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PHOTOGENERATION OF SUPEROXIDE ANION BY IRIS OF THE HUMAN EYE UNDER *IN VITRO* CONDITIONS

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Iris of the human eye controls the amount of light transmitted to the retina. This melanin-containing tissue can be exposed to significant fluxes of ultraviolet and visible light transmitted by the cornea. Although melanin is usually considered to be an important photoprotective pigment, it can also photogenerate reactive oxygen species. To evaluate the importance of such photoreactions that under certain conditions may occur *in situ*, we measured *in vitro* the efficiency of oxygen photoconsumption and photogeneration of superoxide anion (SOA) by iridial melanin. Irradiation of human and bovine iridial homogenates with UV-VIS light led to consumption of oxygen, which was accompanied by generation of SOA. When normalized to equal content of protein and melanin, all human iridial samples, regardless the age of the donors and color of their eyes, photogenerated similar amounts of SOA. Our data suggest that melanin may be a key chromophore responsible for the photoformation of SOA and hydrogen peroxide (HP) in the aqueous humor of the human eye. Ascorbate, typically present at high concentration in the anterior chamber of the human eye, considerably accelerated melanin-mediated photoconsumption of oxygen and the corresponding generation of HP. The latter phenomenon has been explained by photoinduced electron-transfer properties of the iridial melanin.

INTRODUCTION

Human iris is the most exposed part of the uvea, placed between the cornea and lens of the eye. It merges into the ciliary body with its peripherial margin. Iris is in contact, on its both sides, with the aqueous humor, which fills entirely the anterior chamber of the eye (Bochenek & Reicher, 1989; Niżankowska, 1992).

All human individuals, except albinos, have their irides distinctly pigmented. The iris color is determined mainly by the iridial melanin, which is distributed rather inhomogeneously in this ocular tissue. Most of the melanin pigment is present in the iridial pigment epithelium placed on the posterior side of the iris. Pigment-containing melanocytes are also present in the middle layer of the iris called stroma (Bochenek & Reicher, 1989). It is believed that the type of melanin and its aggregation state are responsible for characteristic appearance of the iris pigmentation (Eagle, 1988; Wilkerson, Syed, Fisher, Robinson, Wallow & Albert, 1996; Imesch, Bindley, Khademian, Ladd, Gangnon, Albert & Wallow, 1996). Optical properties of the extracellular matrix components may also play a role in the color appearance of the human iris (Eagle, 1994).

Controlling the amount of light that reaches the retina, iris is exposed to significant fluxes of ultraviolet and visible light transmitted by the cornea. Melanin, the main iridial pigment, absorbs UV radiation and blue light more efficiently than visible light of longer wavelengths. Due to rich vascularization of the uvea (Bochenek & Reicher, 1989; Niżankowska, 1992), iridial melanin may undergo oxygen-dependent photodamage accompanied by the release of reactive oxygen species (ROS) and low-molecular products of melanin degradation. Thus, it can not be ruled out that under certain conditions, melanin can actually contribute to oxidative stress of the pigment tissue.

There are several eye diseases whose incidence may be related to the iridial pigmentation. Thus, individuals with lightly pigmented eyes seem to have a higher incidence of senile macular degeneration (Niżankowska, 1992), called age-related maculopathy (Weiter, Delori, Wing & Fitch, 1985; Weiter, Delori, Wing & Fitch, 1986; Cruickshanks, Klein & Klein, 1993). Such individuals also show a tendency of faster bleaching of their irides, particularly noticeable at older age. In addition, iridial melanoma is found in blue irides more frequently than in darker ones. Moreover, the tumor is more often located in the lower half of the human iris, which is exposed to higher fluxes of the ambient radiation (Żygulska-Mach, 1998). In several studies the dependence of the prevalence of photopic visual acuity (Short, 1975), senile cataract (Zigler & Goosey, 1981; Spector, 1984; Taylor, West, Munoz, Newland, Abbey & Emmett, 1988; Andley & Clark, 1989;

Table 1. Human irides according to age of the donors and color of their eyes

	Color (number of samples)			
	Blue (44)	Intermediate (24)	Medium (48)	Brown (26)
	Age [years] (number of samples)			
Age range symbol	I	II	III	IV
Blue	17-34 (12)	40-54 (38)	56-75 (60)	76-98 (74)
Brown	8-30 (20)	37-54 (22)	57-75 (30)	76-98 (28)

