OPTICAL PROPERTIES OF RAT SERUM AFTER INTRAGASTRIC ADMINISTRATION OF MELANIN

Previous studies of the biological activity of melanin produced by the Antarctic black yeast Pseudonadsoniella brunnea have shown its antioxidant, stress-adaptogenic, dermatotropic, wound-healing and antibacterial effects. However, the primary physicochemical mechanisms of the systemic influence of melanins remain insufficiently studied. Therefore, the aim of the study was to determine effects of the intragastric administration of melanin produced by the Antarctic black yeast Pseudonadsoniella brunnea on the optical properties of a protein component and an aqueous phase of rat serum. White nonbread adult male rats weighing 180–200 g were used in the experiments. The intragastric route of administration of melanin by means of soft gastric catheter at a dose of 3 mg/kg was used. Rats of the control group were administered the physiological solution in the same way. After 1 hour the animals were sacrificed by cervical dislocation and blood serum was obtained for further studies. The absorption spectra of blood serum samples were recorded using Shimadzu Biospec-Mini spectrophotometer in the range of 190–1100 nm. Analysis of the absorption spectra of blood serum in a wide range from UV to near IR indicated that one hour after intragastric administration of melanin to rats at the dose of 3 mg/kg the optical properties of protein component were not changed, whereas the optical properties of the aqueous phase of the blood serum were changed due to statistically significant decrease of an amount of hydrogen bonds. Authors hypothesized that the appearance of substances that destruct the hydrogen bond network in the blood is one of the reasons for such changes. Changes of properties of water as the solvent and the structure-forming factor can have further systemic consequences due to changes in the hydration of biological polymers and low molecular weight metabolites, their solubility and intermolecular interactions, cell membrane permeability, molecular dynamics and functional activity of biomacromolecules, etc.

Keywords: melanin, blood serum, serum proteins, water.

Introduction. Melanins belong to the group of pigments synthesized in living organisms – both in pro- and eukaryotes. It is well known that melanins have a wide range of biological action: antioxidant, cytoprotective, photo- and radioprotective, etc., they can be used as sorbents of a number of radioisotopes and heavy metals [1, 2].

Previous research has shown that the biological activity of melanin produced by the Antarctic black yeast Pseudonadsoniella brunnea showed antioxidant, stress-adaptogenic [3], dermatotropic, wound-healing, antibacterial [4, 5], antiphuttopathogenic [6, 10] effects of melanin. This, in turn, allows us to consider melanin as a promising substance for numerous drugs with many useful properties that also makes it useful in medicine and veterinary medicine. The mechanism of action of melanins on biological processes is primarily associated with their antioxidant properties. However, the biological effects observed in the experiments are mainly systemic in nature. We must state that the primary physicochemical mechanisms of systemic influence of melandinon men and animals remain insufficient. Therefore, the aim of this study was to determine the effects of intragastric administration of melanin on the optical properties of the protein component and the aqueous phase of the blood serum of rats.

Materials and methods. The white nonbread adult male rats raised in the animal house of the Institute of Biology and Medicine of Taras Shevchenko National University of Kyiv and weighing 180–200 g were used in experiments. Animals were kept on a standard diet in an accredited animal house in accordance with standard rules for the organization, equipment and maintenance of experimental biological clinics (animal houses). Animals were divided into two groups: control (n = 5) and experimental (n = 5). The experimental procedures were conducted according to international recommendations for conducting biomedical research using animals in accordance with the European Convention. The intragastric route of administration of melanin by means of soft gastric catheter at a dose of 3 mg/kg was used. Rats of the control group were administered the physiological solution in the same way. After 1 hour the animals were sacrificed by cervical dislocation and blood serum was obtained for further studies. Melanin was obtained from a strain of black yeast-like fungi Pseudonadsoniella brunnea (Basidiomycota, Agaricomycotina, Agaricomycetes, Meripilaceae) 470 FCKU, isolated from Antarctic rock samples of Fr. Galindez. Strain P. brunnea 470 FCKU is stored in the Collection of Microscopic Fungi of Institute of Biology and Medicine of Taras Shevchenko National University of Kyiv (international acronym of the FCKU collection), registration number P. brunnea in the Depository of the State Research and Control Institute of Biotechnology and Strains of Microorganisms is N 607 [6]. In order to obtain biomass and synthesis of melanin the cultivation of the strain P. brunnea 470 FCKU was carried out by means of deep method using liquid nutrient media. The composition of nutrient media was chosen according to results of our previous studies [7–9]. Malt extract broth and Saburo medium (manufactured by HiMedia Laboratories, India and Conda, Spain) were used for biomass accumulation. Isolation of melanin from the culture medium of P. brunnea 470 FCKU was carried out in accordance with the Regulation “Obtaining polyphenol-carbon complex from Antarctic black yeast-like fungi Pseudonadsoniella brunnea” Melanin “on the basis of Specification U 15.9-30034243-004: 2005 with changes in name stain and additions and changes in p. 2.2.1, 2.2.3, 5.9.3-2017”. For the cultivation of P. brunnea 470 FCKU in order to obtain melanin we used barley-malt extract (YASE No 3, produced by “Starch Products of Ukraine”, Specification U 15.8 – 32671885-001: 2011) (4.6 % by hydrometer-sugar meter AST-2) with the addition of 0.05 % L-tyrosine and 1 % enzymatic peptone.

