Saudi Journal of Medical and Pharmaceutical Sciences
Scholars Middle East Publishers
Dubai, United Arab Emirates
Website: http://scholarsmepub.com/

ISSN 2413-4929 (Print)
ISSN 2413-4910 (Online)

A Scientific and Technical Approach to the Development of Device for the Extraction of a Nucleotide-Peptide Substance from the Yeast *Saccharomyces cerevisiae*, an Activator of Ca^{2+}-Dependent NO Synthase

Marakhova AI*, Zhuravleva MA, Stanishevsky Ya M, Kezimana P
Peoples' Friendship University of Russia (RUDN University), 117198, Miklukho-Maklaya str, 6, Moscow, Russia

Original Research Article

***Corresponding author**
Marakhova AI

Article History
Received: 07.11.2018
Accepted: 17.11.2018
Published: 30.11.2018

DOI:
10.21276/sjmps.2018.4.11.5

Abstract: A device for the isolation of nucleotide complex from biomass of *Saccharomyces cerevisiae* cells was developed, and the biological active substances in the extract were analyzed. The isolated nucleotide preparation can be used to stop oxidative stress and improve immunity. The developed device allows extraction of biologically active substances from *Saccharomyces cerevisiae* cells in series by extractants: distilled water under the action of an alternating voltage with a frequency of 10^5 Hz, a solution of hydrochloric acid with pH = 1.0-1.5 without using voltage. After acid extraction, the extract was neutralized with sodium hydroxide solution. The extracts obtained were combined and dried. The technical result of the device is a more complete two-fold extraction of the nucleotide complex from native *Saccharomyces cerevisiae* biomass. The use of our constructed device makes extraction of biologically active substances easier and complete. The content of biologically active substances, such as adenine nucleotides, amino acids, vitamins, micro-, macro- elements in the obtained extract was analyzed. A comparison of the activating effect of the isolated nucleotide preparation on Ca^{2+}-dependent NO - synthase with T-activin was performed. Experiments showed that the nucleotide preparation, obtained by new device has a pronounced activating effect on Ca^{2+} -dependent NOS from rat thymocytes.

Keywords: device for isolation of nucleotide complex preparation, *Saccharomyces cerevisiae*, alternating voltage, NO-synthase.

INTRODUCTION

It is known that Ca^{2+}-dependent NO-synthase is one of the key enzymes of antioxidant defense of the organism [1]. Reduced activity of Ca^{2+} -dependent NO-synthase and other antioxidant defense enzymes accompanies the emergence and development of a number of diseases such as diabetes, atherosclerosis, myocardial infarction; heart failure [2, 3]. In this regard, particular relevance search activators Ca^{2+} -dependent NO-synthase (NOS), among which the most promising for use as medicaments adenine derivatives obtained from various biological objects, in particular from yeast *Saccharomyces cerevisiae* [4]. As shown by Orlova [5], the biologically active substances of *Saccharomyces cerevisiae* are represented by amino acids, vitamins, micro-macroelements and other useful metabolites. For the industrial preparation of a nucleotide complex from the biomass *Saccharomyces cerevisiae*, it is necessary to create a device that provides efficient extraction. At present, devices for electro-pulsed extraction of biologically active substances of their medicinal plant material are known [6, 7]. However, the proposed installations are not without shortcomings. For example, in Patent No.105290, a high-voltage electrode is used that provides impulse processing of raw materials at elevated temperature, which makes it necessary to include a cooling radiator in the device to prevent the destruction of biologically active substances (BAS). Disadvantages of the device [7] are that prior to extraction it is necessary to suspend the biomass beforehand. Extraction in this device is possible only under the influence of one factor - impulse processing.

In addition, the above constructions do not provide for multiple extraction by different extractants of the same raw materials. The disadvantage of the installation [6] is the need to use a paper-fibrous membrane, which is not suitable for extraction from raw yeast biomass.

All this makes device development actual for industrial production of biomass nucleotide complex from *Saccharomyces cerevisiae* - Ca^{2+}-dependent activator NO-synthase.

