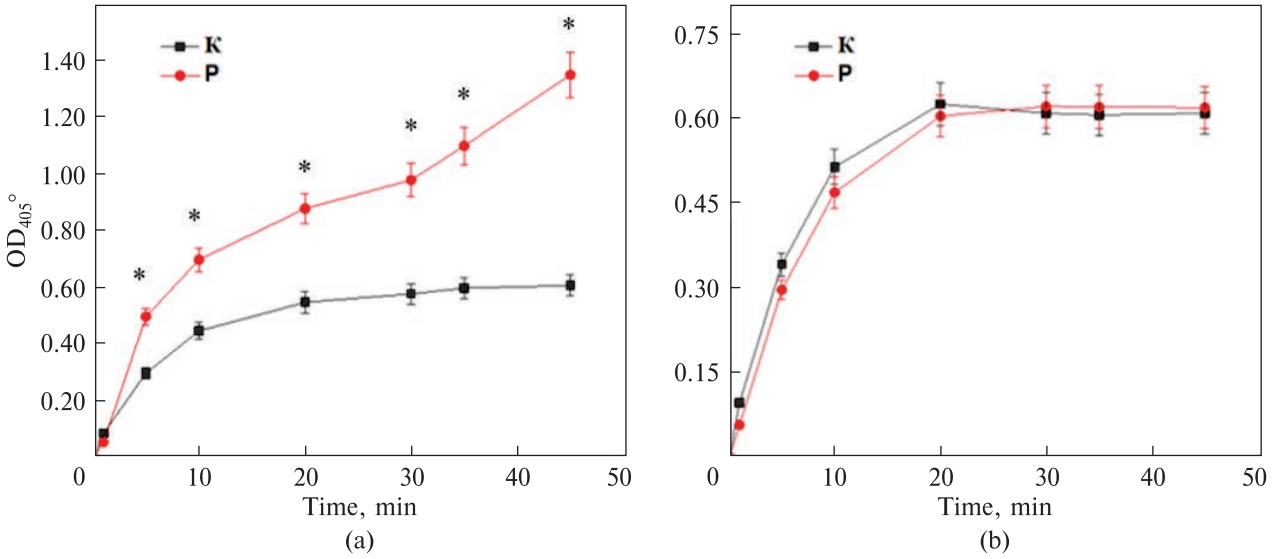

Figure 3. The effect of the hydrolytic (a) and endogenous (b) peptides from *Euphausia superba* on thrombin activity: K – the control sample; P – the peptide fraction (100 μg). Data is expressed as optical density (OD) of samples at 405 nm. * $p < 0.05$ – significantly different from the control sample

hydrolytic peptides. Both endogenous peptides and peptides obtained by hydrolysis of hydrobiont tissue have the greatest reducing activity – this figure for endogenous peptides was 64%, and for the fraction of hydrolytic peptides – 37%.

It should be noted that the DPPH scavenging activity and the reducing power of *E. superba* peptides were significantly lower than that of the ascorbic acid sample. On the contrary, the nitric oxide radical scavenging activity was higher than that of ascorbic acid – 2.8 times for the hydrolytic peptide fraction and 3.4 times for the fraction of endogenous peptides.

3.3 The influence of hydrolytic and endogenous peptides from *Euphausia superba* on ADP-induced platelet aggregation in platelet-rich plasma

The potential effect of hydrolytic and endogenous peptides from *E. superba* on ADP-induced platelet aggregation was evaluated (Fig. 2). The results indicate a multidirectional effect of the studied peptides. So, the fraction of hydrolytic peptides inhibited ADP-induced platelet aggregation (from 60% in the control sample to 34%). It should be mentioned that hydrolytic peptides did not induce spontaneous platelet aggregation. In contrast, the endogenous peptides, when added to PRP, had a slight pro-aggregation effect, which was manifested in a concentration-dependent increase in the degree of platelet aggregation – addition of peptides at a concentration of 0.4 μg and 40 μg per 1 mL of PRP caused an increase in the degree of platelet aggregation by 5% and 20%, respectively.