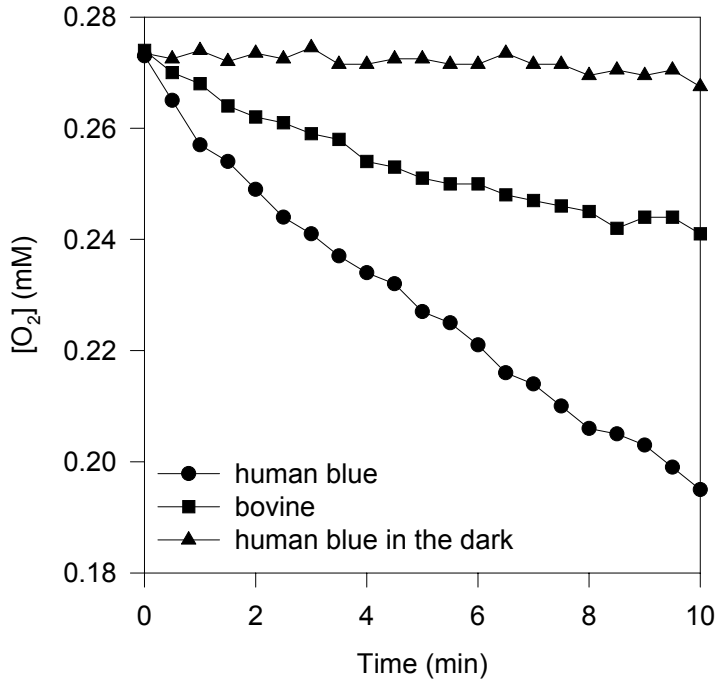

Fig.1. Oxygen photoconsumption in iridial homogenates (melanin concentration: 0.15 mg/ml)

ley & Clark, 1989; Niżankowska, 1992) or glaucoma (Scheie & Cameron, 1981), intraocular malignant melanoma (Tucker, Shields, Hartge, Augsburger, Hoover & Fraumeni, 1985; Gallagher, Elwood, Rootman, Spinelli, Hill, Threlfall & Birdsell, 1985; Regan, Judge, Gragoudas & Egan, 1999) on color of the iris, has been analyzed. However, the exact role of melanin in the etiology of the diseases remains unclear. It has been speculated that melanin may act as a two-edged sword being both a potential antioxidant and pro-oxidant (Hill, Li, Xin & Mitchell, 1997). While the photoprotective effect of iridial melanin has been documented in some studies, its pro-oxidant abilities still remain rather controversial.

In spite of considerable efforts to determine the composition and contents of the iridial melanin (Menon, Persad, Haberman, Kurian & Basu, 1982; Menon, Basu, Persad, Avaria, Felix & Kalyanaraman, 1987; Hu, Ritch, McCormick & Pelton-Henrion, 1992; Menon, Wakeham, Persad, Avaria, Trope & Basu, 1992; Hu, McCormick, Orlow, Rosemblat, Lin & Wo, 1995; Imesch *et al.*, 1996; Wilkerson *et al.*, 1996; Prota, Hu, Vincensi, McCormick & Napolitano, 1998; Novellino, Napolitano & Prota, 2000), very little is known about the structure – function relationship of the uveal

Tab.2. Initial rates of oxygen photoconsumption (in μM/min) in iridial homogenates (melanin concentration: 0.15 mg /ml)

Color			
Blue	Intermediate	Medium	Brown
9.7±3.4	7.4±4.2	12.5±1.5	8.6±1.8

Age				
Age range symbol	I	II	III	IV
Blue	9.1±1.7	9.0±6.1	9.2±3.0	6.0±2.4
Brown	10.3±1.3	6.7±2.2	7.4±1.8	10.9±2.2

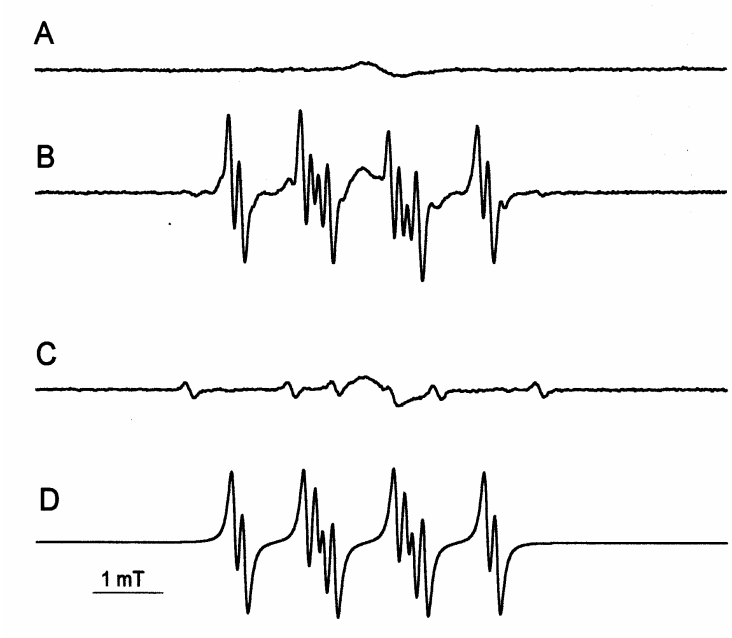

Fig.2. EPR spectra of samples containing human iridial homogenates and DMPO in 80% DMSO; A: in the dark before irradiation; B: after 18 min. irradiation of aerated suspension; C: after 18 min. irradiation of deaerated suspension; D: simulated EPR spectrum of DMPO-OOH spin adduct (a_N=13.1, a_H=10.5, a_H=1.5)

