ENZYMIC AND NONENZYMIC SYSTEMS PROTECTING PHOTORECEPTOR MEMBRANES AGAINST ACTIVE FORMS OF OXYGEN AND LIPID PEROXIDES

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Activity of the enzyme systems protecting membrane structures of photoreceptor cells (the outer and inner segments of the retinal rods) against active forms of oxygen (superoxide dismutase) and lipid peroxide (glutathione peroxidase, glutathione reductase) was shown to be low. At the same time a high concentration of α-tocopherol (vitamin E) was demonstrated in these membranes. It is concluded that the polyene lipids of the retinal photoreceptors are protected against the harmful action of lipid peroxidation by the vitamin E system.

KEY WORDS: superoxide dismutase; glutathione peroxidase; glutathione reductase; α-tocopherol; membranes of photoreceptors.

Modification of the polyene lipids of biomembranes by active forms of oxygen (O_2^-, $^1O_2$, $^\cdot OH$) is accompanied by accumulation of peroxidation products and gives rise to marked disturbances of the structural and functional organization of biomembranes. Generators of active forms of oxygen and lipid peroxides in the cell are under the control of enzymic and nonenzymic regulatory systems, the most important of which are considered at the present time to be superoxide dismutase (SOD), glutathione peroxidase (GP), and vitamin E [2].

Membranes of retinal photoreceptors have an extremely high content of polyunsaturated phospholipids [3] and for that reason they readily undergo autooxidation by molecular oxygen both in vitro (during "aging," and the action of visible light [4], in the presence of catalytic systems inducing peroxidation of lipids (POL [3, 4]), and in vivo (during the development of alimentary avitaminosis E [5], and during the action of high-intensity light [6]). The study of systems regulating POL in photoreceptor cells is thus extremely important.

This paper gives the results of an investigation of the distribution of activity of SOD, GP (and also glutathione reductase – GP), and the content of α-tocopherol in membranous structures of the rods of the bovine and frog retina: fractions of outer segments (OS) and inner segments (IS).

EXPERIMENTAL METHODS

The OS and IS fractions were isolated from the frog (Rana temporaria) and bovine retinas by centrifugation in a sucrose density gradient [7, 8]. SOD activity was determined in retinal homogenates, photoreceptor membranes, or extracts of membranes in aqueous ethanol – after preliminary treatment of the membrane suspension with a mixture of chloroform and ethanol (3:5) by the method described in [9] – using the xanthine oxidase–xanthine system to generate the superoxide anion radical (O_2^-). Triton X-100 was added to the cuvette (to a concentration of 1%) during measurement to produce solubilization of the membrane material. The rate of formazan formation was recorded on the Shimadzu MPS 50L spectrophotometer.

GP and GR activity was determined by the method described in [10], using tert-butyl hydroperoxide as the substrate for the peroxidase reaction.

The tocopherol concentration in ethanol–hexane (1:4) extracts of the membrane suspension for saponification was determined after preliminary thin-layer chromatography (in a system of hexane:diethyl ether:glacial

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TABLE 1. SOD Activity in Frog and Bovine Photoreceptor Membranes and Retina (M ± m)

Object	SOD activity*
Frog	
Outer segments:	
membranes	0,18±0,02
extract	0,21±0,01
Inner segments:	
membranes	0,18±0,01
extract	0,17±0,01
Retina	0,16±0,01
Retina after removal of photoreceptors†	0,14±0,02
Ox	
Outer segments:	
membranes	0,15±0,01
extract	0,17±0,01
Inner segments:	
membranes	0,14
extract	—

*50% Inhibition of rate of formazan formation from nitro-BT taken as the unit of SOD activity (in the control at 560 nm the rate was 0.025 optical density unit/min·mg protein at 25°C).

† The rhodopsin content in the retinal homogenate before and after removal of the photoreceptors was 0.43 ± 0.07 and 0.09 ± 0.01 nmole rhodopsin/mg protein respectively.

TABLE 2. GP and GR Activity and α-Tocopherol Content in Frog and Bovine Photoreceptor Membranes (M ± m)

Object	GP activity	GR activity*	Content of α-tocopherol, mg/mg protein
Frog			
outer segments	0,28±0,02	0,32±0,04	0,21±0,02
inner segments	2,86±0,20	1,50±0,30	0,45±0,07
Ox			
outer segments	0,74	0,72	0,28±0,03
inner segments	1,30	1,32	0,16

*Activity of GP and GR extract in nmoles reduced (oxidized) glutathione/min·mg protein.

