ANTIOXIDATIVE ENZYME ACTIVITY AND PEROXIDE METABOLISM IN THE LENS AND THEIR ROLE IN CATARACT FORMATION

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UDC 617.741-004.1-092:[617.741-008.931:577.152.1+617.741-008.939.15-39

KEY WORDS: lens, catalase, superoxide dismutase, glutathione peroxidase, glutathione, cataract, lipid peroxidation.

Transparency of the optical media of the eye is maintained chiefly through the fault-free activity of glutathione-dependent enzyme systems of the lens, maintaining a low H_2O_2 concentration in the aqueous humor and in the lens itself [6, 7]. Disturbance of peroxide homeostasis as a result of oxidative stress leads to the appearance of high-molecular-weight aggregates, causing intensive scattering of light and, consequently, opacity of the optical media of the eye [13]. In particular, an increase in the H_2O_2 concentration in the lens is considered to be one of the most important factors in cataract formation, which is considered to take place through the formation of intermolecular cross-linkages between crystallins [8, 14].

It has recently been postulated that injury to the membrane structures of the lens play a leading role in the initial stages of cataract formation, and that the concrete molecular mechanism of this process consists of activation of lipid peroxidation (LPO) [15]. In fact, activation of LPO in cataract has been proved experimentally [1]. However, the question of the mechanisms of this activation remains unexplained. In particular, there is no information on the role of changes in activity of the enzyme systems regulating LPO in the lens in cataract.

The aim of this investigation was to study antioxidative enzyme activity during cataract formation and the possibility of inducing cataract by the action of LPO products and their effect on the content of reduced thiols (the oxidation-reduction balance) in the lens.

EXPERIMENTAL METHOD

Opacity of the human lens was obtained in the course of the operation of intracapsular cryoextraction of a cataract. Depending on the clinical characteristics of opacity, lenses with a right cataract were selected. Transparent human lenses were obtained from eye donors for corneal grafting. In all cases the integrity of the lens capsule was preserved. To assess the ability of the lenses to decompose H_2O_2 they were placed in 1 ml of Hanks' medium (pH 7.0, 25°C), containing glucose (7 mM) and H_2O_2 (0.1 mM). After assigned time intervals samples were taken from the incubation medium (50 µl) to determine the H_2O_2 concentration, using a highly sensitive chemiluminescence method in a system of luminol–horseradish peroxidase [3]. Chemiluminescence was recorded in medium containing, besides 50 µl of the sample for analysis, 930 µl of luminol (60 µM) in phosphate buffer (100 mM, pH 7.6, 25°C) and 20 µl of horseradish peroxidase (1750 U/ml). The calibration curve of the light sum of chemiluminescence as a function of H_2O_2 concentration was obtained by the use of standard solutions of H_2O_2 (from 1 µM to 0.5 mM). A model of cataract was created in chinchilla rabbits aged 4 months. After anesthesia a microincision was made in the conjunctiva under aseptic conditions 4 mm away from the limbus. The exposed sclera was punctured with a needle-stilet 0.17 mm in diameter and 0.05 ml of liposome suspension, containing 0.4 mg of phospholipids (dilinoleoyl- or dipalmitoyl-lecithin), was injected into the posterior part of the vitreous body from a microsyringe under the control of an indirect binocular opthalmoscope. For strongly oxidized phospholipids the concentration of malonyl dialdehyde (MDA) was 22.2 nmoles MDA/µmole phosphorus, and for weakly oxidized phospholipids it was 2 nmoles MDA/µmole phosphorus.

Moscow Helmholtz Research Institue for Eye Diseases. Institute of Physiology, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria. (Presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 2, pp. 143-146, February, 1987. Original article submitted February 19, 1986.

 0007-4888/87/0002-0156$12.50

TABLE 1. Activity of Antioxidative Enzymes and Rate of Decomposition of H_2O_2 by Transparent Lenses and Lenses Affected by Cataract (M ± m)

Lens	Lens		Lens homogenate			
	area of opacity (1)	rate of H_2O_2 decomposition (2)	catalase (3)	SOD (4)	glutathione peroxidase	
					H_2O_2	TBHP (5)
Transparent (n = 10)	0—0,1	0,27±0,06	7,0±1,8	76,2±20,1	1,3±0,2	0,51±0,04
With right cataract (n = 8)	0,8—1,0	0,07±0,01	6,0±3,4	20,1±7,9	1,1±0,1	0,20±0,04
P		<0,01		<0,05		<0,05

Legend. 1) Ratio of area of zone of opacity to total area of lens, 2) in μmoles H_2O_2/h per lens at 25°C, 3) in μmoles H_2O_2/min per lens at 37°C, 4) in conventional SOD units per lens, 5) in μmoles NADPH/min per lens with H_2O_2 or tert-butyl hydroperoxide (TBHP) as the substrate.

