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Prospects of Using Epidermal Secretion of Scaleless Fish Species as a Source of Peptide Complexes with Antioxidant Activity †

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Abstract: Every year peptide-based preparations are becoming more and more demanded in medicine. Therefore, the identification and study of new biomaterials that are sources of regulatory peptides is an urgent task. This study considers the epidermal secretion of the African catfish (Clarias gariepinus) as a readily available raw material for the preparation of peptide complexes. In the history of peptide drug synthesis, this object was first investigated as a source for obtaining regulatory peptides with antioxidant activity. We applied a new approach to the process of isolation of biologically active peptide fractions, thereby reducing the time and increasing the yield of the target product.

Keywords: peptides; regulatory peptides; antioxidant activity

1. Introduction

Preparations based on peptides are becoming more and more in demand due to their specificity, which, first of all, lies in their functional features. Peptides, getting into the human body, perform the following pharmacological functions: eliminate inflammatory processes, strengthen tissues and bones, restore the process of metabolism, have an immunomodulatory effect on the whole organism. Functional features of regulatory peptides can be very different and are determined by the functions of organs and tissues from which they are isolated.

For example, such a peptide preparation as "Bonomarlot" is used as a cancer prevention agent and promotes the formation of bone system cells, it is isolated from the bone marrow of cattle. Also from the organs of cattle, namely from the parathyroid gland, it is possible to isolate peptides designed to normalize the work of parathyroid gland cells. Such peptides are contained in the preparation "Bonortic", it is prescribed for the prevention of osteoporosis. The drug "Visoluten" can also be referred to a group of drugs derived from biological tissue of animals. Peptide complex isolated from eye tissues of young healthy animals strengthens or partially restores: cells of retina, conjunctiva, visual analyzer. Number of preparations whose active substance is, are regulatory peptides isolated from biological material is large and it is not possible to cover them all [1].

Due to the fact that raw materials for the isolation of peptide preparations are expensive resources, we chose the skin secretion of the African catfish (*Clarias gariepinus*) as a biological material. This object is interesting because the mucus of this scaleless fish species is not only a relatively inexpensive source of peptides, but also the only defense of the African catfish against harmful microorganisms and mechanical damage.

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One of the main advantages of regulatory peptides is the presence of some antioxidant activity, which is manifested to varying degrees in peptide complexes at different concentrations.

In this paper we will highlight the methods of determining the antioxidant activity that regulatory peptides isolated from the skin secretion of the African catfish (*Clarias gariepinus*) should possess.

2. Materials and methods

Obtaining the peptide fraction used as an inhibitor. The regulatory peptide fraction was isolated by acetic acid extraction of Clarias gariepinus epidermal mucus. A 3% acetic acid solution containing 0.1 to 0.3% inorganic salt was used. The inorganic salt used was zinc, calcium and magnesium chlorides. The extraction was carried out in the mode of periodic ultrasonic cavitation treatment on the IL100-6/1 disperser for 48 h at a temperature of 4 °C. The obtained extract was separated by centrifugation for 10 min at 7000 rpm and further purified by adsorption with activated carbon for 24 h under constant stirring. After removal of the adsorbent from the clarified solution, the peptide fraction was isolated by re-precipitation with a 2-fold excess of acetone. The resulting precipitate of the peptide fraction was separated on a Schott filter (16 μm pore width).

Use of UV spectroscopy to analyze antioxidant properties. To determine the antioxidant activity of the samples we used the technique of studying the autooxidation of adrenaline in vitro. The essence of the method is to compare the rate of autoxidation of pharmacy 1% adrenaline solution in the presence (analyzed solution) and in the absence (control solution) of aqueous solution of peptide fraction in sodium-carbonate buffer (pH = 10.65) [2].

To determine the antiradical activity of peptide complexes, 2,2-diphenyl-1-picrylhydrazyl (DPPH) dissolved in ethanol was used. The optical density of the initial DPPH solution was compared with the optical density after the reaction of DPPH with the antioxidant under study. The decrease of optical density values at a wavelength of 515nm indicates the presence of antioxidant activity of the studied compound.

The following reagents were used: 1% adrenaline solution; salts—$ZnCl_2$; $MgCl_2$ $CaCl_2$; DPPH, L-glutathione reduced, C_2H_5OH (Sigma Aldrich). The peptide fraction was prepared as described above. Sodium-carbonate buffer was prepared from Na_2CO_3 fixed pH value was achieved by adding dry $NaHCO_3$ to the solution to the desired pH = 10.65. All solutions were prepared with bidistilled water.

3. Results and discussions

The assumption about the antioxidant properties of the isolated peptide complexes was made based on the functional features of the epidermal secretion of the African catfish. Therefore, it was of interest to quantify the antioxidant activity of the investigated peptides. For this purpose, two independent spectroscopic methods were used [3].

