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OPTICAL PROPERTIES OF RAT SERUM AFTER INTRAGASTRIC ADMINISTRATION OF MELANIN

Previous studies of the biological activity of melanin produced by the Antarctic black yeast Pseudonadsoniela brunnea have shown its antioxidant, stress-adaptogenic, dermatotropic, wound-healing and antibacterial effects. However, the primary physicochemical mechanisms of the system influence of melanins remain insufficiently studied. Therefore, the aim of the study was to determine effects of the intragastral administration of melanin produced by the Antarctic black yeast Pseudonadsoniela 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 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 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 radionuclides and heavy metals [1, 2].

Previous researchesthat we have conducted to determine the biological activity of melanin produced by the Antarctic black yeast Pseudonadsonielabrunnea showed antioxidant, stress-adaptogenic [3], dermatotropic, wound-healing, antibacterial [4, 5], antiphytopathogenic [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 melaninson men and animals remain insufficient. Therefore, the aim of this study was to determine the effects of intragastral 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 not breed 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). Animal were divided on two groups: control (n_c = 5) and experimental (n_e = 5). The experimental procedures were conduct 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 *Pseudonadsoniellabrunnea* (Basidiomycota, Agaricomycotina, Agaricomycetes, Polyporales, 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 of 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 Pseuonadsoniellabrunnea" 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 № 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.

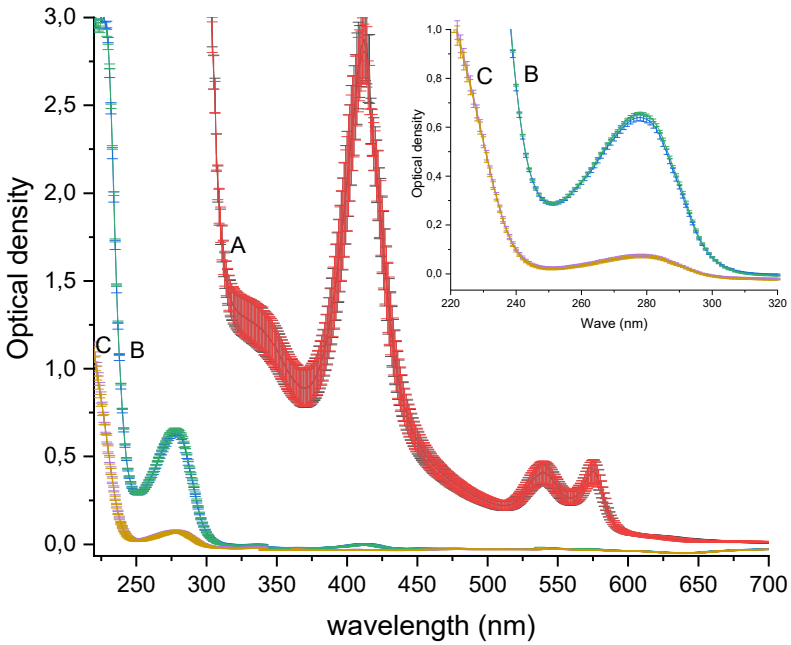

Fig. 1. Absorption spectra of whole blood serum samples in groups of control and experimental rats (line A) and diluted serum 1 : 100 and 1 : 1000 (lines B and C). The mean error of the average value (D ± d) is given for each value of optical density. The averaged absorption spectra for the control and experimental samples actually coincide

The absorption band corresponding to the vibronic overtones of water molecules (2v1 + v2 = 951.5 nm; v1 + 2v2 = 936.3 nm; 2v1 + 2v3 = 982.1 nm; 2v2 + 2v3 = 951.5) 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).

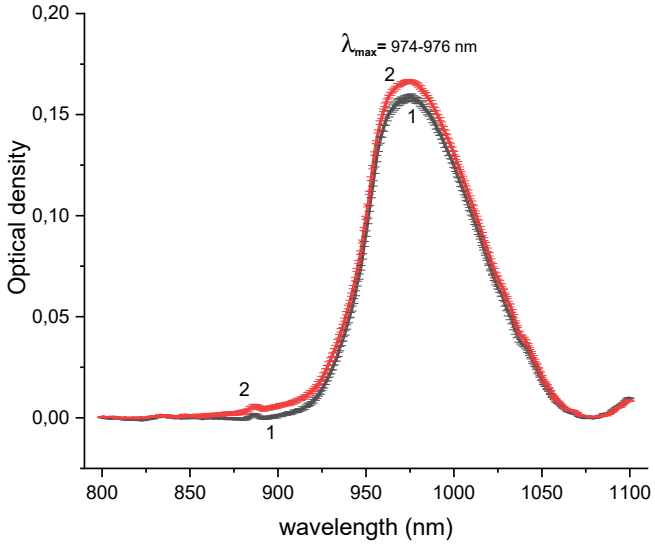

Fig. 2. The normalized absorption spectrum in the near IR range of vibronic overtones of water molecules 900–1070 nm (2v1 + v2 = 951.5 nm; v1 + 2v2 = 936.3 nm; 2v1 + 2v3 = 982.1 nm; 2v2 + 2v3 = 951.5 nm) for undiluted blood serum (1 – control group of rats, 2 – group of rats with the administration of melanin). The mean error of the average value (D ± d) is given for each value of optical density

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 λ_{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 vibronic overtones on 885–895 nm (2v1 + 2v3). 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 $C\ (g/l) = 1.45D_{280} - 0.74D_{260}$, 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.