CH_3COOH, 84:15:1) from the characteristic fluorescence spectra [11]. The protein concentration was measured by the biuret reaction, using bovine serum albumin as the standard.

The reagents used were: xanthine oxidase and xanthine from Reakhim (Olaine Factory), yeast GR from Sigma, NADPH from Boehringer, nitro-BT from Chemapol, reduced and oxidized glutathione from Reanal, and the tert-butyl hydroperoxide was generously provided by Candidate of Chemical Sciences Yu. D. Norikov (Institute of Chemical Physics, Academy of Sciences of the USSR).

EXPERIMENTAL RESULTS

The results given in Table 1 show that SOD activity in the OS and IS fractions of the retinal rods was practically identical. Comparison of the activities of this enzyme in the membrane preparations themselves and in aqueous-ethanol extracts from them shows that the SOD in the photoreceptors is an enzyme, containing copper and zinc [9], and resistant to the action of chloroform. This result is in agreement with the complete inhibition of SOD in OS by cyanide [12]. Although SOD activity in the layer of photoreceptor cells was a little higher than in the retina as a whole, nevertheless these differences in activity in these experiments did not exceed 15-20%. Removal of the photoreceptor cells from the retina led only to a small decrease in enzyme

activity in the homogenate. These results are contrary to those of Hall and Hall [12], who found a 200-400-fold increase in SOD activity in OS compared with retinal homogenates. Such a difference in SOD activity in OS and in the retina as a whole nevertheless seems extremely improbable. In fact, if the SOD activity in the retina and in OS stated in the paper cited above [12] is accepted, a simple calculation shows that the protein contained in OS of the photoreceptors ought to account for not more than 0.25-0.50% of the total quantity of protein in the retina, which does not agree with many sources in the literature (see, for example [13]). Meanwhile SOD activity in the retina measured in the present experiments is in good agreement with the results obtained by Fried and Mandel [14]. Hence, low SOD activity is found in the retina and its photoreceptors, close to the activity of this enzyme in the brain [14], which is a homologous tissue, but much lower than in the organelles of the liver and in erythrocytes [9].

GP and GR activity in OS and IS also was extremely low (Table 2), although significantly higher in the OS than in the IS fraction. The possibility cannot be ruled out that the activity of these two enzymes measured in OS was due to contamination of the OS fraction with the IS fraction, and that the glutathione-peroxidase–glutathione-reductase system is virtually absent in the OS themselves. This result becomes perfectly clear if it is recalled that reduction of glutathione in the reductase reaction and, consequently, the regeneration of reduced glutathione – the substrate for the glutathione-peroxidase reaction – is an NADPH-dependent process, just like the retinol-dehydrogenase reaction, catalyzed by a membrane-bound enzyme (retinol dehydrogenase) in the disks of OS. Clearly competition between these two reactions for NADPH could give rise to extremely unfavorable conditions for regeneration of the visual pigment rhodopsin in the photoreceptors.

Consequently, the activity of the enzyme systems controlling the formation of active forms of oxygen (SOD) and regulating the content of lipid peroxides (GP) in the retinal photoreceptors was found to be very low. How is a stationary level of generation of active forms of oxygen and of lipid peroxides in these membrane structures maintained?

It follows from the results given in Table 2 that vitamin E is present in OS and IS fractions in concentrations 5 to 10 times greater than in the mitochondria and microsomes of the liver. A high concentration of α-tocopherol in OS of the photoreceptors also was found by Farnsworth and Dratz [15]. In this connection it should be pointed out that α-tocopherol (vitamin E), with its high antiradical activity, is an effective physical and chemical quencher of singlet oxygen and, at the same time, it interacts with high velocity with superoxide anion radicals [2]. Meanwhile, in α-tocopherol deficiency dystrophic changes develop in the retina, accompanied by intensive accumulation of POL products [5]. It can thus be concluded that the main load as regards protection of polyene lipids from active forms of oxygen and POL in the membrane structures of the photoreceptor cells of the retina falls on the vitamin E system.

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