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DETECTION OF MYELOPEROXIDASE IN HUMAN EYE TISSUE

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Myeloperoxidase (MPO; EC 1.11.1.7) is an enzyme which is usually detected in peripheral blood neutrophils [5, 9]. Its main function is to produce the bactericidal hypochlorite iom (ClO^-) by interaction between hydrogen perioxide and chlorine ions [4]. Unlike the various metabolites constantly found in the animal body, ClO^- is a powerful oxidizing agent [2]. The presence of MPO in the eye tissues has not previously been discussed in the literature, although there are certain facts which may suggest that this is so. For instance, it has been shown that the retina possesses phagocytic properties, without which it would be impossible today to imagine regeneration and the working of the photoreceptor apparatus of the eye [11]. The more superficial parts of the eye are "armed" against infectious agents in the following way: the secretions of the lacrimal fluid contain lysozyme and the aqueous humor contains hydrogen peroxide [6]. However, there are no data on the presence of myeloperoxidase activity in the eye tissues. The investigation described below was carried out to study this problem.

EXPERIMENTAL METHOD

Eyes from previously healthy individuals dying from chest injury or alcohol poisoning and eyes from persons with senile cataract were used for the investigation. The lenses were extracted from the eye and kept until required for analysis at −8°C. The various eye structures were homogenized in 0.15 M NaCl solution. Enzyme activity was determined with *o*-dianisidine reagent at 20°C by the standard method [7]. For this purpose, to 50 μl of extract or MPO ($0.9 \cdot 10^{-8}$ M) was added 500 μl of phosphate buffer, pH 6.0, containing 0.6 mg/ml of *o*-dianisidine hydrochloride. Specific MPO inhibitors, quercetin and morin [10], were used in a concentration of 10^{-4} M. The velocity of the MPO enzyme reaction was measured during the first 30 sec after formation of the reaction mixture, by determining the formation of the product staining at 460 nm. Next H_2O_2 was added to the cuvette up to a final concentration of $1.4 \cdot 10^{-4}$ M. The appearance of a color was recorded on a "DU-7" spectrophotometer (Beckman, Austria). MPO isolated from human blood leukocytes as in [3] was used as enzyme for calibration in the enzyme test. The ratio between coefficients of absorption at 430 and 280 nm, which gives a measure of the degree of purity of the enzyme [12], was 0.60 and enzyme activity according to the *o*-dianisidine test was 200 units/mg.

For immunochemical identification of MPO in the tissue extract, the immunodiffusion test in gel was carried out by the method in [8] in the modification in [1]. A monospecific antiserum to human MPO, obtained by standard immunization of rabbits, was used.

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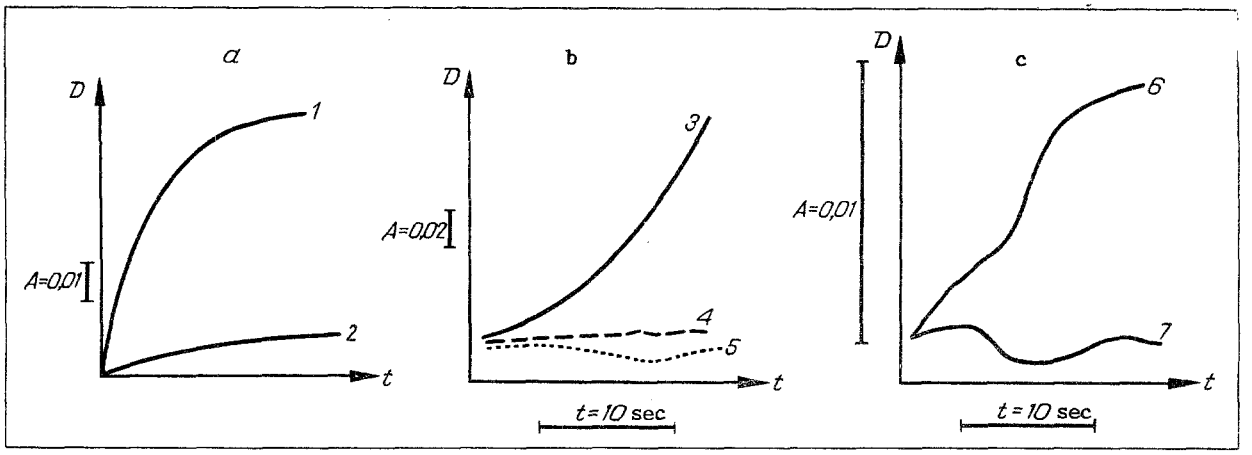

Fig. 1. Kinetics of change in optical density of MPO solutions and various eye tissue extracts in the presence of H_2O_2 and inhibitors. Abscissa, incubation time (in sec); ordinate, optical density at 420 nm (in optical density units). 1) MPO + H_2O_2, 2) MPO + H_2O_2 + quercetin, 3) retinal extract + H_2O_2, 4) retinal extract + H_2O_2 + quercetin, 5) retinal extract + H_2O_2 + morin, 6) extract of transparent lens + H_2O_2, 7) extract of transparent lens + H_2O_2 + quercetin.

TABLE 1. MPO in Various Tissue Extracts of the Human Eye

Source of MPO	MPO activity, optical density units (according to [7])	Enzyme concentration	
		in extract, μg/ml	in tissue, μg/mg
Blood serum	1,6±0,3 (n=3)	1,20	—
Retina	53±22 (n=4)	10,0	0,03
Lens			
Transparent	4,5±1,3 (n=6)	1,0	0,007
With senile cataract	2,8±0,9 (n=4)	Not detected serologically	

EXPERIMENTAL RESULTS

Changes in optical density of the reaction mixture at 420 nm, evidence of enzymic production of ClO^- ions, is shown in Fig. 1a. Clearly during accumulation of these ions the rate of work of the isolated enzyme diminishes. Specific inhibitors of MPO, namely morin and quercetin, almost completely blocked the formation of the colored product. The data given in Fig. 1b demonstrate that of all the eye structures it is the pigmented layer of the retina which has the highest MPO activity. Morin inhibits the course of the enzyme reaction (just as in the case of MPO from leukocytes) when different eye tissue homogenates are used.

The water-soluble fraction of lens proteins also possessed myeloperoxidase activity (Fig. 1c), and it could also be found in opaque lenses (Table 1). Table 1 summarizes the results of MPO determination in the different structures of the eye. It will be noted that the ciliary body, vitreous, aqueous humor, and retina did not possess any appreciable activity. Weak MPO activity was characteristic of the ciliary body and the iris (not shown).

The results of spectrophotometric determination of myeloperoxidase activity in the different eye structures confirmed the immunochemical data. Table 1 shows that a characteristic feature of extracts of the retina and transparent lenses was a higher MPO concentration, but conversely, in the presence of senile cataract, the enzyme could not be detected immunochemically in extracts from the lenses. This likewise was impossible for other eye tissues in which, according to the spectrophotometric measurements, myeloperoxidase activity was very low or could not be detected at all (Table 1).

The discovery of MPO in the retina, which performs a phagocytic function [11], is not an unusual phenomenon. The enzyme is evidently necessary for the working of the visual system. Conversely, activity of the enzyme in the lens is a completely unexpected fact. Only a few suggestions can be put forward regarding its function.

First, the enzyme may undertake detoxication of the hydrogen peroxide in the aqueous humor, the concentration of which may reach 0.1 mM in cataracts [6]. Second, MPO may be involved in processes of renewal of the lens proteins and lipids. This is an essential function, for throughout life of the individual the lens is constantly subjected to the action of solar radiation. But unlike, for example, epithelium, it possesses no substitutive repair systems.

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