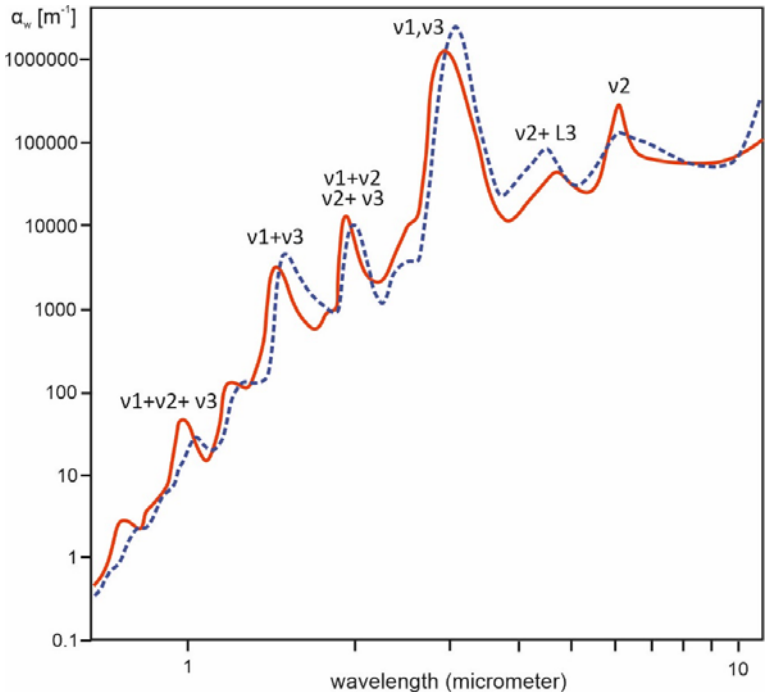

Fig. 3. Generalized light absorption spectra for liquid water (1, red solid) and ice (2, blue dotted line) [11–14]

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, radioprotective 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 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.

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ОПТИЧНІ ВЛАСТИВОСТІ СИРОВАТКИ КРОВІ ЩУРІВ ПІСЛЯ ІНТРАГАСТРАЛЬНОГО ВВЕДЕННЯ МЕЛАНІНУ

Попередні дослідження біологічної активності меланіну, продуцентами якого є антарктичні чорні дріжджоподібні гриби Pseudonadsoniela brunnea, показали, що меланін виявляє антиоксидантну, стрес-адаптогенну, дерматотропну, ранозагоювальну та антибактеріальну дію. Однак первинні фізико-хімічні механізми системної дії меланіну залишаються недостатньо дослідженими. У зв'язку із цим метою дослідження було з'ясування ефектів інтрагастрального введення меланіну на оптичні властивості білкової компоненти і водної фази сироватки крові щурів. У дослідах використовували білих нелінійних статевозрілих щурів-самців із масою 180–200 г. Застосовували інтрагастральний шлях уведення препарату меланіну катетером м'яким шлунковим у дозі 3 мг/кг (10-кратна терапевтична). Щурам контрольної групи таким саме чином уводили дистильовану воду. За 1 год тварин умертвляли методом цервікальної дислокації та отримували сироватку крові, яку використовували для подальших досліджень. Реєстрацію спектрів поглинання зразків сироватки крові проводили на спектрофотометрі "Shimadzu Biospec-mini" в діапазоні 190–1100 нм. Аналіз спектрів поглинання сироватки крові у широкому діапазоні від УФ до ближнього ІЧ свідчить про те, що за годину після підшкірного введення щурам меланіну в дозі 3 мг/кг оптичні властивості білків у крові не змінюються, однак достовірно змінюються властивості водної фази крові в бік зменшення кількості водневих зв'язків. Автори припустили, що поява речовин, які руйнують мережу водневих зв'язків у крові, є однією із причин таких змін. Зміни властивостей води як розчинника і структуроутворюючого фактора можуть мати подальші системні наслідки через зміни гідратації біологічних полімерів і низькомолекулярних метаболітів, їхньої розчинності та міжмолекулярних взаємодій, проникності клітинних мембран, молекулярної динаміки та функціональної активності біомакромолекули тощо.