The absorption spectra of blood serum samples from each animal were recorded using Shimadzu Biospec-Mini spectrophotometer in the range of 190–1100 nm. Light absorption by serum samples was measured in quartz cuvettes with an optical path length of 1 cm against air that allowed to investigate the optical properties of not only the protein component but also spectral features of water, which is the main component of blood serum and other biological tissues.
ANOVA statistic was used for analysis of experimental data using Origin Pro that licensed for Taras Shevchenko National University.

**Results and discussion.** The blood serum of the rats had a light pink color due to the presence of small impurities of hemoglobin which got into the serum due to the mechanical destruction of erythrocytes during preparation of blood samples. The absorption spectra clearly show the typical absorption bands of oxyhemoglobin (Sore band 350–450 nm, as well as two bands in the range 500–600 nm) (Fig. 1, line A).

The statistically significant differences in the absorption spectra in the range of 300–700 nm of serum samples in groups of control and experimental animals were not detected (Fig. 1, line A). Analysis of the optical properties of serum proteins in the range of 230–320 nm, that characterizes absorption of aromatic and sulfur-containing amino acids, was possible after diluting blood serum 1 : 100 and 1 : 1000 (Fig. 1, lines B and C). In this case there were also not statistically significant differences between the control and experimental groups for this spectral range.

The absorption band corresponding to the vibronic overtones of water molecules \((2\nu_1 + \nu_2 = 951.5 \text{ nm}; \nu_1 + 2\nu_2 = 936.3 \text{ nm}; 2\nu_1 + \nu_3 = 982.1 \text{ nm}; 2\nu_2 + \nu_3 = 951.5 \text{ nm})\) was observed in the near infrared (IR) range of 900–1070 nm. In order to eliminate the effects of nonspecific light scattering in the different samples of blood serum, the absorption spectrum of each sample for the specified range was normalized relative to the baseline calculated by using procedure "Peak and Baseline" in Origin Pro. Then statistical processing was performed and spectral lines were averaged over animal groups taking into account the mean error for every average value of optical density (Fig. 2).
The analysis of absorption spectra in the range of 900–1070 nm shown the statistically significant (p < 0.05) increase in optical density by 6 % at \( \lambda_{\text{max}} = 974–976 \) nm in serum samples of rats administered with melanin, compared to samples of control animals. This indicates that despite the absence of spectral changes of the protein component, the state of the aqueous phase of blood serum was changed in some way. An additional confirmation of the real changes in the water state is the statistically significant increase of light absorption of near-IR light in the range of higher-order vibrionic overtones on 885–895 nm (2\( \nu_1 + 2\nu_3 \)). In addition, the absorption maximums of the spectral bands in the control and experimental serum samples are respectively 976 nm and 974 nm that indicates a spectral shift to the blue region of the water spectrum in serum samples of experimental group of animals. A natural question arises as to the nature and causes of such spectral changes.

Water is the main component of the blood serum, so the increase of its optical density in near IR range can be explained by an increasing its content in the biological samples. According to this assumption we should expect a decrease of the content of other components in blood serum, especially the proteins as the main component. The protein concentration for serum samples is easy to calculate using the Kalckar’s formula \( C (\text{g/l}) = 1.45D_{280} – 0.74D_{260} \), where \( D_{280} \) and \( D_{260} \) are the optical density at the respective wavelengths [15]. Using this formula, the serum protein concentration was calculated to be 64 and 66 g/l that corresponds to a protein concentration of 6.4 % and 6.6 %, respectively, for control and experimental groups of animals. These values are usual for the protein concentration indicies in the blood serum of rats and the difference does not exceed 3 % compared to the serum samples of control animals and it is not statistically significant value, which is clearly seen in Fig. 1. Even if we take this value as an extremely weak tendency to increase the concentration of protein in the blood serum of experimental rats, it contradicts the accepted assumption of increasing the water content in the blood serum samples. Thus, the most plausible explanation for the detected spectral shifts is the changes in the state of the aqueous phase in the serum samples of experimental animals.

Fig. 3 shows the generalized absorption spectra of liquid and solid phases of water. It is clear that when freezing water, when it is structured by the formation and stabilization of hydrogen bonds, there is a red shift (shift of absorption maxima in the region of greater wavelengths) and hypochromism (decrease in optical density) in the spectral range corresponding to higher overtones of water. In our case, the opposite effect was detected, namely the increase of optical density and the shift of the absorption maximum to the blue region. Thus, based on data on the optical properties of water and ice (Fig. 3), we can conclude that increase of optical density of water and the shift of the spectrum to the blue region in the near IR range indicates an increase of free molecules of water due to destruction of hydrogen bond network. This fact, in turn, allow us to suggests the changes of ratio of low molecular weight substances and electrolytes, which are structurants or destructors of the hydrogen bond network and affect the thermal motion of water molecules [16, 17] in the blood of experimental animals administered with melanin, that, in turn, should affect the solubility of substances of different nature in the blood and the hydration of biological molecules. The nature of such substances in blood serum and their properties require separate verification.

