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QUANTITATIVE CHANGES IN Cu,Zn-ACTIVATED SUPEROXIDE DISMUTASE AND CATALASE IN THE LIVER OF RATS WITH ALLOXAN DIABETES

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UDC 616.379-008.64-092.9-07:616.36-008.931:577.152.193

KEY WORDS: alloxan diabetes; superoxide dismutase; catalase

In rats with alloxan diabetes (AD) activity of superoxide dismutase (SOD) and catalase in the tissues is reduced, along with activity of other enzymes involved in antiradical protection of the cell [1, 9, 11]. On the other hand, elevation of the glucose level leads to an increase in the concentration of superoxide anions ($O_2^{\cdot-}$) in experiments not only *in vitro* [5], but also *in vivo* [12].

Administration of exogenous SOD has a protective effect in AD, for it lowers the blood glucose concentration to normal and inhibits excessive lipid peroxidation [4, 8].

The aim of this investigation was to study the spectral properties and activity of Cu, Zn-activated SOD (Cu,Zn-SOD) and catalase, isolated from rat liver and purified in order to discover the causes of their inactivation in AD.

EXPERIMENTAL METHOD

Experimental AD was induced in Wistar rats weighing 150-200 g by a single intraperitoneal injection of alloxan in a dose of 15 mg/100 g body weight. The rats were decapitated on the 5th day.

Cu,Zn-SOD and catalase were isolated from 60 g of rat liver by treatment with acetone [13]. The proteins were purified by ion-exchange chromatography of DE-52 cellulose (Whatman, England) and by gel-filtration on Toyopearl-55 (Japan).

SOD activity was studied by the method in [14], with determination of the quantity of $O_2^{\cdot-}$ undergoing dismutation in a certain time. As the source of $O_2^{\cdot-}$ generation an aqueous solution of H_2O_2 (0.5 M), pH 10.5, was used; the reaction mixture was incubated at 90°C for 2 min.

Department of Biochemistry, Erevan Medical Institute. Laboratory of Physical Chemistry of Proteins, Institute of Biochemistry, Academy of sciences of the Armenian SSR. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Orekhovich.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 3, pp. 306-308, March, 1987. Original article submitted August 22, 1985.

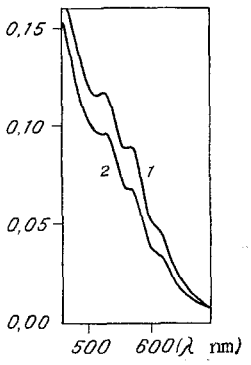

Fig. 1

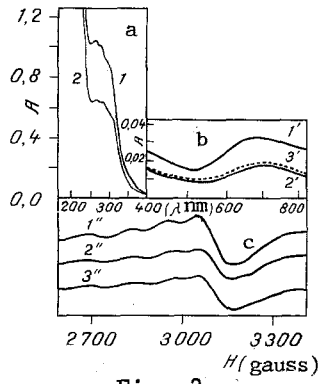

Fig. 2

Fig. 1. Adsorption spectra (a, b) and EPR spectra (c) of Cu,Zn-SOD isolated from rat liver. 1, 3 and 6) Control; 2, 5, and 7) diabetes. 4, 8) Aeration of solution of SOD from liver of experimental rats with oxygen. Condition of recording EPR spectra: time constant 0.3 sec, scanning time 2 min, sensitivity $2 \cdot 10^3$, microwave power and frequency 10 mM and 9.08 GHz, respectively) amplitude of modulation 6.3 G.

Fig. 2. Absorption spectra of catalase, isolated from rat liver: 1) control; 2) diabetes. Abscissa, wavelength, in nm; ordinate, optical density.

Catalase activity was determined by a spectrophotometric method [7], by measuring the quantity of H_2O_2 decomposed per minute and comparing it with the quantity of H_2O_2 decomposed by a known concentration of catalase at 25°C. The reaction mixture contained: $5 \cdot 10^{-3}$ M H_2O_2 ($\varepsilon_{240} = 61$ M^{-1} cm^{-1}), $5 \cdot 10^{-9}$ M catalase ($\varepsilon_{405} = 5 \cdot 10^4$ M^{-1} cm^{-1}) and 0.05 M phosphate buffer, pH 7.0.

