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THE EFFECT OF MELANINS ON OXIDATION OF LECITHIN IN LIPOSOMAL MEMBRANES *

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Lecithin peroxidation in liposomal membranes induced by UV light was studied in the presence of natural eye melanin and synthetic melanins prepared from various precursors. It was shown that melanins inhibited lecithin photooxidation, and that the extent of this effect strongly depended on the type and concentration of melanin. Comparative study indicated that melanin obtained from adrenolutin was the most effective antioxidant. The ability to inhibit lipid peroxidation depends both on the concentration of paramagnetic centers in the melanin polymer and the accessibility of these centers for free radicals formed during irradiation of liposomes.

It is generally believed that melanins are heterogenous polymers derived from products of oxidative tyrosine metabolism such as 3.4-dihydroxyphenylalanine (DOPA), dopamine, noradrenaline, and adrenaline. Physicochemical properties of melanins and, in particular, the ability to transform electromagnetic radiation to heat, stable paramagnetism and redox capacity predestinate these biopolymers to the role of natural radio-and photoprotectors [1, 2, 3]. It was observed that the retinal pigment epithelium of pigmented animals is much more resistant to lipid photooxidation than that of albino animals. This effect depends on the presence of melanin in the pigmented tissue [4]. The inhibitory action of eye melanosomes and synthetic DOPA-melanin on lipid peroxidation was also demonstrated [5]. The purpose of this study was to compare the effect of different types of melanin on UV light induced lipid peroxidation in lipsomal membranes. The investigations were carried out on natural eye melanin and model polymers synthesized from various precursors.

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MATERIALS AND METHODS

Model melanins were obtained by oxidative polymerization of DOPA, adrenolutin (3,5,6-trihydroxy-1-methylindole) and dopamine in the absence or presence of copper ions according to the procedure described previously [6]. Natural melanin was prepared from melanosomes isolated from pigmented tissues of bovine eyes and purified by the use of differential centrifugation method, as reported previously [7]. After solubilization of melanosomal proteins with 2% sodium dodecylsulphate/5% mercaptoethanol, the melanin granules thus obtained were treated with chloroform-methanol mixture (2:1, v/v) to extract lipids and phospholipids, and additionally hydrolyzed with 6 M HCl to remove proteins. Multilamellar liposomes from commercial egg lecithin (Merck) were prepared by the method described by Bangham *et al.* [8]. Chloroform solution of lecithin (240 mg/ml) was evaporated under nitrogen. The obtained thin film of lecithin was shaken with 50 mM Tris/HCl buffer at pH 7.4 (control liposomes) or with melanin suspension in 50 mM Tris/HCl buffer. Lecithin peroxidation in liposome membranes was induced by UV light. The accumulation of peroxidation products was determined by the use of thiobarbituric acid test [9].

ESR measurements were performed at X band frequencies and 100 kHz field modulation using the ESR spectrometer type S"X"-2542 (Radiopan, Poland) with a TE_{102} rectangular reflection-type cavity. Spectra were recorded at liquid nitrogen temperature.

RESULTS AND DISCUSSION

The kinetics of lecithin peroxidation in liposomal membranes in the presence and absence of DOPA-melanin is shown in Fig. 1. For control liposomes a linear relationship between the accumulation of lipid oxidation products (among which malonic dialdehyde is dominant) and illumination time was observed (curve 1). The addition of melanin at the stage of liposome formation inhibited the photooxidation of lecithin. It was shown that this inhibiting effect strongly depended on the kind of melanin, and was enhanced by increased melanin concentration (Fig. 2). Among the analyzed polymers, melanin prepared from adrenolutin was the most effective antioxidant. At low concentration of melanin in the reaction medium (50 μg/ml), adrenolutin-melanin inhibited lecithin peroxidation by about 40%, whereas melanins prepared from dopamine did not show any significant effect. Natural bovine eye melanin, at low concentration, inhibited lipid peroxidation similarly as observed for synthetic DOPA-melanin. Under the experimental conditions used the concentration of natural melanin higher than 50 μg/ml did not affect the rate of lecithin photooxidation, and the extent of the inhibition.