3.4 The influence of hydrolytic and endogenous peptides from *Euphausia superba* on thrombin activity

In the next stage of the study, we analyzed the effect of peptides from *E. superba* on the activity of thrombin in *in vitro* experiments using active enzyme and a chromogenic substrate – S2238. Endogenous peptides had no effect on the activity of thrombin (Fig. 3). In contrast, pre-incubation of thrombin with the fraction of hydrolytic peptides led to a pronounced increase in the thrombin activity (Fig. 3). After 5 minutes, the optical density of the sample containing thrombin, pre-incubated with hydrolytic peptides, exceeded the control value by 1.6 times.

4 Discussion

There are many sources of bioactive peptides, and marine organisms are among the most attractive. The same species of hydrobionts can be a source of endogenous peptides, which represent a unique set of molecules typical for a certain organism, and at the same time serve as raw materials for the obtaining of hydrolytic peptides, which are not present in the body in free form. In the latter case, biologically active peptides may be hidden in the structure of the parent proteins and can be released by enzymatic hydrolysis, which, at present, is the most popular and effective method for obtaining bioactive peptides (Cruz-Casas et al., 2021). Enzymatic hydrolysis has several advantages over chemical hydrolysis, including minimal damage to the nutritional value of the protein and the absence of amino acid modifications typical for acid hydrolysis.

The biological effects of bioactive peptides strictly depend on their unique amino acid composition. Moreover, the amino acid sequence and configuration, as well as the molecular weight and length of the peptides, determine the type of biological activity and also influence the effectiveness of the peptides (Zou et al., 2016). For example, the antioxidant activity of peptides has been linked to the presence of lysine, valine, tyrosine, alanine, histidine, leucine, methionine, proline, cysteine, and tryptophan (Xu et al., 2017), while angiotensin-converting enzyme (ACE) inhibitory peptides are rich in valine, alanine, and proline (Daskaya-Dikmen et al., 2017). According to the literature, hydrolysis under controlled conditions (duration of hydrolysis, temperature, pH, enzyme-to-substrate ratio, the use of a single enzyme or enzyme mixture) may be a way to produce peptides with the desired functional properties and activities (Zhang et al., 2012; Akbarian et al., 2022). Since our goal was to obtain a total peptide fraction that contains peptide molecules of different structures and biological activity, we used an approach that included trypsin hydrolysis and further purification to obtain peptides with a molecular weight below 5 kDa. To obtain peptides with a given molecular weight, ultrafiltration using low molecular weight membrane cutoffs was applied. This approach allowed obtaining the peptide fractions that were similar in their molecular weight but different in amino acid composition.

Next, the fractions of hydrolytic and endogenous peptides were analyzed for their antioxidant potential and ability to influence the activity of several hemostasis factors. Depletion of the endogenous antioxidant defense system, combined with augmented production of reactive oxygen species, leads to disruption of the antioxidant-oxidant balance and can cause oxidative stress, which is a significant factor contributing to the development of complications in many diseases (Halliwell, 2007; Fujii et al., 2022). Moreover, excess production of free radicals triggers a number of diseases, in particular pathologies associated with oxidative stress. In this case, regularly consuming antioxidants may be part of a strategy to prevent the harmful effects of free radicals on the body. However, the literature has reported that long-term consumption of synthetic antioxidants is associated with some potential health risks, prompting the search for natural antioxidants (Martemucci et al., 2022).

