Microbiology, Vol. 71, No. 4, 2002, pp. 410–416. Translated from Mikrobiologiya, Vol. 71, No. 4, 2002, pp. 482–490.

EXPERIMENTAL ARTICLES

The Photochemical and Surface-Active Properties of Melanins Isolated from Some Black Fungi

I. I. Turkovskii and N. A. Yurlova[1]

St. Petersburg State Academy of Pharmaceutical Chemistry, Russia

Received December 18, 2000; in final form, January 17, 2002

Abstract—The absorption spectra of melanins isolated from some black ascomycetes, as well as of synthetic melanin and natural melanin from *Sepia officinalis*, were recorded in the long-wavelength ultraviolet region A ($320 \text{ nm} < \lambda < 400 \text{ nm}$) and in the blue–violet region of the electromagnetic spectrum at illumination intensities varying from 0.02 to 1 mW/cm^2. The photochemical properties of fungal melanins were found to be dependent on both the producing strain and the conditions of its cultivation. The fungal melanins are more susceptible to photomodification and more biologically active than the synthetic melanin, indicating that these properties may be related. The data obtained suggest that the fungal melanins susceptible to photomodification possess higher biological activity than commercial melanins.

Key words: black fungi, *Aureobasidium pullulans*, melanin, photomodification.

Natural pigment melanins possess photo- and radioprotective properties and anticancer activity [1–4]. All animal melanins (eumelanins) are synthesized from tyrosine via dioxyphenyl alanine (DOPA), whereas fungal melanins (allomelanins) can be synthesized either via DOPA (as in basidiomycetes and basidiomycetous mitospore-producing fungi) or from 1,8-dihydroxynaphthalene via the pentaketide pathway [5]. Harvey described an eumelanin obtained by the genetic engineering technique [6].

The physicochemical properties of melanins have been well studied. Melanins are used as components of drugs and cosmetics for human protection against excess electromagnetic, including ultraviolet, irradiation [7].

Black fungi growing under extreme conditions (at low or high temperatures, under intense insolation, at high radioactivity) synthesize great amounts of melanins [4]. The main biological function of melanins (protection of cells from the detrimental effect of various types of radiation) is related to their specific chemical structure, characterized by the presence of conjugated double bonds and the easiness of formation of paramagnetic centers [1, 4], due to which melanins are able to convert the energy of ultraviolet and infrared radiation into heat and to scavenge cytotoxic free radicals.

Some authors believe that the biological activity of melanins is due to their antioxidant and radical-scavenging properties [8, 9], whereas others argue that eumelanins possess high prooxidant activity [10]. This discrepancy can be attributed to the different experimental conditions used by various researchers, in particular, the different molar concentrations of the melanins used. It turned out that it is a difficult task to compare the concentrations of melanins in the experiments described in the literature, since melanins are heterogeneous in molecular mass, and the reported weight concentrations of melanins [8, 10, 11] cannot be converted to their molar concentrations. At the same time, many properties of substances depend on their molar concentration. In particular, there is evidence that some substances, which exert antioxidant action at certain concentrations, may exert prooxidant action at lower concentrations [12].

In an attempt to overwhelm the difficulty related to an uncertain molecular mass of melanins, we invoked their spectral properties. Taking into account the known redox properties of melanins [10, 13], there must be a strong absorbance in the visible spectral region (due to the low ionization potential of melanins) and photolability upon exposure to visible light at a sufficiently high intensity (due to the initiation of a cascade of photochemical reactions). In this case, we took advantage of the work of Dontsov *et al.* [10], who showed that eumelanin possesses prooxidant properties under illumination at 100 mW/cm^2 or more, while it possesses antioxidant properties at 5.3 mW/cm^2. These data cannot obviously militate against the photoprotective effect of melanins, since the illumination intensity 100 mW/cm^2 exceeds the intensity of the Sun rays in outer space. (It should, however, be noted that Sarna and Sealy [14] reported on the prooxidant activity of melanins at an illumination intensity as low as 0.9 mW/cm^2.) In any case, there is no doubt that the physicochemical properties of melanins change under illumination.