Accumulation of free-radical products of lecithin peroxidation in the absence and presence of melanins was monitored by the use of ESR

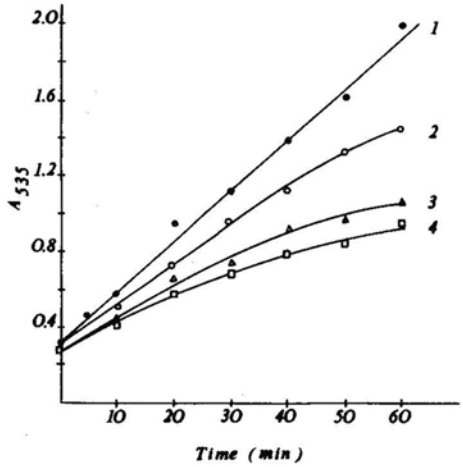

Fig. 1. UV light induced oxidation of lecithin in liposomal membranes in the absence (1) and presence of DOPA-melanin at concentration of 100 μg/ml (2), 200 μg/ml (3) and 400 μg/ml (4). Lecithin concentration, 14.4 mg/ml. Accumulation of lecithin peroxidation products was determined using thiobarbituric acid test and expressed as the increase of absorbance at 535 nm

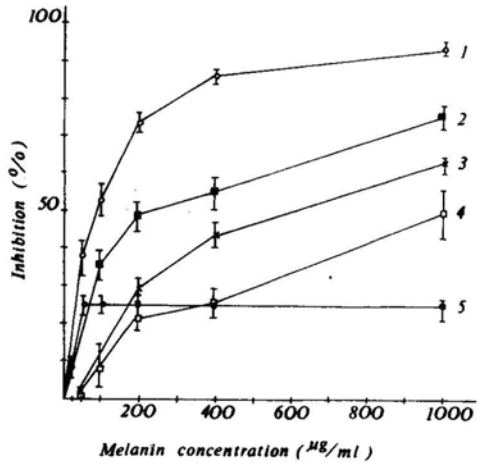

Fig. 2. The inhibitory effect of synthetic melanins obtained from adrenolutin (1), DOPA (2), dopamine/Cu^{2+} mixture (3), dopamine (4) and natural melanin isolated from bovine eyes (5) on lecithin peroxidation in liposomal membranes. Lecithin oxidation was induced by irradiation with UV-lamp for 30 min; concentration of lecithin – 14.4 mg/ml; buffer Tris/HCl (50 mM, pH 7.4)

spectroscopy. It was shown that a broad ESR signal from radical species formed during irradiation of lecithin was suppressed in the presence of all melanins examined. The highest suppression was observed for melanin obtained from adrenolutin. DOPA-melanin and natural eye melanin were less effective.

It was found that before irradiation the radical concentration of melanin polymers was in the range from $2 \cdot 10^{18}$ to about $7 \cdot 10^{18}$ spin/g, and decreased in the order: DOPA-melanin $\approx$ eye melanin > adrenolutin-melanin > dopamine--melanin obtained in the presence of copper ions > dopamine-melanin prepared without copper ions. UV light caused the increase of free radical concentration in all analyzed melanins. The level of photoinduced radicals depended strongly on the type of precursor used for melanin synthesis. The highest rise in the ESR signal intensity during irradiation was observed for melanin prepared from dopamine in the presence or absence of copper ions (about 17 and 12-fold, respectively), the lowest for DOPA-melanin (about 5-fold).

The presented results clearly indicate that the ability to inhibit lipid peroxidation depends on both the concentration of paramagnetic centers in the melanin polymer and the accessibility of these centers to free radicals formed during irradiation of liposomes.

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