Our data allow us to consider *E. superba* a possible source of peptides with moderate antioxidant activity. The DPPH radical scavenging activity of *E. superba* peptides, combined with their reducing ability, suggests that the peptides may reduce the intensity of free radical reactions, thereby reducing the development of oxidative stress. Our results are consistent with the results of Wang et al. (2021) and Zhang et al. (2021), who also showed the presence of antioxidant activity in peptides from Antarctic krill hydrolysate. It should be emphasized that the less pronounced antioxidant potential of Antarctic krill peptides compared to the widely used antioxidant ascorbic acid is rather a positive characteristic of peptides since it allows the long-term use of such peptides without harm to the body. This is especially important given the results of studies showing that systematic intake of highly active antioxidants can trigger tumor progression (Sayin et al., 2014).

It was revealed that endogenous peptides and hydrolytic peptides differ in antioxidant effectiveness, which may be a consequence of differences in the amino acid composition of the peptides. We can speculate that the higher antioxidant activity of endogenous peptides, namely radical scavenging activity, may be due to the high proportion of hydrophobic and aromatic amino acids, which is considered a key factor in the radical scavenging ability of peptides. Since the antioxidant activity of protein hydrolysates is to some extent determined by the hydrolysis conditions, particularly the enzyme used, the lower antioxidant activity of hydrolytic peptides may be partly explained by the use of trypsin. It is well known that trypsin cleaves peptide bonds formed by arginine or lysine residues, resulting in the formation of hydrolysates rich in positively charged peptides, which are less effective as antioxidants but, according to numerous studies, can exhibit significant antimicrobial activity (Zou et al., 2016).

The next part of the work was devoted to studying the influence of *E. superba* peptide fractions on a key factor of hemostasis, namely thrombin, which is an important enzyme of the blood coagulation system, and platelets, which are necessary in the initial stages of blood clot formation. Thrombosis is currently recognized as a leading cause of morbidity and mortality all over the world. In many cardiovascular pathologies, it is a major constituent (Wendelboe & Raskob, 2016). Several anticoagulant drugs are used to control blood clotting. Despite their effectiveness, most of them usually have unwanted side effects. In this regard, there is increasing research interest in the search for natural agents active against specific hemostasis factors to prevent thrombotic events. According to the obtained data, the hydrolytic and endogenous peptides have different effects on thrombin activity, possibly due to differences in the compositions of peptides within the fraction. It seems that peptides obtained by trypsin hydrolysis of *E. superba* tissue proteins do not have much clinical promise as thrombin inhibitors since they increase the amidolytic activity of thrombin (by some unclear mechanism). We can speculate that changes in thrombin activity may result from changes in the local structure of the enzyme, es-

pecially the conformation of the loops surrounding the active site – a process commonly referred to as structural or enthalpic allostery (Cargnelutti et al., 2012). Nevertheless, hydrolytic peptides can be useful for an in-depth study of the mechanisms of thrombin regulation and its interaction with other hemostasis factors. Moreover, the hydrolytic peptides would be considered potential candidates for external use to enhance blood clotting or to stimulate wound healing.

The difference in the composition of endogenous peptides and peptides obtained by hydrolysis can be indirectly confirmed by hydrolytic peptides inhibiting ADP-induced platelet aggregation. In contrast, endogenous peptides cause platelet aggregation by themselves. The results on the ability of hydrolytic peptides to inhibit ADP-induced platelet aggregation open up the prospect of a more detailed study of *E. superba* peptides for the development of new and effective antiplatelet peptide-based drugs with fewer side effects. This is especially important given that the use of antiplatelet drugs, including aspirin, clopidogrel, and glycoprotein IIb/IIIa antagonists, is limited due to an increased risk of bleeding and antiplatelet drug resistance. Since the fraction of hydrolytic peptides contains part of the molecules with Arg residues as a result of the action of trypsin on tissue proteins, it can be assumed that the antiplatelet effect of the peptides is realized through the binding Arg-containing peptides to a certain subpopulation of platelet receptors. It has been reported that peptides containing Gly-Pro-Arg-Pro, Arg-Gly-Asp, and Arg-Gly-Asp-Ser motifs can bind to platelet receptors, thus preventing platelet-platelet, platelet-subendothelium interactions as well as the interaction of certain factors with platelets (Chiang et al., 1995; Rengasamy et al., 2019). In general, the ability of endogenous peptides to slightly induce platelet aggregation in combination with moderate antioxidant activity and the lack of influence on the activity of the key coagulation enzyme thrombin makes these peptides a possible component of wound healing drugs.