The first one consisted of inhibition of the adrenaline autooxidation reaction by the peptide complex. To determine the antioxidant activity of the obtained peptides, the concentration of adrenaline oxidation products was determined by the optical density readings of the solutions. According to its properties and chemical structure, adrenaline is an electron donor, this function is realized in the process of quinoid oxidation. Autoxidation of adrenaline into adrenochrome occurs in the process of dehydrogenation and cyclization with the formation of intermediate products: adrenaline-semiquinone, adrenalinequinone, leucoadrenochrome, adrenochrome-semiquinone. This process is accompanied by one-electron reduction of oxygen and is associated with further formation of superoxide radicals (O2-.), which in turn oxidize and destroy nucleic acids, proteins, polysaccharides and biomembranes.

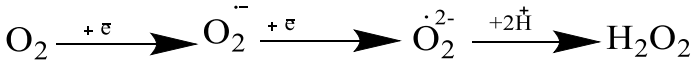

Figure 1. Scheme of oxygen reduction and superoxide radical formation.

In this work, the ability of zinc, calcium and magnesium chlorides to regulate the deposition of major proteins of the epidermal secretion of Clarias gariepinus epidermal secretion was compared. Controlled sedimentation allows to change the total peptide composition of the fractions. This directly affects the most important properties of the obtained peptide complexes [4].

To quantify the inhibitory effect of the peptide fraction, we determined the ratio of the rate constants of the autoxidation reaction of adrenadine without an inhibitor and in the presence of the peptide complex:

$$W(\text{inhibition}) = \frac{k_0}{k_п}$$

where, W(inhibition)-fraction of inhibition of adrenaline autooxidation

k0—rate constant of adrenaline autooxidation without peptide

kp—adrenaline autoxidation rate constant in the presence of peptide

Calculation of the inhibitory effect of peptide complexes is presented in Table 1.

Table 1. Inhibition of adrenaline autooxidation k0/kp.

	Antioxidant			
Antioxidant concentration Mkg/mL	Timalin	Precipitated peptide complex $ZnCl_2$	Precipitated peptide complex $CaCl_2$	Precipitated peptide complex $MgCl_2$
5	1.15	3.98	1.23	1.46
10	0.77	1.47	1.11	1.13
20	0.66	0.88	0.89	0.83

Thus, the inhibitory effect of peptides precipitated by biogenic metal salts is many times higher or comparable to the similar effect of the pharmacopoeial drug Timalin. The presented data allow us to assume the presence of sulfhydryl and/or phenolic functional groups, which are responsible for the antioxidant properties of regulatory peptides [5].

The second method for the determination of antioxidant activity is based on spectrophotometric determination of the optical density of the reaction product of isolated regulatory peptides with 2,2-diphenyl-1-picrylhydrazyl (DPPH) in an organic solvent (ethanol). L-glutathione reduced was used as a reference antioxidant. We used it as a reference for comparison of antioxidant properties. The quantitative measure of antiradical activity in this method was used as the value of decrease in optical density of 0.004% DPPH solution after reaction with antioxidant—ΔD. Comparison of antioxidant properties of glutathione and peptide complexes isolated by different salts is presented in Table 2.

Table 2. Anti-radical activity using DPPH radical—ΔD.

Antioxidant Concentration Mkg/mL	**Antioxidant**		
	L-Glutathione	**Precipitated Peptide Complex $CaCl_2$**	**Precipitated Peptide Complex $ZnCl_2$**
10	0.372	1.09	1.03
20	0.341	1.09	1

ΔD decrease in optical density after addition of antioxidant to DPPH solution.

The results obtained confirm the antioxidant activity of peptide complexes consisting in direct binding of free radicals. Obviously, such activity of peptides does not depend on

the choice of salt taken for their isolation. Although it markedly exceeds the analogous activity of glutathione. The difference in the results obtained by the two methods can be explained by the multicomponent nature of the peptide fractions. Probably, the concentration of individual peptides capable of reacting with superoxydradical (in adrenaline autooxidation) is higher in the case of using $ZnCl_2$ for isolation. While the amount of peptides capable of interaction with DPPH is approximately the same in all fractions, regardless of the nature of the salt taken for precipitation [6].

4. Conclusions

The nature of the salt used for precipitation of major proteins from the epidermal mucus of the African catfish plays a determining role in the formation of the properties of the peptide preparation. It is shown that among the biogenic metal chlorides chosen for the study it is $ZnCl_2$ that allows the isolation of the peptide fraction with the highest antioxidant activity. This activity is almost 4 times higher than the activity of the preparation "Timalin" and 2 times higher than the activity of the analogous fraction isolated using $CaCl_2$ and $MgCl_2$. At the same time, the ability of the studied peptide fractions to inhibit the process of adrenaline autooxidation is manifested when they are added to the substrate in concentrations of 5 Mg/mL

Comparison of the obtained results on determination of antioxidant activity using the mentioned methods showed that the highest value of antioxidant activity is observed in the range of low concentrations of antioxidants, i.e., obtained peptide complexes. Thus we can state that the isolated complexes of regulatory peptides have antioxidant activity, hence they can prevent oxidation of nucleic acids, proteins, polysaccharides and biomembranes by superoxide radicals [7]. This, in turn, makes them promising drugs for the treatment of severe forms of wound processes.

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