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SPECTRAL PROPERTIES OF TWO WATER-SOLUBLE MELANINS

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Aim. The work was purposed to study the spectral properties of two types of water-soluble melanins to clarify the nature of the optical absorption and emission centers in them.

Materials and Methods. Studied are the spectral properties of two types of water-soluble melanins: melanin obtained from black yeast-like fungi Pseudonadsoniella brunnea 470 FCKU, dissolved in an aqueous NaOH solution with pH 11, and plant melanin obtained from black tea according to German patent DE102004003801A1, which is added to drinking water for medicinal purposes.

In the spectral region of 200–900 nm, optical absorption spectra, spectra, and fluorescence excitation spectra are measured at room temperature.

Results. The optical density of both studied samples monotonically decreases with increasing radiation wavelength. Fluorescence spectra represent one broadband, the peak position of which depends on the excitation wavelength. The fluorescence excitation spectrum consists of at least two bands and does not coincide with the absorption spectrum.

Conclusions. The shape of the absorption spectra may indicate that the aqueous solutions of melanins under study contain many absorption centers with different resonance frequencies. Fluorescence spectra represent a superposition of several elementary bands with varying spectra of excitation. The different excitation spectra corresponding to fluorescence at different wavelengths most likely indicate that each of the melanin solutions under study contains several of its species.

The spectral range of absorption, as well as the low fluorescence intensity, make further studies of water-soluble melanins as components of complex nanosystems for photoacoustics and photothermal therapy promising.

The use of producer microorganisms for melanin production provides a cost effective biotechnological process.

Key words: melanin, aqueous solution, optical absorption, fluorescence.

Melanins are a class of high-molecular-weight organic compounds that act as pigments (natural dyes) in the simplest organisms, such as bacteria, as well as in plants, animals, and humans. They exhibit an irregular structure and complex chemical composition. The term "melanin" does not convey specific information about the chemical structure of these pigments, other than indicating they are polymers with relatively high molecular weight. Any given melanin sample is almost certainly composed of a mixture of macromolecular species. Furthermore, different melanins, such as those from various living organisms, are constructed from different monomeric units.

The situation is further complicated by the potential for polymer chain branching and cross-linking between chains [1].

From chemical and physical perspectives, melanins are aromatic organic compounds, meaning they are π-electron systems capable of absorbing and emitting light in the ultraviolet and visible regions of the spectrum ($\lambda \geq 200$ nm). Although there is a substantial body of literature on the spectral properties of melanins of both natural [2] and synthetic origins [3, 4], the nature of the absorption and emission centers in melanins remains unresolved to this day.

Melanins exhibit a broad spectrum of biological activities, including antioxidant, immunomodulatory, antimicrobial, antifungal, antiviral, anti-inflammatory, antistress, antitumor, dermatotropic, cytoprotective, photoprotective, and radioprotective effects [5, 6]. Additionally, microbial melanins can function as sorbents for radionuclides and heavy metals [7, 8]. Microscopic fungi are recognized as valuable producers of pigments, including melanin. Fungal melanins are actively utilized in the development of a new class of biologically active high-tech materials [8–11]. Among melanin producers, black yeast-like fungi hold a special place due to their potential applications in medicine [12, 13]. A key characteristic of these black yeasts is their ability to synthesize not only intracellular melanin but also to excrete most of the melanin into the culture medium. It has been established that melanin produced by the strain *Pseudonadsoniella brunnea* 470 FCKU exhibits antioxidant, anti-phytopathogenic [14], dermatotropic, wound-healing [15, 16], and stress-adaptive [17] activities, making it a promising candidate for pharmaceutical applications.

Another water-soluble melanin, derived from plants [18], is currently widely used in medicine.

Objective and Research Goals

The objective of this study was to examine the spectral properties of two water-soluble melanins (isolated from black yeast-like fungi and black tea) to investigate the nature of the optical absorption and emission centers in them. The black yeast-like fungi *Pseudonadsoniella brunnea*, isolated from Antarctic rock samples from Galindez Island, have been the subject of our long-term research [19]. The melanin derived from black tea was provided by LLC "Plant Polymer Melanin" (Kyiv, Ukraine).

In this work, we analyzed the absorption spectra, fluorescence spectra, and fluorescence excitation spectra of these two types of water-soluble melanins at room temperature.