[1]Corresponding author. E-mail: yurlova@ss5777spb.edu

0026-2617/02/7104-0410$27.00 © 2002 MAIK "Nauka / Interperiodica"

The effect of 3-min illumination (at intensities of 0.1 and 1 mW/cm^2) on the spectral characteristics of an aqueous solution of the melanin isolated from the *A. pullulans* var. *aubasidani* strain F-448 grown on Czapek–Dox medium

Wavelength, nm	310	320	330	340	350	360	370	380	390	400
Dark spectrum:	0.84	0.42	0.71	1.35	1.17	1.02	0.95	0.85	0.76	1.20
0.1-mW/cm^2 illumination										
1-mW/cm^2 illumination	0.85	0.96	0.71	1.35	1.36	1.28	1.01	0.92	0.76	1.19
	0.88	0.93	0.71	1.35	1.14	0.94	0.87	0.94	0.72	1.19

The aim of the present work was to study the spectral behavior of melanins isolated from some black ascomycetes, as well as of synthetic melanin and natural melanin from the marine mollusk *Sepia officinalis*, under illumination at intensities varying from 0.02 to 1 mW/cm^2 and to investigate the surface-active properties of aqueous melanin solutions in order to develop an approach to the estimation of the mean molecular mass of melanins and the molar concentrations of their aqueous solutions.

MATERIALS AND METHODS

Experiments were carried out using the black ascomycetous fungi *Aureobasidium pullulans* var. *aubasidani* VKPM F-448, obtained from the All-Russia Collection of Industrial Microorganisms (VKPM), *A. pullulans* var. *pullulans* SPAPC 129(11), SPAPC 2275, and SPAPC U, obtained from the collection of St. Petersburg Academy of Pharmaceutical Chemistry (SPAPC), and *Nadsoniella nigra* var. *hesuelica* VKM F-2137, obtained from the All-Russia Collection of Microorganisms (VKM).

The fungi were grown at 25 ± 1°C for 14 days on a shaker (220 rpm) in flasks containing 200 ml of either Czapek–Dox medium or 4% wort. Alternatively, they were grown on the same agar-solidified media under the same conditions. Cells were separated from the growth medium, washed thrice with sterile distilled water, dehydrated with ethanol and then acetone, and dried to constant weight.

Melanins were extracted from the dry biomass with 0.5 N NaOH at 121°C for 1 h and then purified as described elsewhere [1, 16]. Aqueous solutions of the isolated melanins, as well as of the authentic synthetic eumelanin and natural melanin from the marine mollusk *Sepia officinalis* purchased from ICN, were prepared by the Malama and Bulanov method [16] modified as described in the textbook [17].

The intensity of illumination was measured using an FDK-227 photosensitive diode with an integral sensitivity of 7.2 μA/W and calibrated monochromatic sensitivity. The photocurrent from the diode was measured with an M-2005 microampermeter.

The photoelement was calibrated at noon on 22–27 June using sunlight as the energy source and a set of standard light filters, whose absorption spectra were recorded with an SF-46 spectrophotometer. The photospheric temperature of the Sun was taken to be 5600 K instead of 6000 K because of a strong absorption of long-wavelength ultraviolet light.

The absorption spectra of melanins were recorded with the SF-46 spectrophotometer. The surface tension of melanin solutions was determined by the liquid drop method [17].

The film thickness of high-molecular-weight surface-active compounds was determined at 20°C by the quasioptical method [17, 18], using the complex coefficient of reflection of a 62-GHz wave from the leicosapphire–tested liquid interface at the open end of the probing waveguide. Such an arrangement of the waveguide provided for an unimodal irradiation and allowed the phase difference and the amplitude ratio between the incident and the reflected signals to be determined and then the modulus and the argument of the complex reflection coefficient to be calculated. The film of a surface–active compound (surfactant) formed between the solution and the leicosapphire increased the phase shift at the waveguide end due to the wave reflection from two interfaces, the leicosapphire–surfactant interface and the surfactant–solution interface, whereas the wave intensity at the frequency used virtually did not fall. Knowing the complex coefficient of reflection and the probing wavelength, the thickness of the surfactant film could be easily calculated. The energy density of the probing radiation did not exceed 1 μW/cm^2.

RESULTS AND DISCUSSION

The Absorption Spectra and the Surface Tension of Aqueous Melanin Solutions at Different Levels of Illumination

The most typical changes in the absorption spectra of melanins induced by daylight at intensities of 0.1 and 1.0 mW/cm^2 are shown in the table. The data refers to the melanin isolated from the *A. pullulans* var. *aubasidani* F-448 grown in the synthetic Czapek–Dox medium.