melanin. In particularly, it remains to be determined what are the differences in the structure and amount of iridial melanin in human eyes of different color. Earlier studies of human iridial melanin did not reveal any significant differences in its photobiological properties regardless the iris color (Menon *et al.*, 1982; Menon *et al.*, 1987). Indeed, using a conventional assay for superoxide anion (SOA), those authors could not detect any formation of SOA by the irradiated iridial melanin, which photogenerated only small quantities of hydrogen peroxide (Menon *et al.*, 1987).

In this study, we reexamined the ability of irradiated human iridial melanin to produce superoxide anion using the EPR spin trapping method. In addition, we analyzed aerobic photoreactivity of iridial melanin obtained from donors with different eye color and different age.

MATERIALS AND METHODS

Chemicals

All chemicals, at least reagent-grade, were used as obtained, unless otherwise stated. KCl, $Na_2HPO_4 \cdot 12H_2O$, KH_2PO_4, NaCl, Na_2CO_3, NaOH, Na-K tartrate were purchased from Polskie Odczynniki Chemiczne (Gliwice, Poland) and $CuSO_4 \cdot 5H_2O$ from PC Odczynniki (Lublin, Poland). Melanin from *Sepia officinalis*, 5,5-dimethyl pyrroline-N-oxide (DMPO), dimethyl sulphoxide

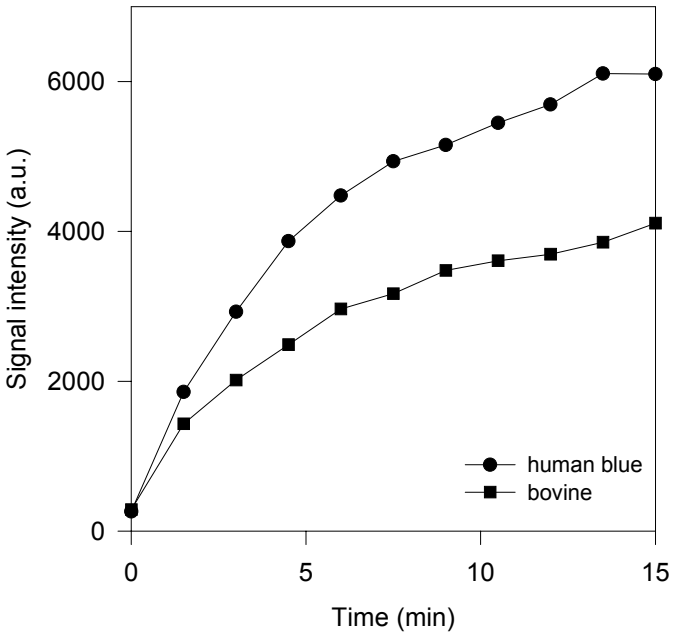

Fig.3. Superoxide radicals photogeneration by iridial homogenates (melanin concentration: 0.075 mg/ml)

Table 3. Initial rate of photogeneration of DMPO-OOH (in a.u./min) by iridial homogenates (melanin concentration: 0.075 mg/ml)

Color			
Blue	Intermediate	Medium	Brown
775±55	706±78	866±57	712±89

	Age			
Color	I	II	III	IV
Blue	753±78	916±105	1148±84	557±74
Brown	587±113	612±66	693±76	623±57

(DMSO), bovine albumin (Fraction V), catalase from bovine liver (Thymol-free), superoxide dismutase (SOD) from bovine erythrocytes (lyophilized powder), glutathione (GSH), α-tocopherol, NADH and Chelex-100 were obtained from Sigma (Poznań, Poland). Folin-Ciocalteu's phenol reagent, benzene for liquid chromatography, activated charcoal and ascorbate ($AscH^-$) were purchased from Merck (Warsaw, Poland). 4-protio-3-carbamoyl-2,2,5,5,-tetraperdeuteromethyl-3-pyrroline-1-yloxy (mHCTPO) was a gift from Prof. H.J. Halpern.

DMPO was purified to minimize the content of paramagnetic impurities prior to use as described elswhere (Barr, 2000).

Phosphate-buffered saline (PBS) (KCl 0.20g/l, KH_2PO_4 0.26g/l, $Na_2HPO_4 \cdot 12H_2O$ 2.9g/l, NaCl 8.1 g/l) was treated with Chelex-100 prior to use, and only double-distilled water from a glass apparatus was used. All glassware was rinsed with 1 N HCl and several times with double-distilled water to remove metal ions.