Absorption and EPR spectra were recorded on Beckman 26 and Varian E-4 (USA) instruments, respectively. A K-70 centrifuge (East Germany) and MPW-302 homogenizer (Poland) also were used.

To isolate Cu,Zn-SOD and catalase from rat liver the method mentioned above was modified so as to obtain catalase, in a sufficiently purified form, as well as Cu,Zn-SOD. In particular, the acetone powder of the liver was mixed with 0.04 M K-phosphate buffer (later the buffer was used in different molarities, pH 7.4) and the supernatant was withdrawn and, after centrifugation (at 600 rpm, 30 min) it was treated a second time with acetone. The resulting residue was dissolved in water and dialyzed against water, after which the supernatant was treated on DE-52 cellulose, equilibrated with 0.04 M buffer. Cu,Zn-SOD was eluted with 0.01 M and catalase with 0.2 M buffer from DE-52. After concentration on DE-52 the solutions were passed through separate columns with Toyopearl-55 (2 × 80 cm) and the active fractions were again purified and concentrated on DE-52 cellulose (2 × 5 cm). As a result, 2.5 ml of Cu,Zn-SOD ($D_{680} = 0.03$ and $D_{260}/D_{280} = 36$) and catalase ($D_{545} = 0.12$ and $D_{280}/D_{410} = 1.6$) was obtained. The resulting proteins were sufficiently pure to enable their absorption and EPR spectra and also their activity to be studied. Altogether four isolations were carried out from the liver of the experimental and control animals.

EXPERIMENTAL RESULTS

Absorption and EPR spectra of Cu,Zn-SOD and catalase, isolated from the liver of intact animals, did not differ quantitatively from those of animals with diabetes (Figs. 1 and 2). Consequently, SOD and catalase underwent no qualitative changes during the development of AD. However, in the terminal stages of the disease the intensity of optical absorption at 680 nm (Fig. 1a, b) and the integral intensity of the EPR spectrum of SOD (Fig. 1c) were reduced by 40% ($P < 0.05$). On aeration the copper of the enzyme from the liver of the experimental animals was oxidized by only 8-10%, so that the main cause of the decrease in SOD activity in AD was a decrease in the quantity of this enzyme. Only 8-10% of the copper of SOD was in the reduced form, and this was reflected in SOD activity (Table 1).

TABLE 1. Dismutation of $O_2^{\cdot}$ Generated during Decomposition of H_2O_2 by Cu,Zn-SOD Isolated from Liver of Intact Rats and Rats with Diabetes

Source of SOD	Number of $O_2^{\cdot}$ undergoing dismutation, M
Liver of control rats (10^{-8} M)	$8 \cdot 10^{-4}$
Liver of rats with diabetes ($6 \cdot 10^{-9}$ M)	$3{,}9 \cdot 10^{-4}$
Liver of experimental rats after aeration of enzyme solution with oxygen for 2 h, 5°C	$4{,}4 \cdot 10^{-4}$

Legend. SOD concentration shown in parentheses.

The decrease in the intensity of optical absorption of catalase (at 545 nm) was 15% ($P < 0.05$). Compared with the control, catalase activity was reduced by the same amount.

It was shown that inhibition of activity of SOD and other enzymes of antiradical protection of the cell in AD leads to spontaneous dismutation, with the formation of singlet oxygen which, since it reacts readily with unsaturated fatty acids, causes the formation of hydroperoxide and leads ultimately to a marked increase in both ascorbate-dependent and NADP-dependent lipid peroxidation [2]. On the other hand active forms of oxygen, inducing destruction of β-cells, can inhibit SOD and catalase [10] irreversibly [15]* or reversibly [6], and can also induce DNA degradation and can disturb normal functioning of RNA [3].

The compensatory protective role of exogenous Cu,Zn-SOD and catalase administered in experimental diabetes is evident from these results.

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*As in Russian original. No reference No. 15 given. — Publisher.