It was found that daylight at intensities up to 1 μW/cm^2 affects the absorbance of aqueous melanin solutions at certain wavelengths (Figs. 1–13). Figures 1 and 2 present the absorption spectra of melanins isolated from the *N. nigra* var. *hesuelica* VKM F-2137 cells grown on 4% wort agar and in liquid Czapek–Dox

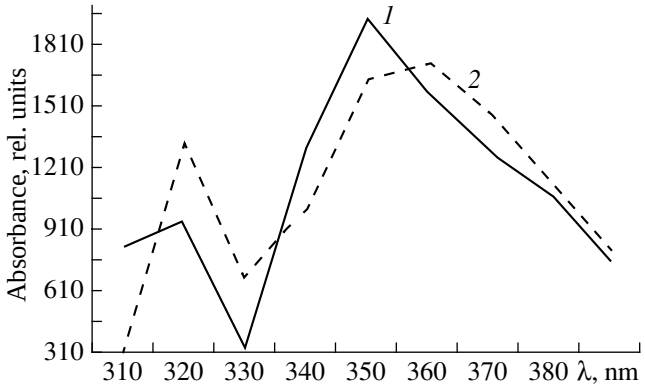

Fig. 1. The absorbance of an aqueous solution of the melanin isolated from the black fungus *N. nigra* var. *hesuelica* VKM F-2137 grown on 4% wort agar (*1*) before and (*2*) after illumination at an intensity of 1 mW/cm^2 for 3 min. Absorbance in Figs. 1–13 is given in relative units defined as $10^3 \times \log I_i/I_t$, where I_i is the intensity of the incident light beam and I_t is the intensity of the transmitted light beam.

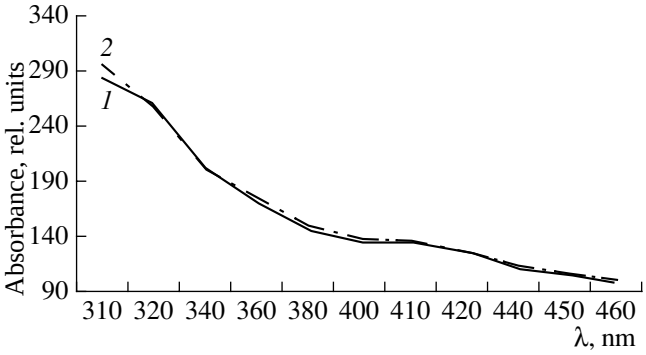

Fig. 3. The absorbance of an aqueous solution of the melanin isolated from the black fungus *A. pullulans* var. *pullulans* SPAPC 129(11) grown on 4% wort agar (*1*) before and (*2*) after illumination at an intensity of 0.02 mW/cm^2 for 3 min.

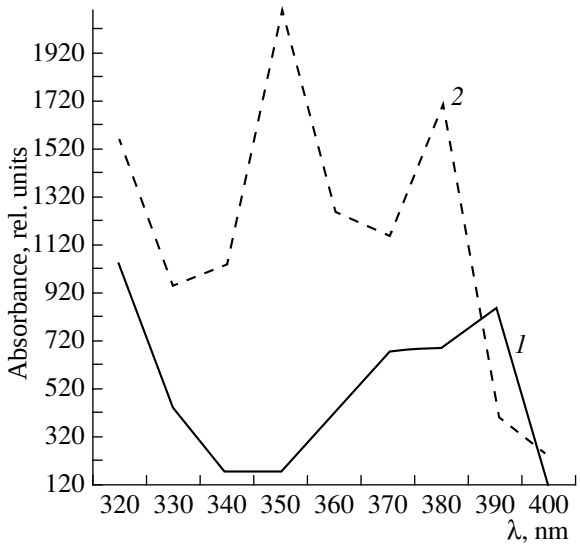

Fig. 2. The absorbance of an aqueous solution of the melanin isolated from *N. nigra* var. *hesuelica* VKM F-2137 grown in liquid Czapek–Dox medium (*1*) before and (*2*) after illumination at 1 mW/cm^2 for 3 min.

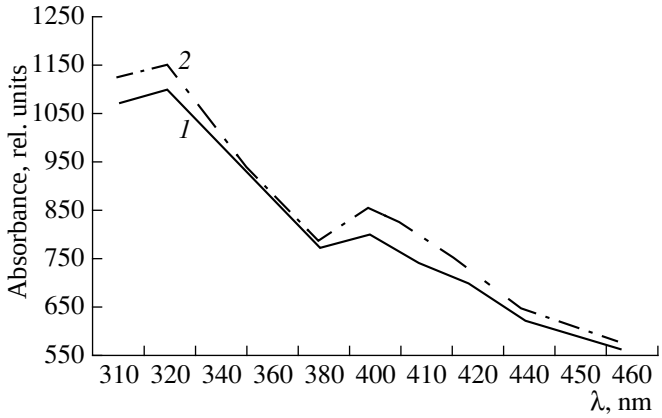

Fig. 4. The absorbance of an aqueous solution of the melanin isolated from *A. pullulans* var. *pullulans* SPAPC 129(11) grown in liquid Czapek–Dox medium (*1*) before and (*2*) after illumination at 0.02 mW/cm^2 for 3 min.

medium, respectively, which were recorded in the dark and after illumination at an intensity of 1 μW/cm^2 for 3 min. It can be seen that the illumination increased the absorbance of the melanin preparations at certain wavelengths, indicating their photomodification. The absorption spectra of the melanins and, hence, their physicochemical properties, depended on the cultivation conditions of the fungi from which they had been isolated.