Irides

Human eyes were obtained from Wisconsin Lion Eye Bank (Milwaukee, Wisconsin, USA). Before extraction from eyes of white donors and freezing, the irides were divided into four selected groups, based on visual inspection of the color of the donors eyes. In addition, the specimens were grouped according to the donors age. The irides were then transported to Poland on dry ice and stored at –70°C until homogenization and measurements.

Bovine irides, used as a control, were obtained from a local slaughter house in Krakow.

426 human irides were divided into four color groups (Tab. 1): blue, intermediate (blue-green, light green), medium (dark green, green-brown,

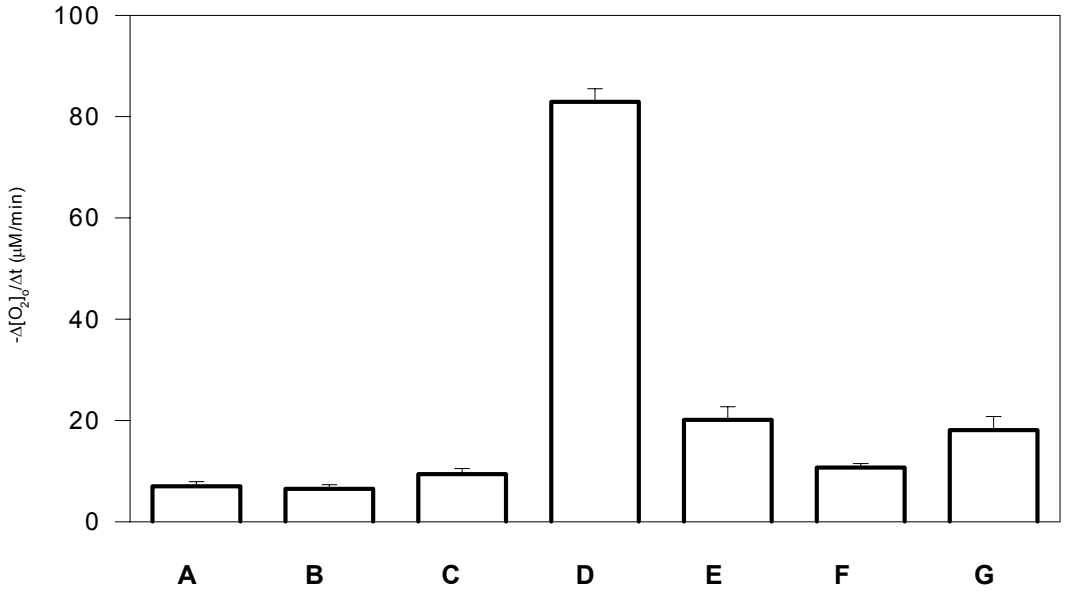

Fig.4. Initial rates of oxygen photoconsumption by blue iridial homogenate (melanin concentration: 0.15 mg/ml) in the presence of exogenous enzymes or reductants; A: control, B: catalase (0.15 mg/ml), C: SOD (0.1 mg/ml), D: $AscH^-$ (0.2 mM), E: GSH (0.2 mM), F: NADH (0.2 mM), G: α-tocopherol (0.2 mM)

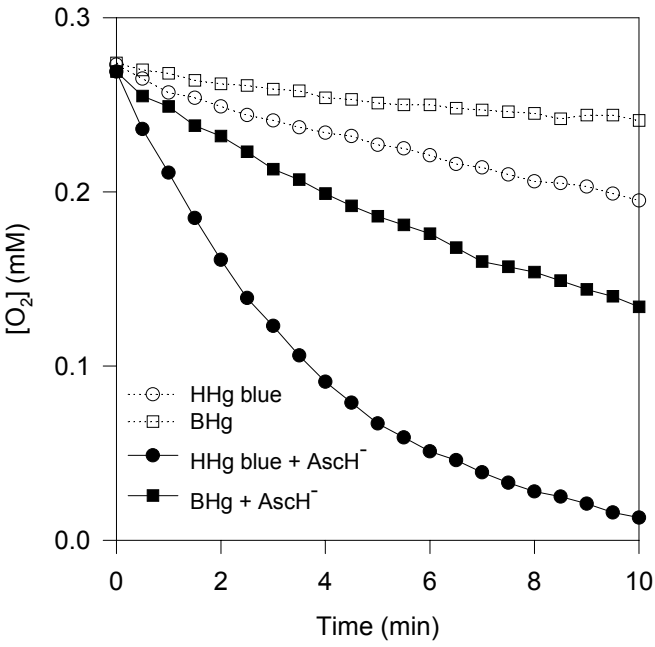

Fig.5. Oxygen photoconsumption in iridial homogenates (melanin concentration: 0.15 mg/ml) in the absence or presence of ascorbate (0.1 mM)

hazel) and brown. An average age of the donors of irides in these groups was: 68.5 ± 4.9, 51.6 ± 22.3, 63.3 ± 17.0, 72.5 ± 10.5 respectively. For the two extreme colors of the irides we distinguished four age groups (Tab. 1).