Materials and Methods

The study focused on the spectral properties of two types of water-soluble melanin:

- **Melanin A:** Derived from black yeast-like fungi, dissolved in an aqueous NaOH solution with a pH of 11.
- **Melanin B:** Plant-derived melanin extracted from black tea, produced according to German Patent DE102004003801A1 [18], used as a dietary supplement in drinking water for therapeutic purposes.

To obtain **Melanin A**, the strain *Pseudonadsoniella brunnea* 470 FCKU (Basidiomycota, Agaricomycotina, Agaricomycetes, Polyporales, Meripilaceae) was used. This strain was isolated from Antarctic rock samples from Galindez Island and is maintained in the Collection of Microscopic Fungi at the ESC "Institute of Biology and Medicine" of Taras Shevchenko National University of Kyiv (FCKU collection, international acronym), with registration number 607 in the Depository of the State Scientific Control Institute of Biotechnology and Strains of Microorganisms 19 . The Pseudonadsoniella brunnea 470 FCKU strain was cultivated in a liquid nutrient medium for the purpose of melanin production. To prepare the medium, barley malt extract (YSE No. 3, produced by "Krochmalprodukti Ukrainy") [20] was used as a source of carbon (carbohydrates) at a concentration of 4.0% according to the areometer-sugar meter AST-2. The medium also contained L-tyrosine (0.05%) and enzymatic peptone (1%). The pH of the medium was adjusted to 1–1.5 using sulfuric acid. Pseudonadsoniella brunnea was cultivated by submerged culture at a temperature of +24±2 °C for 10–14 days. The extraction of melanin from the culture medium of *Pseudonadsoniella brunnea* was carried out in several sequential stages, including multiple adjustments of the culture medium's acidity. In particular, for the alkaline hydrolysis stage of the biomass, a sodium hydroxide solution with a pH of 10–11 was used, added in a 1:5 ratio. Melanin precipitation was carried out at a pH of 1–2, using hydrochloric acid to acidify the medium. In the final stages, the formed

and compacted precipitate containing melanin was washed twice with warm (30–35 °C) water in a 1:10 ratio. After settling, the precipitate with the purified melanin was dried at a temperature of 27–30 °C in a Venticell drying oven.

The absorption spectra of melanin solutions were measured at room temperature using a single-beam spectrophotometer UV1900PC (Macy Instruments Inc., China), while the fluorescence and fluorescence excitation spectra at room temperature were measured using a fluorescent spectrophotometer Cary Eclipse (Varian, Australia) [21].

Results and Discussion

Absorption Spectra

Figure 1 shows the absorption spectra of both types of melanin solutions (melanin A and melanin B) at room temperature. Melanin A was dissolved in an aqueous NaOH solution with a pH of 11, while melanin B was dissolved in drinking water. The concentration of melanin in both solutions was 0.1 mg/mL. For convenience in comparing the spectra, the optical density values for the melanin A solution were reduced by a factor of 10. The optical density of each melanin solution was measured relative to the corresponding solvent.

From the figure, it can be seen that the optical density of the studied samples monotonically decreases with increasing wavelength of radiation. This type of absorption spectrum suggests that the solutions contain many absorption centers with different resonance frequencies. Due to the monotonic decline of the spectrum and the absence of characteristic peaks, it is impossible to determine the aforementioned absorption frequencies.

Figures 2 and 3 show the fluorescence and fluorescence excitation spectra of the solutions of both types of melanin at room temperature. It should be noted that the fluorescence of melanin A at room temperature was significantly weaker than the fluorescence of melanin B.

As seen in Figure 2, with an increase in excitation wavelength (315, 370, and 415 nm), the fluorescence spectrum peak of melanin A shifts towards the longer wavelengths. Additionally, the fluorescence excitation spectra at emission wavelengths of 424 and 475 nm differ—at the latter, an additional band appears with a peak around 380 nm. Thus, it can be suggested that the melanin A solution contains several distinct emission centers.

Fluorescence of melanin B was excited by light at wavelengths of 240, 315, and 380 nm. The analysis of the spectra reveals the following:

- The spectra excited at 240 nm and 315 nm are almost identical in shape and position, with a peak around 422 nm, but differ only by intensity. This suggests that these two fluorescence bands likely correspond to the same fluorescence center in melanin.
- The band excited by 380 nm shows a long-wave shift, indicating a different fluorescence center is responsible for this emission.