The same is evident from the spectra presented in Figs. 3 and 4. Namely, illumination at an intensity of 0.02 μW/cm^2 insignificantly influenced the absorption spectrum of the melanin isolated from the *A. pullulans* var. *pullulans* SPAPC 129(11) cells grown on 4% wort agar (Fig. 3), whereas the absorbance of the melanin isolated from the same strain grown in liquid Czapek–Dox medium was increased by illumination at wavelengths of 380–420 nm by 5–7% as compared with the dark spectrum (Fig. 4).

Similar spectral changes were observed for the melanins isolated from the *A. pullulans* var. *pullulans* SPAPC 2275 cells grown on 4% wort agar (Fig. 5), in liquid Czapek–Dox medium (Fig. 6), and in liquid 4% wort (Fig. 7). The first melanin somewhat changed its extinction after illumination at an intensity of 0.1 μW/cm^2 (Fig. 5). Analogous changes in the spectral properties of the second melanin at wavelengths of 310–380 nm were observed after illumination at a lower intensity (0.06 μW/cm^2) (Fig. 6). The greatest increase in absorbance at some wavelengths within a range of 310–420 nm was observed for the third melanin illuminated at the intensity 0.1 μW/cm^2 (Fig. 7).

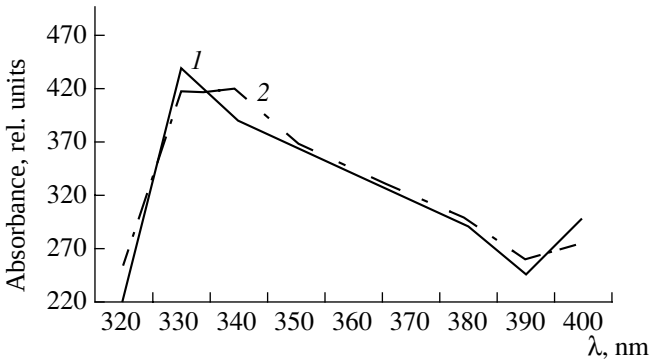

Fig. 5. The absorbance of an aqueous solution of the melanin isolated from the black fungus *A. pullulans* var. *pullulans* SPAPC 2275 grown on 4% wort agar (*1*) before and (*2*) after illumination at an intensity of 0.1 mW/cm^2 for 3 min.

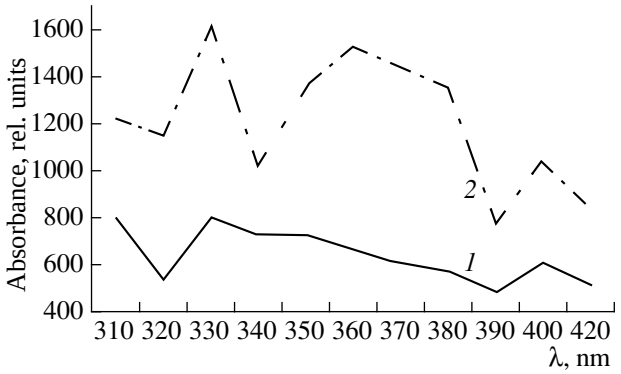

Fig. 7. The absorbance of an aqueous solution of the melanin isolated from *A. pullulans* var. *pullulans* SPAPC 2275 grown in 4% wort (*1*) before and (*2*) after illumination at 0.1 mW/cm^2 for 3 min.

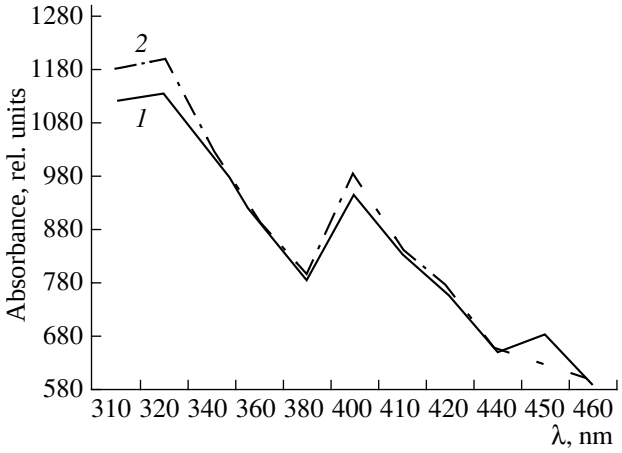

Fig. 6. The absorbance of an aqueous solution of the melanin isolated from *A. pullulans* var. *pullulans* SPAPC 2275 grown in liquid Czapek–Dox medium (*1*) before and (*2*) after illumination at an intensity of 0.06 mW/cm^2 for 3 min.