Because of limited availability of the human material and low yield of the melanosome isolation, we used iridial homogenates in our experiments. Human and bovine irides were homogenized mechanically at 0°C in a glass homogenizer and then suspended in PBS.

Protein content in samples was determined by the Lowry assay (Lowry, Rosenbrough, Farr & Randall, 1951), using albumin as a standard.

EPR spectroscopy

Electron paramagnetic resonance spectroscopy measurements were carried out using a Bruker

Table 4. Initial rate of ascorbate (c_o=0.45 mM) photooxidation (in μM/min) by iridial homogenates (melanin concentration: 0.15 mg/ml)

Color (number of samples)			
Blue	Intermediate	Medium	Brown
39.3 ± 4.2	38.0 ± 5.8	35.9 ± 4.6	19.4 ± 7.2

	Age			
Color	I	II	III	IV
Blue	46.9 ± 15.6	46.0 ± 3.2	51.2 ± 2.9	30.7 ± 13.4
Brown	23.6 ± 3.0	23.5 ± 3.5	35.7 ± 7.5	35.1 ± 5.4

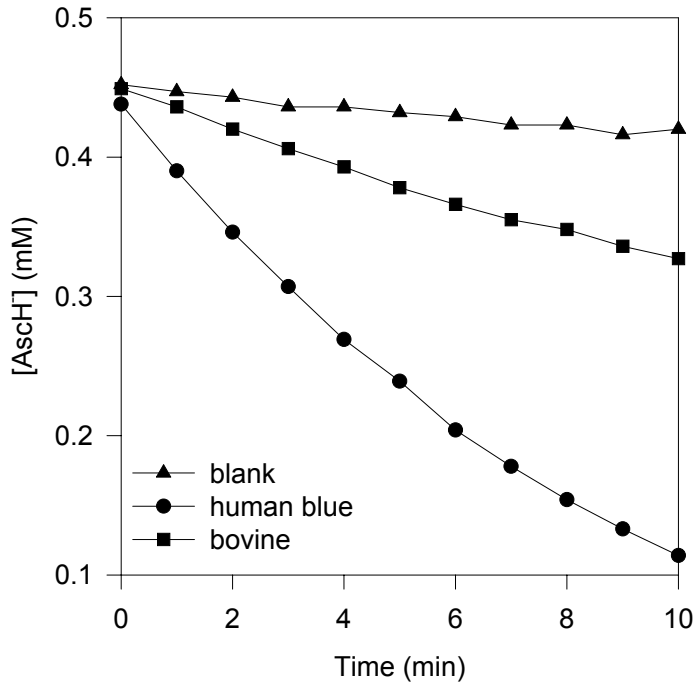

Fig.6. Photooxidation of ascorbate (c_o=0.45 mM) by iridial homogenates (melanin concentration: 0.15 mg/ml)

ESP 300 E spectrometer operating at X-band and equipped with 100 kHz field modulation.

Melanin content in samples was determined by the EPR assay (Pilas & Sarna, 1985; Enochs, Nilges & Swartz, 1993). EPR signals of the melanin radicals were run at 77 K. For quantitative determination of melanin in the samples, their signal intensities (after double integration) were compared with that of known amount of *Sepia* melanin used as a standard.

To determine photochemical properties of melanin, EPR measurements were carried out at room temperature using quartz flat cell and *in situ* irradiation of the samples (Różanowska, Ciszewska, Korytowki & Sarna, 1995).

Sample irradiation

Samples were irradiated with polychromatic light 300-660 nm using a high pressure compact-arc xenon lamp (Photomax 150 W, Oriel) equipped with condensing quartz lenses and $CuSO_4$ filter (35 g/l) in water.

For monochromatic irradiation of EPR samples, a high pressure compact-arc xenon 450 W lamp was used equipped with a condensing quartz lens and narrow-band interference filters.

Radiation intensity was regulated with a light reducing metal reticule and was routinely measured with a calibrated silicon photodiode (Hamamatsu, Photonics, K.K., Japan).

Oxygen photoconsumption

Oxygen consumption, induced by irradiation of the samples, was determined by EPR oximetry (Hyde & Subczyński, 1989) using 0.1 mM mHCTPO as a nitroxide probe (Halpern, Peric, Nguyen, Spencer, Tercher, Lin & Bowman, 1990; Różanowska, Bober, Burke & Sarna, 1997).