Ключові слова: меланін, сироватка крові, білки сироватки, вода.

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ОПТИЧЕСКИЕ СВОЙСТВА СЫВОРОТКИ КРОВИ КРЫС ПОСЛЕ ИНТРАГАСТРАЛЬНОГО ВВЕДЕНИЯ МЕЛАНИНА

Предыдущие исследования биологической активности меланина, продуцентами которого являются антарктические черные дрожжеподобные грибы Pseudonadsoniela brunnea, показали, что меланин проявляет антиоксидантное, стресс-адаптогенное, дерматотропное, ранозаживляющее и антибактериальное действие. Однако первичные физико-химические механизмы системного действия меланина остаются недостаточно исследованными. В связи с этим целью исследования было выявление эффектов интрагастрального введения меланина на оптические свойства белковой компоненты и водной фазы сыворотки крови крыс. В опытах использовали белых нелинейных половозрелых крыс-самцов с массой 180–200 г. Применяли интрагастральный путь введения препарата меланина катетером мягким желудочным в дозе 3 мг/кг (10-кратная терапевтическая). Крысам контрольной группы таким же образом вводили дистиллированную воду. Через 1 час животных умерщвляли методом цервикальной дислокации и получали сыворотку крови, которую использовали для дальнейших исследований. Регистрацию спектров поглощения образцов сыворотки крови проводили на спектрофотометре "Shimadzu Biospec-mini" в диапазоне 190–1100 нм. Анализ спектров поглощения сыворотки крови в широком диапазоне от УФ до ближнего ИК свидетельствует о том, что через час после подкожного введения крысам меланина в дозе 3 мг/кг оптические свойства белков в крови не изменяются, однако достоверно изменяются свойства водной фазы крови в сторону уменьшения количества водородных связей. Авторы предположили, что появление веществ, разрушающих сеть водородных связей в крови, является одной из причин таких изменений. Изменения свойств воды как растворителя и структурообразующего фактора могут иметь дальнейшие системные последствия в результате изменений гидратации биологических полимеров и низкомолекулярных метаболитов, их растворимости и межмолекулярных взаимодействий, проницаемости клеточных мембран, молекулярной динамики и функциональной активности биомакромолекул и др.

Ключевые слова: меланин, сыворотка крови, белки сыворотки, вода.

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РЕЄСТРАЦІЯ ІОННИХ СТРУМІВ КРІЗЬ LCC-КАНАЛИ ЯДЕРНОЇ МЕМБРАНИ: ХРОНОБІОЛОГІЧНИЙ АСПЕКТ

Протягом семи років досліджень транспортних систем ядерних мембран із застосуванням методу patch-clamp спостерігали певну закономірність: узимку ефективність роботи цим методом значно знижувалась. Оскільки різні сезони/пори року характеризуються різними світловими і температурними показниками, то ми вирішили перевірити ймовірний вплив останніх на успішність виконання досліджень. Тому метою цієї роботи було перевірити вплив таких сезонних факторів, як зміна тривалості світлового дня, температура, атмосферний тиск, кількість опадів і хмарність на якість patch-clamp-реєстрацій іонних струмів крізь LCC-канали ядерної мембрани кардіоміоцитів і нейронів Пуркіньє мозочка. Ми припустили, що зі зменшенням тривалості світлового дня і зниженням температури зменшуються якісні та кількісні показники patch-clamp-реєстрацій. Для перевірки цього припущення застосовано кореляційний аналіз Пірсона із вихідними даними про тривалість світлового дня, метеорологічні умови та розрахованою успішністю реєстрацій (%) за конкретний день. На основі результатів такого аналізу встановлено, що існує пряма виражена лінійна залежність якості та кількості реєстрацій від тривалості світлового дня (r = 0,6) і температури (r = 0,6), а також слабка обернена залежність від хмарності (r = 0,3). На основі дисперсійного аналізу (ANOVA) підтверджено достовірно більшу успішність реєстрацій, виконаних у літній період, порівняно із зимовими того самого року. Отримані результати можуть стати основою для оптимізації дослідницької діяльності робочих груп, які вивчають функціонування внутрішньоклітинних транспортувальних систем електрофізіологічними методами, зокрема patch-clamp.

Ключові слова: біоритми, хронобіологія, метеорологічні умови, patch-clamp, ядерна мембрана, іонні канали, LCC-канали.

Вступ. Ядро є однією із ключових органел еукаріотичної клітини: воно зберігає, реалізує та передає генетичну інформацію, регулює синтетичні процеси у клітині тощо. Від цитоплазми, у якій містяться решта органел, ядро відмежоване ядерною оболонкою, яка утворена зовнішньою і внутрішньою мембранами та перинуклеа-