MATERIALS AND METHODS

The objects of the study were strains of yeast *Saccharomyces cerevisiae,* which were used to create a

new device to extract nucleotide-peptide substance. To receive the nucleotide preparation, five strains of the yeast *Saccharomyces cerevisiae* race 14 were used, which were grown on a nutrient medium of the following composition: macroelements (g/l) KH_2PO_4 - 1.0; K_2HPO_4 - 0.13; $MgSO_4 \cdot 7H_2O$ - 0.7; NaCl - 0.1; $(NH_4)_2SO_4$ - 3.5; microelements (mg / l) $ZnSO_4 \cdot 6H_2O$ - 0.18; $CuSO_4 \cdot 5H_2O$ - 0.15; $MnSO_4 \cdot 5H_2O$ - 0.15; $CoCl_2$-0.18; vitamins (mg / l) thiamine - 0.4; biotin - 0.1. The source of carbon nutrition was glucose is 10.0 g/l. The activity of Ca^{2+} - dependent NOS in thymocytes of Wistar rats was determined by the method of Hwang [8] with some modifications. The basis is the determination of the activity of NOS by the final product - NO, which, after conversion to NO^{-2}, is quantitatively determined using a Griss reagent. The Grissa reagent was prepared from sulfanilic acid and α-naphthylethylamine, in a ratio of 1:1 (by weight). This mixture (10 g) was dissolved in 90 ml of 12% acetic acid. To 800 To 800 µl of the supernatant (thymocytes) were added in strict order, first 100 µl of 20 mM K_2-phosphate buffer (pH 7.4), followed 50 µl L-arginine and at the end 50 µl of 5,4 • 10^{-4} M NADPH (Sigma, USA).

NADPH "starts" the enzymatic reaction, so a disturbance of the ordere of introduction the reagents into the mixture can lead to incorrect results (8). The kinetic parameters of enzymatic reactions are calculated by the Michaelis-Menten equation using the SPLab4 software package. The values of the apparent Michaelis constant (K_m^{app}) and the unit velocity (V_{un}) of the NOS-catalyzed reaction were determined [13]. The prepared reaction mixture was incubated in a water bath with vibration at a temperature of 37°C for 10 minutes. The reaction was then stopped by the addition of 300 µl of Griess reagent (Sigma). Samples were removed from the water bath and left at room temperature (20°C) for 30 minutes for maximum color development [9].

The NO_2^- concentration was determined on a Beckman DU-65 (USA) spectrophotometer at a wavelength of 548 nm by the intensity of the violet red azo complex formed from the reaction between sulfanilic acid, NO_2^- and α-naphthylethylamine. The sensitivity of the method is 10^{-7} M NO_2^-.

The composition and content of amino acids in the preparation were determined on the amino acid analyzer Varian (USA).

Micro- and macro-elements were determined by atomic-adsorption spectroscopy using the Enujiugha V.N. method [9] using an atomic adsorption spectrometer from Varian (USA).

Vitamins, nucleic acids and carbohydrates were determined using a liquid chromatograph from Waters (USA). Symmetry C18 (Waters) columns were used to analyze water-soluble vitamins (B1-thiamine, B2-riboflavin, B3-niacin, vitamin PP, B4-choline, B5-pantothenic acid, B6-pyridoxine, B9 folic acid, H-biotin) and carbohydrates.

Columns Nucleosil C18 from Macherey-Nagel (Germany) was used to determine the content of fat-soluble vitamins E (tocopherol) and K (phylloquinone, prenylmene quinone). A reversed-phase column C-8 (Shimadzu, Japan) was used to determine fatty acids. For the analysis of nucleic acids, DEAE-anion exchange column Nucleogen 4000-7 (Macherey-Nagel) was used [10].

The device was constructed to isolate the nucleotide preparation. The extraction device is shown in Fig. 1. It consists of a vessel made of stainless steel equipped with an opening valve (1) for loading the yeast cell mass, a steam jacket (4) for maintaining the temperature, opening bottom (7). To the opening bottom (7) and the loading valve (1), plate electrodes are mounted. An alternating voltage is applied to the electrodes from the generator with a frequency of 105 Hz and a power of 4 V. The generator (9) is placed in a separate compartment (8). After opening the bottom, a prefilter (3) with a pore diameter of 2 micrometers is used to separate the extractant and yeast cells.

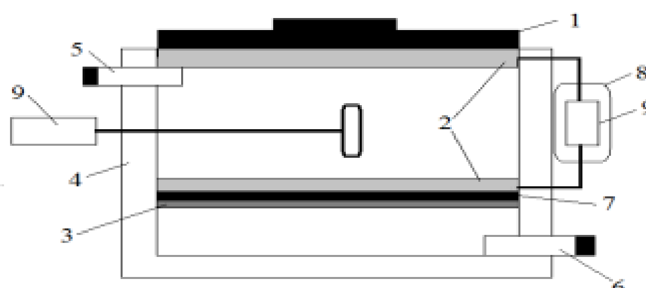

Fig-1: The extraction device

In addition, the design provides for the presence of nozzles: inlet (5) for feeding alternately distilled water, a solution of hydrochloric acid and a solution of alkali; output (6) for discharge of used extractants.