Spin trapping

Photogeneration of superoxide anion by iridial samples in 80% DMSO was monitored by spin trapping using DMPO (0.1M) as a spin trap (Kalyanaraman, 1982; Roberts, Wishart, Martinez and Chignell, 2000). Typical instrumental settings for recording EPR signals of the generated spin adducts were as follows: microwave power 10 mW, modulation amplitude 0.47 G, scan time 84 s, receiver gain 1×10^5, scan range 10 G and time constant 82 ms (to record selected parts of the spectrum when monitoring time-resolved changes) or scan range 100 G and time constant 164 ms (to record complete DMPO-OOH spectrum for its identification).

Ascorbate photooxidation

Photoxidation of ascorbate induced by irradiation of iridial homogenates was monitored spectrophotometrically at 265 nm (Stahl, Liebes, Farber & Silber, 1983) using HP 8453 diode array spectrophotometer.

Kinetics of oxygen photoconsumption and ascorbate photooxidation were determined on the same samples using the approach described previously (Różanowska *et al.*, 1997).

RESULTS

Oxygen photoconsumption

Oxygen concentration in samples of bovine or human iridial homogenates decreased very slowly in the dark. Irradiation of the samples with UV-VIS light led to a relatively fast consumption of oxygen. Normalized to equal amount of melanin (or protein) and equal fluxes of light, human specimen showed higher rates of oxygen photoconsumption than bovine samples (Fig. 1).

We also measured oxygen depletion induced by irradiation of homogenates of irides of different color and age of human donors (Tab. 2). The highest initial rate of oxygen photoconsumption was detected in homogenates of medium-colored irides while the lowest in samples of intermediate-colored irides. However, the differences in the rates of oxygen photoconsumption of the samples studied were not statistically significant. No clear trend in the oxygen photoconsumption rate with either age of the donors or colors of their irides was therefore apparent.

Superoxide anion photogeneration

The consumption of oxygen, induced by irradiation of iridial homogenates, was accompanied by the formation of superoxide anion (Fig. 2). In the dark, the only EPR signal detected in aerated iridial homogenates containing high concentration of DMPO (in 80% DMSO), was that originating from the melanin paramagnetic centers (Fig. 2A). Irradiation of the sample with light resulted in enhancement of the melanin signal intensity and appearance of a new EPR signal that had all features consistent with the spectrum of DMPO-OOH (Fig. 2B, D). Significantly, the latter signal was not detectable under anaerobic conditions even during irradiation of the sample (Fig. 2C).

When normalized to equal content of melanin (or protein) and similar number of incident photons, human irides appeared to photogenerate SOA more efficiently than bovine irides (Fig. 3).

Consistent with oxygen photoconsumption measurements, the initial rates of SOA photoformation by human iridial homogenates were similar regardless color of the eye or age of the donors in blue or brown color groups (Tab. 3).

The effect of exogenous enzymes and reductants on oxygen photoconsumption

We then examined the influence of exogenous antioxidant enzymes such as catalase, SOD and reductants such as $AscH^-$, GSH, NADH and α-tocopherol on oxygen photoconsumption by human iridial homogenates (Fig. 4).

The data indicate that the rate of oxygen uptake during irradiation of the blue iridial homogenate was not modified by the presence of the enzymes. Of all electron donors tested, only ascorbate significantly accelerated oxygen photoconsumption measured in iridial samples. The ascorbate effect was similar in all samples tested (Fig. 5).

For comparison, we also measured rates of aerobic photodepletion of ascorbate, mediated by human and bovine iridial homogenates. As seen in Fig. 6, irradiation of the samples led to photooxidation of the reductant, which was faster for the human specimen.

The rate of ascorbate photodepletion in human homogenates did not exhibit any measurable dependence on irides color. Within experimental error, the rate was comparable for all tested samples except brown iridial samples, in which photooxidation of the antioxidant was slower than in other samples (Tab. 4). Among samples with

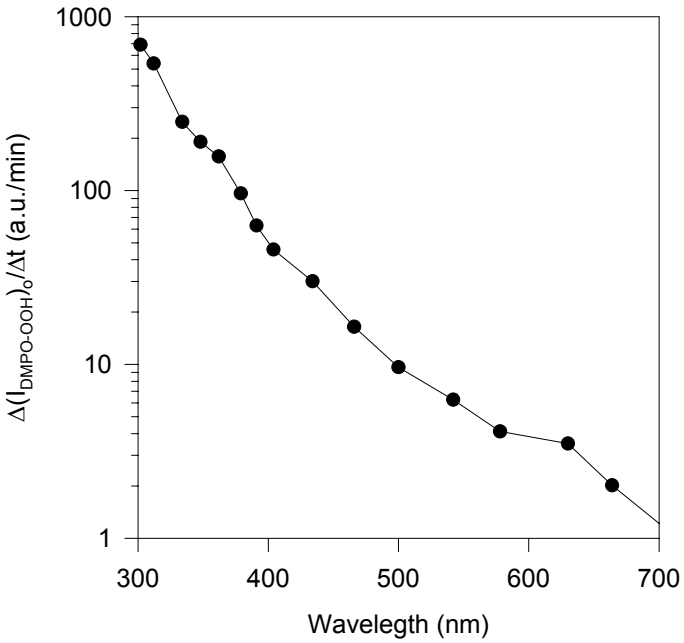

Fig.7. Wavelength dependence of photogeneration of DMPO-OOH by human iridial homogenate (melanin concentration: 0.075 mg/ml)