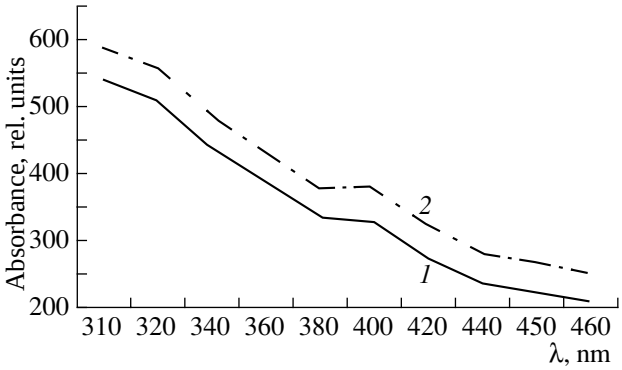

Fig. 8. The absorbance of an aqueous solution of the commercial (ICN) melanin isolated from the marine mollusk *Sepia officinalis* (*1*) before and (*2*) after illumination at an intensity of 0.02 mW/cm^2 for 3 min.

The electron paramagnetic studies of these melanins showed that they contain different amounts of paramagnetic centers (1×10^{18}, 4×10^{18}, and 6.6×10^{18} per g dry preparation, respectively).

The photoinduced changes in the spectral properties of melanins suggest a rearrangement of their molecular structure due to excitation of the electrons of conjugated π bonds and indicate the low ionization potential of the melanin molecules. Furthermore, it can be suggested that the melanins isolated from the fungal cells grown in the liquid growth medium with continuous mixing (Figs. 2, 4, 6, 7) will possess the highest biological activity.

The aqueous solutions of natural melanin from *Sepia officinalis* (Fig. 8) and synthetic eumelanin (Figs. 9–11) showed different responses to illumination. The synthetic melanin exhibited insignificant changes in the spectral region 320–400 nm in response to illumination at 0.06 μW/cm^2 for 3 min (Fig. 9). At the same time, natural melanin showed more significant changes in this spectral region in response to illumination at a lower intensity (0.02 μW/cm^2) (Fig. 8).

It should be noted that the absorption spectra of the synthetic melanin presented in Figs. 9–11 were recorded at three different melanin concentrations (0.1, 0.02, and 0.01 mg/ml). The absorbance of the melanin solutions was almost proportional to the melanin concentration. Consequently, this melanin obeys the Bouguer–Lambert–Beer law, implying that melanin molecules are uniformly distributed in the solution. The surface tension of a 0.1% solution of synthetic melanin at 200°C (72 ± 1 J/m^2 from the results of 4 measurements) did not differ from that of distilled water (72 J/m^2). For comparison, the surface tension of the melanin isolated from strain F-2137 grown in liquid Czapek–Dox medium was found to be 69 ± 1 J/m^2.

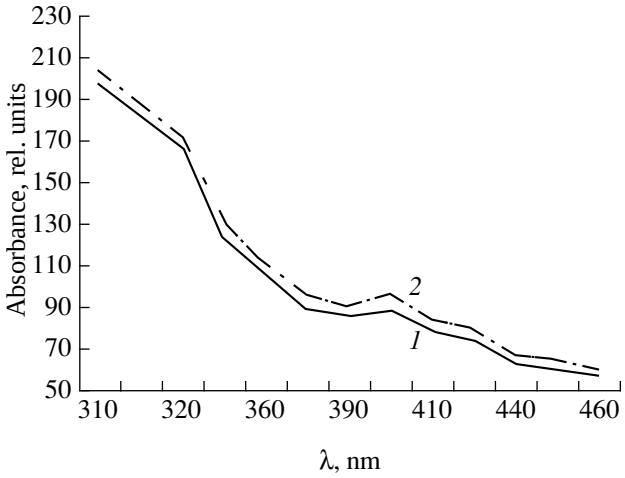

Fig. 9. The absorbance of an aqueous solution (0.015 mg/ml) of the commercial (ICN) synthetic melanin (*1*) before and (*2*) after illumination at an intensity of 0.06 mW/cm^2 for 3 min.