The spectra of fluorescence excitation at wavelengths of 425, 470, and 520 nm, as

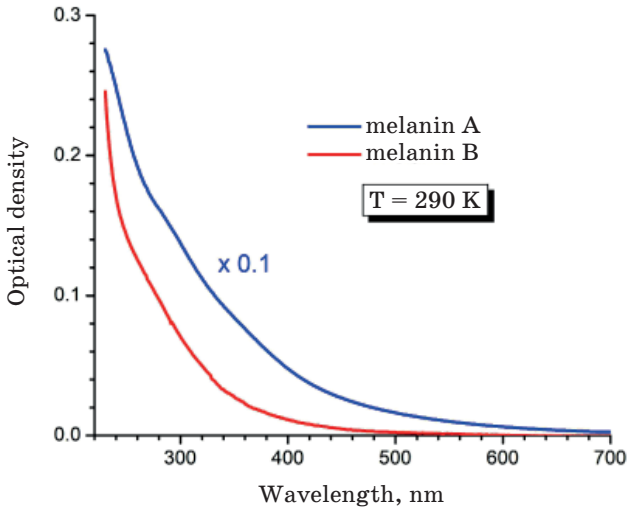

Fig. 1. **Absorption spectra of melanin A and melanin B solutions**

shown in the same figure, exhibit interesting features. Specifically:

• The excitation spectra consist of at least two bands.

• The longer-wavelength band shifts towards the longer-wavelength region of the spectrum as the fluorescence emission wavelength increases from 425 nm to 520 nm.

The following preliminary conclusions can be drawn from the obtained results.

Firstly, the excitation fluorescence spectra differ from the absorption spectra, which may indicate that not all absorption centers of melanin are emission centers.

Secondly, the fluorescence spectra represent a superposition of several elementary bands with different excitation spectra.

And thirdly, the different excitation spectra corresponding to fluorescence at different wavelengths most likely indicate that each of the studied melanin solutions contains several species of melanin.

Thus, we have studied the spectral properties of two water-soluble melanins. The absorption spectral range, as well as the low fluorescence intensity, make them promising for further research as components of complex nanosystems for photoacoustics and photothermal therapy.

Finally, it should be noted that melanins belong to the so-called biologically active compounds (BACs). Biologically active compounds synthesized and accumulated by extremophilic microorganisms are widely used in various fields of human activity: biotechnological production, medicine, agriculture, and more [22–25]. It has been established that microscopic fungi, which grow under the extreme conditions of Antarctica, are powerful sources of metabolites with antimicrobial, antifungal, anticancer, and antioxidant activity and can be objects of the pharmaceutical industry [26, 27]. It has also been shown that Antarctic yeasts, *Candida antarctica*, can be used for the effective synthesis of indolysin, derivatives of which are used as anticancer, antituberculosis, analgesic, and antioxidant agents, among others [28]. Among the microorganisms that produce biologically active compounds, melanins are a special category. The use of microorganisms for melanin production is an important research direction, as there is growing interest in safer, environmentally friendly products [29, 30]. The use of melanin-producing microorganisms ensures an economically advantageous biotechnological process, as melanin synthesis by microorganisms can be carried out using inexpensive culture media and is not subject to seasonal limitations, among other advantages [31, 32].

Conclusions

The form of the absorption spectra may indicate that the studied aqueous solutions of melanins contain many absorption centers with different resonance frequencies. The fluorescence spectra represent a superposition of several elementary bands with different excitation spectra. The different excitation spectra corresponding to fluorescence at different wavelengths most likely suggest that each of the studied melanins contains several melanin species.

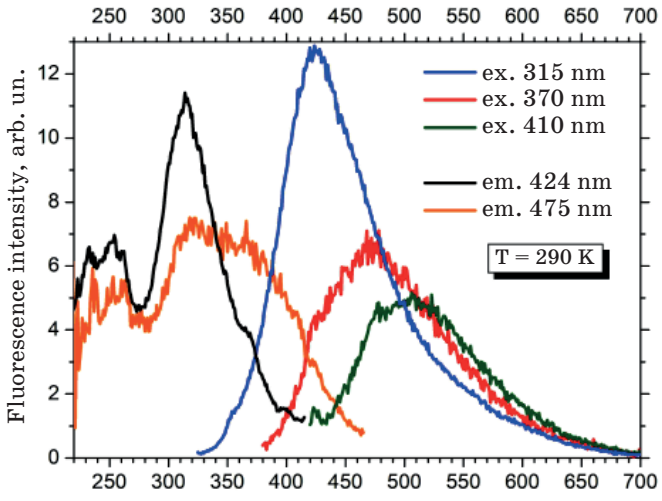

Fig. 2. **Fluorescence and fluorescence excitation spectra of the melanin A solution**