5 Conclusion

To our knowledge, this is the first report examining the biological activity of endogenous and hydrolytic peptides obtained from the same species, namely hydrobiont of the Antarctic region. Our findings suggest that *E. superba* could be used as a source of bioactive peptides with molecular weight up to 5 kDa. It was found that the endogenous peptides possess moderate antioxidant activity and may be exploited in treating disorders associated with oxidative stress development. The peptides produced by hydrolysis with trypsin showed less pronounced antioxidant activity but were active against key factors of hemostasis – the peptides caused an increase in the amidolytic activity of thrombin and inhibited ADP-induced platelet aggregation. Further studies should be done to fractionate the obtained peptide fractions and isolate peptides with targeted activity to elucidate the mechanisms of action of peptides from *E. superba*. More detailed research on physiological functions, pharmacological effects, and structure-activity relationships of the purified peptides will also be needed.

Author contributions. N.R. carried out the experiments; wrote the article; T.H. carried out the experiments; manuscript review; T.V. carried out the experiments; O.S. experimental design, data interpretation; T.B. and L.O. supervised the research.

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Conflict of Interest. The authors declare no conflict of interest.

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Вивчення потенціалу біологічно активних пептидів з антарктичного криля *Euphausia superba*

Реферат. Метою дослідження було одержати з *Euphausia superba* фракції ендогенних та гідролізних пептидів і дослідити їх антиоксидантний потенціал і вплив на функціонування деяких факторів системи гемостазу. Для отримання фракції ендогенних пептидів застосовували метод поетапного осадження білків перхлоратною кислотою та етиловим спиртом. Задля одержання пептидів з молекулярною масою не вище 5 кДа використовували метод ультрафільтрації. Гідролізні пептиди отримували шляхом ферментативного гідролізу біомаси криля за участі трипсину. Чистоту отриманих пептидних фракцій та молекулярну масу пептидів оцінювали методом електрофорезу за присутності додецилсульфату натрію. Антиоксидантну активність пептидів *in vitro* оцінювали за результатами загальної антиоксидантної активності, редукуючої здатності та здатності знешкоджувати радикали нітроген (II) оксиду. Здатність пептидів впливати на АДФ-індуковану агрегацію тромбоцитів перевіряли у плазмі, збагаченій на тромбоцити. Для визначення впливу пептидів на амідолітичну ак-

тивність тромбіну, фермент попередньо інкубували з пептидними фракціями та надалі визначали активність тромбіну, використовуючи хромогенний субстрат S2238. Відповідно до отриманих результатів, як ендогенні, так і гідролізні пептиди виявляли помірну антиоксидантну активність, причому ендогенні пептиди виявляли більш виражені антиоксидантні активності, ніж пептиди, отримані шляхом ферментативного гідролізу. Також було виявлено здатність пептидів з *E. superba* впливати на деякі фактори системи гемостазу. Так, встановлено інгібування АДФ-індукованої агрегації тромбоцитів гідролітичними пептидами (у 1,76 рази), тоді як ендогенні пептиди, навпаки, викликали агрегацію тромбоцитів у плазмі, збагаченій на тромбоцити. Отримані результати щодо відмінностей в активностях та ефективності пептидів можуть бути наслідком різного амінокислотного складу. З огляду на отримані результати, *E. superba* може слугувати потенційним джерелом для одержання пептидів з помірними антиоксидантними властивостями та пептидів, здатних впливати на активність ключових факторів системи гемостазу.

Ключові слова: антарктичний гідробіонт, антиоксидантна активність, гемостаз, гідролітичні пептиди, ендогенні пептиди