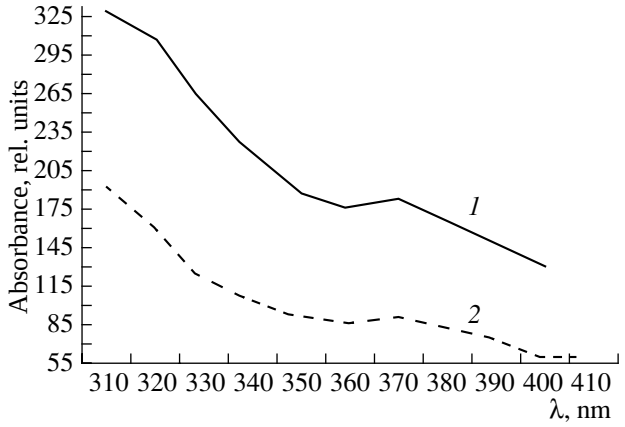

Fig. 11. The dark absorbance of aqueous solutions containing (*1*) 0.02 and (*2*) 0.01 mg/ml commercial synthetic melanin.

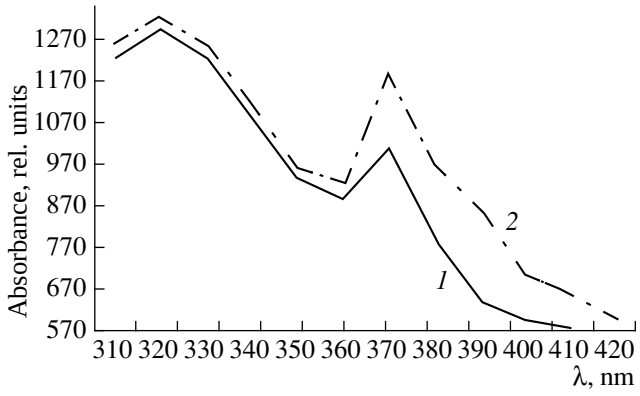

Fig. 10. The absorbance of an aqueous solution (0.1 mg/ml) of the commercial synthetic melanin (*1*) before and (*2*) after illumination at 0.06 mW/cm^2 for 3 min.

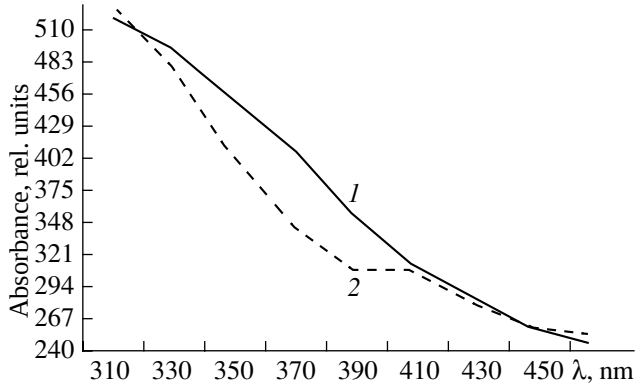

Fig. 12. The absorbance of an aqueous solution containing 0.1 mg/ml melanin isolated from the black fungus *A. pullulans* var. *pullulans* SPAPC U grown on 4% wort agar (*1*) before and (*2*) after illumination at an intensity of 0.02 mW/cm^2 for 3 min.

The melanin isolated from the *A. pullulans* var. *pullulans* SPAPC U cells grown on 4% wort agar showed the dependence of its absorbance on illumination (an indication of its photomodification) and a deviation from the Bouguer–Lambert–Beer law (Figs. 12 and 13). The deviation from this law indicates that the molecules of this melanin are distributed nonuniformly in solutions due, for instance, to the formation of a colloid or the adsorption of melanin molecules on the walls of the measuring cuvette. The aggregation of melanin molecules into micelles is unlikely, since, if large micelles had been formed, the melanin solution would have been turbid, which is not the case. As for small micelles with diameters less than 500 nm, their formation is hardly probable for the reason of the inappropriate molecular size. The surface tension of a 0.1% solution of the melanin from strain SPAPC U grown on 4% wort agar was 67 ± 1 J/m^2 at 20°C, i.e., 7% lower than the surface tension of distilled water.

The Molecular Mass of the A. pullulans var. pullulans SPAPC U Melanin Estimated from the Thickness of Its Langmuir Films

This melanin was chosen because of the high surface tension of its aqueous solutions. According to the Fisher theory [17], an aqueous solution of a high-molecular-weight surfactant can exist only if its molecules are linear and do not fold in a globule. The diameter of the polar head of the high-molecular-weight surfactant was estimated by Fisher to be 4 Å.