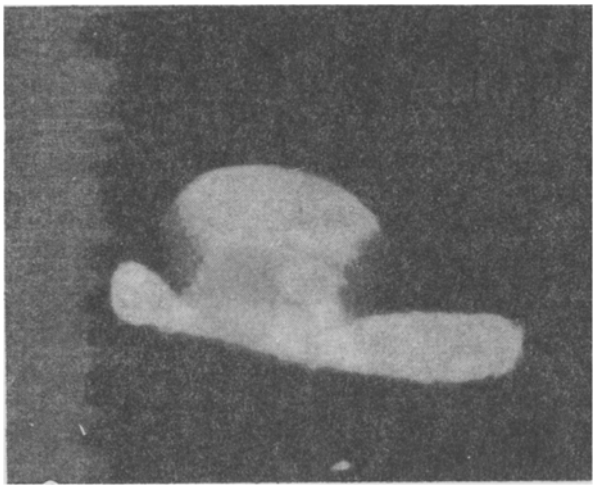


Fig. 1. Lens after injection of strongly oxidized phospholipids. Dilinoleoyl-phosphatidylcholine, 0.4 mg, 22.2 nmoles MDA/mg lipids. White reflection indicates zone of opacity of the lens.

Glutathione peroxidase (I and II) activity was determined by the method in [9]. Other enzymes whose activity also was measured were glutathione peroxidase I (selenium-containing), using H_2O_2 as the substrate, and glutathione peroxidase reducing organic peroxides (in the presence of tert-butyl hydroperoxide) [9]. Activity of superoxide dismutase (SOD) was measured by the method in [4] and catalase by the method in [10]. The concentration of reduced thiols in the lenses was determined by the reaction with 5,5-dithio-bis-2-nitrobenzoic acid [12].

EXPERIMENTAL RESULTS

In the experiments of series I the ability of transparent and opaque human lenses to catalyze the decomposition of exogenous H_2O_2 was studied by means of a highly sensitive chemiluminescence method [3]. Transparent lenses were shown to be able to decompose H_2O_2 effectively (Table 1). The rate of H_2O_2 destruction by normal lenses, determined by the chemiluminescence method, was found to correspond to data in the literature [7]. The effectiveness of H_2O_2 decomposition by lenses with cataracts was reduced, and this was particularly true in the case of right cataracts (Table 1). It can accordingly be concluded that systems controlling the oxidation-reduction balance are disturbed in lenses affected by cataract, and this may be reflected in their reduced ability to decompose H_2O_2. This disturbance may be based both on a decrease in activity of any of the enzymes controlling the redox balance and also on a deficiency of cofactors for enzymic and nonenzymic antioxidative reactions (glutathione, for example). We know that the main enzymes of the antioxidative system in the lens are catalase, SOD, and the two forms of glutathione peroxidase: I (containing selenium) and II (not containing selenium).

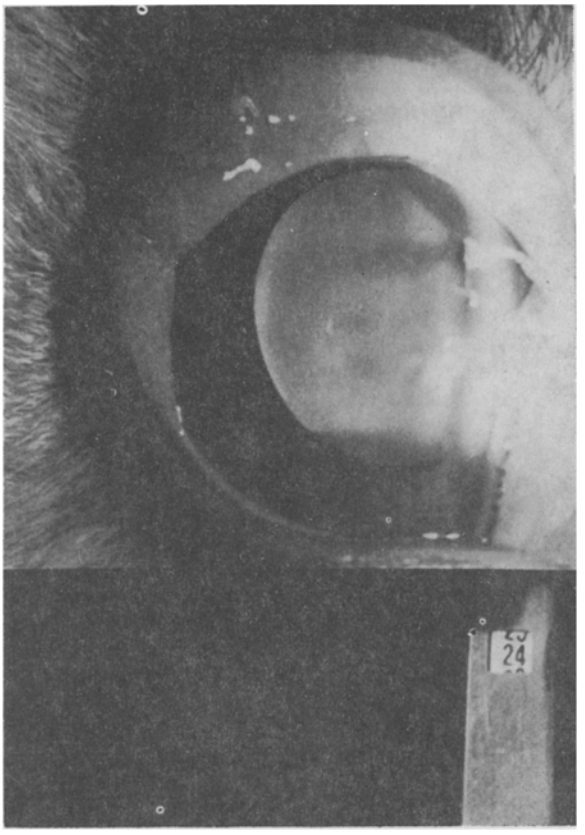


Fig. 2. Rabbit lens after injection of dipalmitoyl-lecithin into vitreous body.