The nozzles are provided with thermostatically controlled valves. The extraction mixture is stirred using a paddle mixer (9).

The device for extracting raw materials works as follows

Through the opening valve (1), the biomass Saccharomyces cerevisiae is placed in the vessel, which is poured in water with distilled water through the inlet pipe (5) until the upper electrode (2) is completely submerged. From the generator (8) electrodes (2) are supplied with a voltage of 10^5 Hz and a power of 4V. Extraction is carried out for 30 minutes with stirring with a stirrer (8). During this time, in these conditions, the permeability of yeast cell membranes and the weakening of the bonds between BAS inside the cell increase. After the extraction is complete, the bottom (7) opens and extract A is drained through the outlet (6). The yeast mass remains on the prefilter (3). The bottom is closed and a solution of hydrochloric acid with pH = 1.0-1.5 is fed into the extraction vessel through the inlet nozzle (5) by approximately 1/2 the volume of the vessel. The steam jacket provides a temperature regime of 80-85 ° C. Extraction with acid is carried out for 1 hour with stirring. After the extraction is completed, a solution of sodium hydroxide is introduced into the extraction vessel through the inlet nozzle (5) to neutralize the acid to pH = 6-7 (extract B). After combining the extracts A and B, a BAS complex (nucleotide preparation) is obtained, separated by centrifugation from the aqueous phase. The separated mass was dried on a lyophilic drier.

Evaluation of the effect of the obtained sample of the nucleotide preparation on the ability to activate Ca^{2+} -dependent NOS in comparison with the T-activin immunostimulator was carried out on thymocytes of Wistar rats. At the same time, T-activin was used in effective therapeutic doses, and the test samples of the nucleotide preparation were comparable to those recommended for sodium nucleates [11-14]. Also, in the obtained nucleotide preparation the main biologically active substances were determined: amino acids, vitamins, micro- and macro-elements, fatty acids.

RESULTS AND CONCLUSIONS

Experiments have shown that the nucleotide preparation with a triple intraperitoneal administration of a dose of 10 mg/kg exerts a pronounced activating effect on Ca^{2+} -dependent NOS from rat thymocytes (Table 2).

Table-1: Composition of the complex nucleotide preparation, mg / 100 g of dry substance

Components of complex nucleotide preparation							
Adenine nucleotides (AN), amino acids		Vitamins		Macro and microelements		Fatty acids	
AN	3200	B1 (thiamine)	0,58	Phosphorus	130	Myristic acid	0,023
Amino acids:		B2 (riboflavin)	7,15	Potassium	294	Myristo-leinic	0,015
lysine	0,93	B3 (niacin, vitamin PP)	10,8	Sodium	36,5	Pentadecane	0,045
Histidine	0,45	B4 (choline)	253,0	Calcium	43,8	Palmitic	4,2
Arginine	0,88	B5 (pantothenic acid)	73,57	Magnesium	47,6	Palmito-leinic	17,94
Aspartic acid	1,52	B6 (pyridoxine)	0,59	Copper	0,2	Heptadecene	0,83
Threonine	0,76	B9 (folic aci)	0,83	Cobalt	0,16	Stearic	2,54
Serin	0,58	B12 (cyanocobalamin)	0,08	Iron	1,03	Olein	12,55
Glutamic acid	5,32	H (biotin)	0,12	Manganese	0,32	Linoleum	0,73
Proline	0,61	K (phylloquinone)	0,91	Zinc	0,19	Linolenic	0,89
Glycine	1,09	E (tocopherol)	0,64	Nickel	0,17	Arachine	0,52
Alanin	1,77			Chromium	0,23	Gadolein	0,85
Valine	0,94			Molybdenum	0,35	Arachidonic	0,49
Methionine	0,42					Bezen	0,12
Isoleucine	0,64					Heptadecene	0,83
Leucine	0,61						
Tyrosine	0,74						
Phenylalanine	0,45						
Cysteine	0,78						

Table-2: Comparative analysis of the effect on the kinetic parameters of the Ca^{2+} -dependent NOS nucleotide preparation from the yeast Saccharomyces *cerevisiae* and T-activin

Investigated objects	The investigated kinetic parameters for Ca^{2+} -dependent NOS	
	K_m, millimole	V, micromol NO_2^-/min/mg of protein
Control - intact thymocytes	0,0620±0,0008*	6,300±0,040*
Nucleotide preparation (10 mg / kg)	0,0030±0,0004**	73,210±0,025**
T-activin (0.25 mg / kg)	0,0080±0,0003**	65,100±0,021**