blue irides, the oldest specimen showed a slightly reduced tendency to photooxidize ascorbate compared to the younger ones.

Wavelength dependence

The aerobic photoprocesses, described above, were also studied as a function of the radiation wavelength in the biologically relevant spectral range 300-700 nm. As seen in Fig. 7, the efficiency of the superoxide anion photogeneration decreased monotonically at longer wavelengths.

Similar dependence on the irradiation wavelength was observed for oxygen photoconsumption. Addition of ascorbate to the iridial samples resulted in a proportional increase in the rate of oxygen photoconsumption at all wavelengths tested (Fig. 8).

DISCUSSION AND CONCLUSIONS

Among pigmented tissues of the human eye, its iris is subjected to highest fluxes of solar radiation. In normal eyes, iris absorbs significant amounts of the incident light and, hence, protects the inner parts of the eye from damage that could be induced by excessive fluxes of light. However, light absorption by the iris may lead to an *in situ* photoformation of superoxide anion. We have shown that aerobic irradiation of human and bovine iridial homogenates with UV-VIS is accompanied by a consumption of oxygen and formation of SOA.

Our data clearly indicate that melanin is the key iridial pigment responsible for aerobic photoreactions of this eye tissue. Significantly, wavelength dependence of oxygen photoconsumption and superoxide anion photogeneration, detected in iridial samples, show striking similarity with the action spectra of photoformation of melanin free radicals and oxygen photoconsumption reported previously for both synthetic and natural melanins (Sarna & Sealy, 1984; Sarna & Swartz, 1998; Nofsinger, Forest & Simon, 1999).

By analogy with the photoprocesses described for the synthetic and natural melanin, interaction of the photoexcited iridial melanin with oxygen can be summarized as follows:

$$\mathrm{Mel} + h\nu \rightarrow \mathrm{Mel}^*$$
$$\mathrm{Mel}^* + O_2 \rightarrow \mathrm{Mel}_{ox} + O_2^{\bullet -} \rightarrow \mathrm{Mel}_{ox} + H_2O_2$$

Where Mel, Mel* and Mel_{ox} stand for melanin in its ground state, melanin in its electronically excited state and oxidized melanin, respectively.

Although in an earlier study Menon *et al*. (1987), using the NBT assay for SOA detection, could not observe any formation of superoxide anion following irradiation of iridial melanin, in this study, using the EPR spin trapping and DMPO as a spin trap, we clearly observe photoformation of a spin adduct with EPR parameters consistent with those of DMPO-OOH, confirming the ability of iridial melanin to photogenerate SOA. Dismutation of SOA molecules, or their interaction with melanin, lead to the formation of hydrogen peroxide (H_2O_2). Our experiments with bovine homogenate and melanosomes (data not shown) indicated that irradiated iridial melanin produced H_2O_2. Significantly, we observed hydrogen peroxide

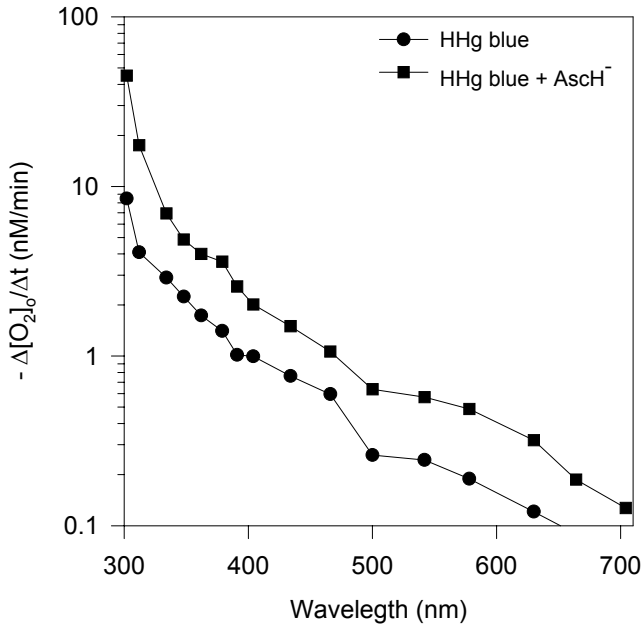

Fig.8. Wavelength dependence of oxygen photoconsumption in human iridial homogenate (melanin concentration: 0.15 mg/ml) in the absence or presence of ascorbate (0.45 mM)

formation even *in situ*, during irradiation of bovine iris pretreated with azide.