Our estimation of the molecular mass of eumelanins, which are synthesized from tyrosine, was based on the Langmuir theory [17, 18], according to which

the thickness of an adlayer, d, the ultimate adsorption G, density r, and the molecular mass M are related as

$$d = GM/r.$$

The ultimate adsorption G can be estimated from the diameter of the polar head of the surfactant molecule. When the packing of surfactant molecules on a surface is dense, a linear surfactant molecule with oligomeric tyrosine as the polar head will have a cross-sectional area of 3×10^{-15} cm^2. In this case, 1 cm^2 of the surface will contain 3×10^{14} molecules or 5×10^{-10} moles of the surfactant, which is in good agreement with experimental estimates.

The thickness of the adlayer was estimated from the interference of 62-GHz (about 5 mm long) waves reflected from the upper and lower surfaces of a melanin film, using the following formula:

$$d = fl/2\pi,$$

where f is the phase shift, and l is the wavelength. The thickness d was estimated to be 8×10^{-5} cm.

The molecular mass of the surfactant corresponding to such a thickness of the adlayer is about 160 kDa, to an accuracy of 50%. The molecular mass of fungal melanins synthesized from 1.8-dihydroxynaphthalene may be underestimated by this approach about 1.5-fold. We failed to estimate the thickness of the surface-active film of synthetic melanin and, hence, its molecular mass by this method but believe that it is lower than 25–30 kDa.

Of interest is to compare the approved optimal dosages of the known oxidants and the probable optimal dosage of melanin from strain SPAPC U estimated from its molecular mass. For instance, the optimal dosage of superoxide dismutase (molecular mass 70 kDa) is 0.5 mg/kg. Provided that the biological activity of melanins is due to their antioxidant properties, the optimal dosage of the SPAPC U melanin will be 0.001 mg/ml or 1 mg/kg. This estimate has been confirmed experimentally (data not presented). At a dosage of 5 mg/kg, the melanin is inefficient, while it is even toxic at a dosage of 10 mg/kg or higher.

Alternate double and single bonds in the melanin molecules are responsible for their low ionization potential [18], which explains not only the absorption of visible and ultraviolet light by melanins but also their photomodification by illumination at intensities higher than 0.1 mW/cm^2.

Photoinduced changes in the absorption spectra of melanins, the increase in the concentration of free radicals in response to the exposure of melanins to 0.9-mW/cm^2 visible light [14], and the inversion of the antioxidant effect of melanins to the prooxidant effect at high-intensity illumination [10] can account for variations in the biological activity of melanins observed under different illumination conditions. Under low-intensity illumination (from 0.1 to 1.0 mW/cm^2), the absorbance of melanins from strains SPAPC U and F-2137 in the long-wavelength ultraviolet and visible spectral regions increased, indicating the retention of a system of conjugated double bonds in the melanin molecules. At the same time, the absorbance of melanin from strain F-448 after illumination at 1 mW/cm^2 decreased. These data suggest that the biological activity of melanins is probably maximum under a low scattered illumination.

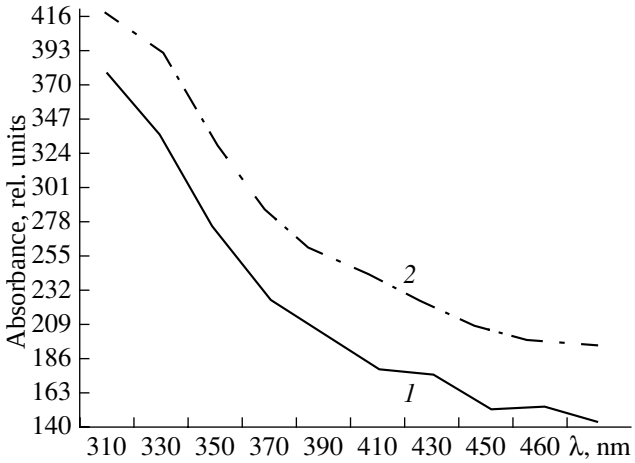

Fig. 13. The dark absorbance of aqueous solutions containing (*1*) 0.1 and (*2*) 0.02 mg/ml melanin isolated from the *A. pullulans* var. *pullulans* SPAPC U cells grown on 4% wort agar.

The closeness of the molar concentrations of melanins exerting the maximum biological effect to the optimal therapeutic concentrations of superoxide dismutase allows the suggestion to be made that the biological activity of melanins is largely due to their antioxidant properties. The dependence of the absorption spectra of melanins on the intensity of illumination implies that they are susceptible to light and, possibly, to oxygen. Consequently, melanin preparations should be stored in the dark under airless conditions, and their storage terms should be limited.

The data presented suggest that the melanins of black fungi possess lower ionization potentials, are more susceptible to photomodification, and are biologically active at lower molar concentrations than the commercial synthetic melanin purchased from ICN.