The authors draw attention to the fact that the antitumor effect of cisplatin-based drugs is accompanied by an increase in the self-diffusion rate of water molecules in both sensitive and cisplatin-resistant tumors of Guerin’s carcinoma, which generally indicates an increase in motility of water molecules under the influence of this drug [18]. Possibly, radiotherapeutic and anticancer effects of melanin drugs are associated not only with their high antioxidant activity [19, 20], but also withinfluence on the physicochemical properties of the aqueous phase that primarily depend on the dynamics of the hydrogen bond network.

Conclusions. Analysis of the absorption spectra of blood serum in a wide range from UV to near IR indicated that one hour after intragastral administration of melanin to rats at the dose of 3 mg/kg the optical properties of
protein component were not changed, but the properties of the aequous phase of the blood serum were changed due to statistically significant decrease of an amount of hydroxyproline.

Authors hypothesized that the appearance of substances that destruct the hydrogen bond network in the blood is one of the reasons for such changes. Changes of properties of water as the solvent and the structure-forming factor can have further systemic consequences due to changes in the hydration of biological polymers and low molecular weight metabolites, their solubility and intermolecular interactions, cell membrane permeability, molecular dynamics and functional activity of biomacromolecules, etc.

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ОПТИЧНІ ВЛАСТІВОСТІ СИРОВАТКИ КРОВІ ЩУРІВ ПІСЛЯ ІНТРАГАСТРАЛЬНОГО ВВЕДЕНИЯ МЕЛАНІНА

Попередні дослідження біологічної активності меланіну, продуцентами якого є антарктичні чорні дріаджеподібні гриби Pseudonatronella bulla, показали, що меланін виявляє антиоксидантні, стрес-адаптаційні, дерматопротивне, ранозахисне та антибактеріальні дії. Однак перші фізико-хімічні механізми системної дії меланіну залишаються недостатньо дослідженими. У зв’язку з цим метою дослідження було здійснено вплив ефектів інтергастрального введення меланіну на оптичні властивості білкової компоненти і водної фази сироватки крові щурів. У досліді використовували білки прінципальних щурів-самців із масою 180–200 г. Застосовувались інтергастральні шиї введення препарату меланіну капеетом’ю шпинцем у дозі 3 мкг (10-кратна терапевтична). Щудря контрольної групи таким самим чином удосконалила воду. За 1 год тварин утверджували методом цервикальної дислокації і отримували сироватку крові, яку використовували для подальших досліджень. Результат струп’ям поглинули на зразки сироватки крові проведено на спектрофотометри "Shimadzu Biospec-mini" в діапазоні 190–1100 нм. Аналіз спектрів поглину
них сироватки крові у широкому шиї введення проведено на електрофотометри "Shimadzu Biospec-mini" в діапазоні 190–1100 нм. Аналіз спектрів поглинуних сироватки крові в широкому діапазоні у ФД до більшого ФИ свідчить про те, що за годуні після підштовхуваного введення щурям меланіну в дозі 3 мкг зміни властивості білків у крові не змінюються, однак достовірно змнюються властивості водної фази крові в бік зменшення кількості водних в’язкостей. Автори припустив, що появляючи мереж водних в’язкостей у крові, є однією із причин таких змін. Зміни властивостей води як розчинника і структуроутворюючого фактора можуть мати пода
льші системні наслідки через зміни діягіазії білкових полімерів і низькомолекулярних метаболітів, їх розподілу різних типів меланіну.

Ключові слова: меланін, сироватка крові, біли кількісні переміни.

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ОПТИЧНІ СВІЙСТВА СИРОВАТКИ КРОВІ КРЯІС ПІСЛЯ ІНТРАГАСТРАЛЬНОГО ВВЕДЕНИЯ МЕЛАНІНА

Протягом семи років досліджень транспортних систем їдерних мембран від засустосування методу patch-clamp автори спостерігали певну закономірність: зміна ефективності роботи цим методом значно знижувалась. Оскільки рагу романські і матеріалом полягають різними світлими i температурними показниками, то ми вирішили перевіт
рить імовірність впливу останніх на успішність виконання дослідів. Тому методо цієї роботи було перевірити вплив таких сучасних факторів, як зміна тривалості світлового дня, температура, атмосферний тиск, кількість опадів i хмарності на який-то patch-clamp-реєстрації іонних струмів крій LCC-каналу щедрий мембрану карідоіміцт і ієроній

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РЕЄСТРАЦІЯ ІОНИЧНИХ СТРУМІВ КРІЙ LSC-КАНАЛІ КДІЄРНОї МЕМБРАНІ: ХРОНОБІОЛОГІЧНИЙ АСПЕКТ

Вступ. Ядро є однією із ключових органел еукарио
тичної клітини: воно зберігає, реалізує та передає гене
тичну інформацію, регулює синтетичні процеси у клітини тощо. Від цитоплазм, у якій містяться решта органел, ядро відділене ядерною оболонкою, яка утворена зовнішніми та внутрішніми мембранами та перинуклеа

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