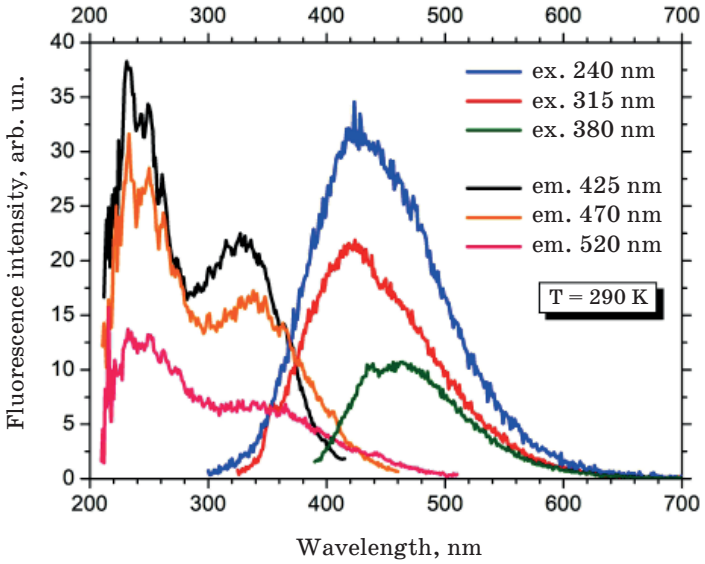

Fig. 3. **Fluorescence spectrum and fluorescence excitation of the melanin B solution**

The spectral absorption range, as well as the low fluorescence intensity, makes further studies of water-soluble melanins as components of complex nanosystems for photoacoustics and photothermal therapy promising.

The use of microorganism producers for melanin production provides a cost effective biotechnological process.

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Authors' contribution

Kravchenko V.M., Losytskyy M.Yu. — spectroscopic measurements, writing original draft, Beregova T.V., Kondratyuk T.O. — melanin extraction, sample preparation, writing original draft, Davidenko S.A., Davidenko E.S., Davidenko S.S. — melanin extraction, sample preparation, Buchatski L.P., Yashchuk V.M. — conceptualization, supervision, editing.

Conflicts of interest

The authors declare no conflicts of interest.

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СПЕКТРАЛЬНІ ВЛАСТИВОСТІ ДВОХ ВОДОРОЗЧИННИХ МЕЛАНІНІВ

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Мета. Аналіз спектральних властивостей двох видів водорозчинних меланінів для з'ясування природи центрів оптичного поглинання і випромінювання в них.

Матеріали та методи. В роботі вивчали спектральні властивості двох видів водорозчинних меланінів: меланіну, отриманого з чорних дріжджоподібних грибів *Pseudonadsoniella brunnea* 470 FCKU, розчиненого у воді з pH 11, та рослинного меланіну, отриманого з чорного чаю згідно з патентом Німеччини DE102004003801A1, що додається до питної води з лікувальною метою.

В спектральній області 200–900 нм було виміряно спектри оптичного поглинання, спектри флюоресценції і збудження флюоресценції при кімнатній температурі.

Результати. Оптична густина обох досліджених зразків монотонно спадає зі зростанням довжини хвилі випромінювання. Спектри флюоресценції являють собою одну широку смугу, положення максимуму якої залежить від довжини хвилі збудження. Спектр збудження флюоресценції складається щонайменше з двох смуг і не збігається зі спектром поглинання.

Висновки. Форма спектрів поглинання може свідчити про те, що в досліджених водних розчинах меланінів присутні багато центрів поглинання, що мають відмінні резонансні частоти. Спектри флюоресценції є суперпозицією кількох елементарних смуг, що мають відмінні спектри збудження. Відмінні спектри збудження, що відповідають флюоресценції на різних довжинах хвиль, скоріш за все, свідчать про те, що кожен із досліджених розчинів меланіну містить у собі кілька його видів. Спектральний діапазон поглинання, а також низька інтенсивність флюоресценції роблять перспективними подальші дослідження водорозчинних меланінів в ролі компонентів складних наносистем для фотоакустики та фототермальної терапії. Застосування мікроорганізмів-продуцентів для отримання меланіну забезпечує економічно вигідний біотехнологічний процес.

Ключові слова: меланін, водний розчин, оптичне поглинання, флюоресценція.