ACKNOWLEDGMENTS

We are grateful to O.A. Yashchurzhinskaya, St. Petersburg Technical University, for the measurements of the electron paramagnetic resonance spectra of melanins.

This work was supported by INTAS grant no. 97-726.

REFERENCES

1. Lyakh, S.P., *Mikrobnyi melaninogenez i ego funktsii* (Microbial Melaninogenesis and Its Functions), Moscow: Nauka, 1981.
2. Borshchevskaya, M.I. and Vasil'eva, S.M., Developing Knowledge of the Biochemistry and Pharmacology of Melanin Pigments, *Vopr. Med. Khim.*, 1999, vol. 45, no. 1, pp. 13–21.
3. Babitskaya, V.G., Shcherba, V.V., Filimonova, T.V., and Grigorchuk, E.A., Melanin Pigments in the Fungi *Paecilomyces variotii* and *Aspergillus carbonarius*, *Prikl. Biokhim. Mikrobiol.*, vol. 36, no. 2, pp. 153–159.
4. Butler, M.J. and Day, A.W., Fungal Melanins: A Review, *Can. J. Microbiol.*, 1998, vol. 44, pp. 1115–1136.
5. Wheeler, M.H., Comparisons of Fungal Melanin Biosynthesis in Ascomycetous, Imperfect, and Basidiomycetous Fungi, *Trans. Brit. Mycol. Soc.*, 1983, vol. 81, no. 1, pp. 29–36.
6. Harvey, B., A Cloned Tyrosinase Turns *Escherichia coli* Black, *Bio/Technology*, 1990, vol. 8, no. 7, pp. 103–107.
7. Genetics Engineering Comes to Sun Tan Lotion, *Biotechnol. News*, 1989, vol. 9, no. 13, p. 6.
8. Blarzino, C., Mosca, L., Foppoli, C., Coecia, R., Marco, C., and Rosei, M.A., Lipoxygenase, HO–catalyzed Oxidation of Dihydroxyindoles Synthesis of Melanin Pigments and Study of Their Antioxidant Properties, *Free Radical Biol. Med.*, 1999, vol. 26, no. 3/4, pp. 446–453.
9. Palumbo, A. and Jackson, I.J., Peroxidase Activity in the Ink Gland of *Sepia officinalis* and Partial Nucleotide Sequence of a Candidate cDNA Encoding the Enzyme, *Biochim. Biophys. Acta*, 1995, no. 21247, pp. 173–178.
10. Dontsov, A.E., Glickman, R.D., and Ostrovsky, M.A., Retinal Epithelium Pigment Granules Stimulate the Photo-Oxidation of Unsaturated Fatty Acids, *Free Radical Biol. Med.*, 1999, vol. 26, no. 11/12, pp. 1436–1446.
11. Shima, T., Sarna, T., Swarts, H.M., Stoppolo, A., Gerlasi, R., and Zecca, L., Binding of Iron to Neuromelanin of Human *Substantia Nigra* and Synthetic Melanin: An Electron Paramagnetic Resonance Spectroscopy Study, *Free Radical Biol. Med.*, 1997, vol. 23, no. 1, pp. 110–119.
12. Vladimirov, Yu.A., Azizova, O.A., Deev, A.I., Kozlov, A.V., Osipov, A.N., and Roshchupkin, D.I., *Svobodnye radikaly v zhivykh sistemakh* (Free Radicals in Living Systems), Moscow: VINITI, 1991.
13. Vladimirov, Yu.A. and Potapenko, A.Ya., *Fiziko-khimicheskie osnovy foto-biologicheskikh protsessov* (Physicochemical Foundation for Photobiological Processes), Moscow: Vysshaya Shkola, 1989.
14. Sarna, T. and Sealy, R.C., Free Radicals from Eumelanins: Quantum Yields and Wavelength Dependence, *Arch. Biochem. Biophys.*, 1984, no. 2232, pp. 574–578.
15. Yurlova, N.A., The Chemical Composition of *Aureobasidium* (*Pululllaria*) *pullulans* Arnaud (de Bary) 1910 Cells, *Cand. Sc. (Biol.) Dissertation*, Leningrad, 1977.
16. Malama, A.A. and Bulanov, P.A., The Effect of Melanin on the Ehrlich ascites tumor cells, *Dokl. Akad. Nauk BSSR*, 1965, vol. 9, no. 9, pp. 627–629.
17. Fridrikhsberg, D.A., *Kurs kolloidnoi khimii* (Textbook of Colloid Chemistry), Leningrad: Khimiya, 1974.
18. Etkins, P., *Molekuly* (Molecules), Moscow: Mir, 1991 (Russian translation).