Therefore, it is likely that iris-dependent photoreactions may in part be responsible for the formation of H_2O_2, which is present in the aqueous humor of both animal and human eyes (Spector & Garner, 1981; Spector, Ma & Wang, 1998). Surprisingly, accumulation of H_2O_2 can reach in normal human eyes a steady-state level of $24 \pm 7\mu M$ (Spector *et al*., 1998).

Due to its ion-exchange properties, iridial melanin can sequester variety of metal ions, including the potentially damaging redox-active iron and copper ions (Bruenger, Stover & Atherton, 1967; Pilas, Sarna, Kalyanaraman & Swartz, 1988; Korytowski & Sarna, 1990a, 1990b; Sarna, 1992; Swartz, Sarna & Zecca, 1992; Enochs, Sarna, Zecca, Riley & Swartz, 1994; Sarna & Różanowska, 1994; Sarna & Swartz, 1998). Because in humans, iridial melanin is mostly formed in the early childhood (Eagle, 1988; Sarna, 1992; Imesch, Wallow & Albert, 1997), it can not be ruled out that the melanin may undergo chemical degradation with age, particularly when chronically exposed to strong light. It can farther be postulated that such age-related changes in iridial pigmentation would modify melanin's ability to sequester iron and copper ions, which can generate hydroxyl radicals via so-called Fenton reaction (Pilas *et al*., 1988; Zaręba, Bober, Korytowski, Zecca & Sarna, 1995; Sarna & Swartz, 1998).

Under the conditions studied, antioxidant enzymes such as catalase and superoxide dismutase (SOD) did not influence significantly the rate of oxygen photoconsumption by human iridial homogenates. On the other hand, ascorbate, a common antioxidant, efficiently accelerated oxygen photoconsumption mediated by iridial melanin. It appears that in the presence of an appropriate electron donor, such as ascorbate, photoexcited iridial melanin induces an efficient electron-transfer between ascorbate and oxygen molecules, generating superoxide anion, according to the reaction scheme described earlier for synthetic melanin and retinal pigment epithelium melanin (Różanowska *et al.*, 1997):

$$\text{Mel} + h\nu \rightarrow \text{Mel}^*$$
$$\text{Mel}^* + \text{AscH}^- \rightarrow \text{Mel}_{red} + \text{Asc}^{\bullet -}$$
$$\text{Mel}_{red} + O_2 \rightarrow \text{Mel} + O_2^{\bullet -}$$

Where AscH^- and $\text{Asc}^{\bullet -}$ are fully reduced and semi-oxidized ascorbate molecules, respectively. It is possible that such a melanin-mediated photooxidation of ascorbate may also take place in the human iris, which is constantly in contact with the aqueous humor, where ascorbate is present at as high concentration as 1 mM (Spector *et al.*, 1998; Ringvold, Anderssen & Kjønniksen, 1999).

It remains to determined, whether such a photogeneration of H_2O_2, if it occurs to any significant extent *in vivo*, has any physiological significance. Except some differences observed, when bovine iridial samples were compared with human iridial samples and when the human samples from the oldest donors were compared with these of young human donors, no systematic trend in the aerobic photoreactivity of iridial homogenates from human donors of different eye colors and age was observed in this study. Although the differences in

the aerobic photoreactivity of human and bovine irides remain unexplained, different degree of melanin aggregation and accessability of the melanin active sites to oxygen in both samples may play a role in the observed phenomena.

In many model systems, melanin has been shown to act as an efficient antioxidant and photoprotective pigment (Sławińska, Sławiński & Cieśla, 1983; Ostrovsky, Sakina & Dontsov, 1987; Scalia, Geremia, Corsaro, Santoro, Baratta & Sichel, 1990; Porębska-Budny, Sakina, Stępień, Dontsov & Wilczok, 1992; Sarna, 1992; Sarna & Swartz, 1998). Therefore, it seems sensible to postulate such a photoprotective function for the iridial melanin, which is present in an environment exhibiting rather high risk of oxidative stress. However, chronic photooxidation of iridial melanin, caused by its prolonged *in situ* irradiation may lower its antioxidant efficiency and even stimulate its pro-oxidant ability. It is tempting to speculate that any significant reduction in the antioxidant efficiency of the iridial melanin together with an enhanced *in situ* photogeneration of superoxide anion and hydrogen peroxide, may contribute to oxidative stress of the anterior segments of the eye and, ultimately, to cataractogenesis.

Acknowledgement

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