TABLE 2. Changes in Content of Reduced Thiols in Lens after Injection of Phospholipid Liposomes (M ± m)

Parameter	Normal	Solvent	Phospholipids		
			strongly oxidized	weakly oxidized	saturated
Total thiols, μmoles/μmole protein	4,1±0,4	3,0±0,2	3,2±2,4	3,0±0,4	3,0±0,5
Reduced glutathione, nmoles per lens	710±106	454±23	121±62	251±75	420±63
P		<0,1	<0,01	<0,01	<0,1
P_1			<0,01	<0,05	

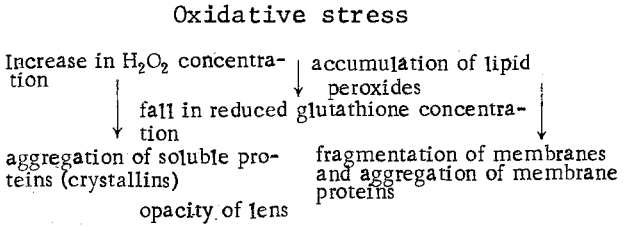
Legend. In each series there were five measurements. Average molecular weight of protein taken to be 20 kilodaltons. P) compared with normal, P_1) compared with solvent.

In the experiments of series II activity of these enzymes was estimated in control lenses and lenses affected by cataract (Table 1). It was found that activity of catalase, and also of glutathione peroxidase I (utilizing H_2O_2) in lenses with cataract did not differ from that in the control. Addition of reduced glutathione to the incubation medium containing lenses affected by cataract increased their ability to decompose exogenous H_2O_2 by almost an order of magnitude. It can accordingly be concluded that the reduced ability of lenses with cataract to decompose H_2O_2 is based, not on reduced catalase or glutathione peroxidase I activity, but on a deficiency of reduced glutathione, in agreement with data obtained by other workers [6, 13]. Meanwhile activity of SOD and glutathione peroxidase, the latter able to catalyze reduction of organic hydroperoxide (including hydroperoxides of lipids [12]), in lenses with cataract was sharply reduced (Table 1). Data on depressed SOD activity are confirmed by the hypothesis [5, 11] that $O_2^{\cdot}$ participates in the formation of intermolecular protein cross-linkages during cataract formation. If the fact that both these enzymes are key enzymes in LPO regulation [4, 5], as well as the accumulation of LPO products observed by the writers previously in

lenses affected by cataract [1] are recalled, it can be tentatively suggested that disturbance of lipid peroxide metabolism is an essential mechanism of cataract formation. In the next series of experiments the possibility of inducing cataract by injecting LPO products into the vitreous body was studied and, at the same time, the state of the thiol system of the lens was monitored. It was found that strongly oxidized phospholipids (MDA content 22.2 nmoles/μmole phosphorus) caused opacity of the lens (Fig. 1). Weakly oxidized phospholipids (2 nmoles MDA/μmole phosphorus) induce a much lesser degree of opacity. To make sure that the opacity of the lens induced by oxidized lipids was not due to penetration of compounds insoluble in water (liposome suspension) into it, control experiments were set up with injection of liposomes composed of saturated phospholipids (dipalmitoyl-phosphatidylcholine) into it. The experiment showed that these substances do not cause opacity of the lens at all (Fig. 2).

One of the characteristic features of disturbance of the redox balance in the lens during cataract is a fall in the concentration of reduced thiols (reduced glutathione) [7, 14]. If LPO products are indeed a significant cataract-forming factor, their injection ought evidently to cause changes in the reduced thiol concentration. It will be clear from Table 2 that as a result of injection of strongly oxidized phospholipids the concentration of reduced glutathione fell, whereas the degree of reduction of high-molecular-weight thiols was virtually unchanged. This confirms the view that activation of LPO plays an essential role in cataract formation and, at the same time, it indicates that LPO participates at the same time in oxidative modification of biomembranes, which is accompanied by their fragmentation [2] and by protein aggregation, which can ultimately lead to opacity of the lens.

It can be concluded from these results that disturbance of the redox balance in the lens may be due not only to disturbances in the system of H_2O_2 metabolism, but also to oxidative stress, induced by activation of free-radical LPO in the membrane structures of the lens. On the basis of these results the role of oxidative stress in cataract formation can be represented by the scheme given below, according to which two interconnected processes — an increase in the H_2O_2 concentration and an increase in the lipid peroxide concentration — trigger aggregation of soluble proteins or fragmentation of the membrane structures of the lens, respectively.



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