* - significant differences in comparison with the control with relative error P <0.05

** - significant differences in comparison with the control with relative error P <0.01

According to the intensity of the activating action, the nucleotide preparation is superior to T-actinin, as evidenced by a 2.7-fold decrease in Km for the drug compared to the K_m reaction of the T-activin with the Ca^{2+} -dependent NOS. From Table 2 that the NOS-catalyzed reaction rate is higher in the nucleotide preparation (73,210-0,025) than in the T-activator. Thus, in the used dose of 10 mg / kg, the nucleotide preparation is the stronger activator of Ca^{2+} -dependent NOS.

In the obtained nucleotide preparation, some biologically active substances were determined: adenine nucleotides, amino acids, vitamins, micro- and macroelements (Table 1).

Thus, a device was constructed that allows extraction of BAS from raw yeast biomass first by water under the action of an electric voltage of 4V and a frequency of 105 Hz, and then, after draining the extract, with acid when the vessel is thermostated to 80-85° C. The resulting drug has an activating effect on Ca2+ -dependent NO-synthase, which plays an important role in the functioning of the immunity system and in the processes of oxidative stress. The nucleotide complex is rich in amino acids, vitamins, micro- and macro-elements, and fatty acids.

ACKNOWLEDGEMENTS

This work was carried out with the financial support of the Ministry of Education and Science of the Russian Federation under the Program for the Implementation of Comprehensive Projects for the Creation of High-Tech Production (Government Decree No. 218 of 09.04.2010, code of the contest 2016-218-09), Contract No. 03. G25.310258.

REFERENCES

1. Leone, A., Moncada, S., Vallance, P., Calver, A., & Collier, J. (1992). Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *The Lancet*, *339*(8793), 572-575.
2. Catravas, J. D., Callow, A. D., Gillis, C. N., & Ryan, U. S. (Eds.). (2012). *Vascular endothelium: physiological basis of clinical problems* (Vol. 208). Springer Science & Business Media.
3. Chade, A. R., Rodriguez-Porcel, M., Herrmann, J., Zhu, X., Grande, J. P., Napoli, C., ... & Lerman, L. O. (2004). Antioxidant intervention blunts renal injury in experimental renovascular disease. *Journal of the American Society of Nephrology*, *15*(4), 958-966.
4. Patent of the Russian Federation No. 105290, publ. 10.07.2011
5. Orlova, V. V., Choi, E. Y., Xie, C., Chavakis, E., Bierhaus, A., Ihanus, E., ... & Chavakis, T. (2007). A novel pathway of HMGB1-mediated inflammatory cell recruitment that requires Mac-1-integrin. *The EMBO journal*, *26*(4), 1129-1139.
6. Patent of the Russian Federation No. 142485, publ. 06/27/2014
7. Patent of the Russian Federation No. 164195, publ. 08/20/2016
8. Hwang, S. M., Lopez, C. A., Heck, D. E., Gardner, C. R., Laskin, D. L., Laskin, J. D., & Denhardt, D. T. (1994). Osteopontin inhibits induction of nitric oxide synthase gene expression by inflammatory mediators in mouse kidney epithelial cells. *Journal of Biological Chemistry*, *269*(1), 711-715.
9. Bobadoye, M. F., Bamisi, O. O., & Enujiugha, V. N. (2016). Hypolipidemic and Antioxidative Effects of African Star Apple Juice (Chrysophylum albidum) on Rats Fed on Diets High in Cholesterol and Oil. *Food and Nutrition Sciences*, *7*(10), 825.
10. Nishiyama-Naruke, A., Souza, J. A., Carnelos, M., & Curi, R. (1998). HPLC determination of underivatized fatty acids saponified at 37 C analysis of fatty acids in oils and tissues. *Synthesis and Reactivity in Inorganic and Metal-organic Chemistry*, *31*(14), 2565-2576.
11. Farron-Furstenthal, F. (1979). An inhibitor protein of nuclear protein kinases. *Nature*, *280*(5721), 415.
12. Kim, D. H., Sarbassov, D. D., Ali, S. M., King, J. E., Latek, R. R., Erdjument-Bromage, H., ... & Sabatini, D. M. (2002). mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell*, *110*(2), 163-175.
13. Gasol, J. M., Zweifel, U. L., Peters, F., Fuhrman, J. A., & Hagström, Å. (1999). Significance of size and nucleic acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria. *Applied and Environmental Microbiology*, *65*(10), 4475-4483.