ACTIVITY AND ELECTROPHORETIC MULTIPLICITY OF MOLECULAR FORMS OF SUPEROXIDE DISMUTASE IN HUMAN BLOOD CELLS

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The level of superoxide dismutase (SOD) activity calculated relative to protein in the various human blood cells falls in the following order: platelet > erythrocyte > lymphocyte > granulocyte. During electrophoresis in homogeneous polyacrylamide gel of homogenates of granulocytes, lymphocytes, and platelets three zones of SOD activity were identified. Two fractions of the enzyme, disappearing after treatment with cyanide, were found in lysates of erythrocytes after removal of the hemoglobin. Of the two SOD fractions of platelets, lymphocytes, and granulocytes migrating rapidly toward the anode, the first corresponds in its ability to be inhibited by cyanide or organic solvents to the cytosol isozyme, the second to the mitochondrial isozyme. The third cathode fraction was not identified. The functional role of SOD in specialized blood cells and the cause of heterogeneity of the enzyme are discussed.

KEY WORDS: superoxide dismutase; erythrocytes; platelets; lymphocytes; granulocytes.

The intensity of free radical processes in the cell depends on various factors, among which enzyme systems are particularly important. Superoxide dismutase (SOD), catalyzing the reaction $O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$, has been shown to protect cells against oxidative injury [11]. Reports have also been published regarding the role of SOD and the superoxide anion in metabolic processes [9]. Data on the level of activity and the isozyme composition of SOD in human blood cells are incomplete and contradictory.

This paper describes a study of the distribution of activity and the electrohporetic spectrum of SOD in the various human blood cells.

EXPERIMENTAL METHOD

Human blood cells were obtained by methods described previously [2]*. The cells were washed with 0.14 M NaCl and the cell residue was suspended in 5 mM Tris-HCl buffer (pH 7.8) and disintegrated by treatment with 0.1% Triton X-100 under hypotonic conditions or by freezing three times followed by homogenization. The hemolysate of the erythrocytes was obtained without treatment by detergent and the hemoglobin was precipitated [12] before determination of the SOD activity. SOD activity was determined by inhibition of autooxidation of adrenalin, stimulated by adrenochrome [3], with continuous recording of the reaction velocity on the Specord UV VIS spectrophotometer (East Germany). The protein content was determined by Lowry's method and hemoglobin by the acetone–cyanhydrin method. Electrophoresis of SOD was carried out in homogeneous 7.5% polyacrylamide gel using Tris-borate electrode buffer (pH 8.3). SOD in the gel was detected with nitro-BT under conditions of photochemical generation of O_2^- [5]. Isoelectrode focusing in polyacrylamide gel (PAG) was carried out in the Multiphor apparatus (LKB, Sweden), using plates of 5% gel containing Ampholines

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TABLE 1. SOD Activity in Various Human Blood Cells (M ± m)

Cells	Number of experiments	SOD activity, units/mg protein
Erythrocytes*	16	1,3±0,22
Platelets	5	1,9±0,25
Lymphocytes	5	0,56±0,02
Granulocytes	5	0,13±0,04

*Activity calculated per milligram hemoglobin.

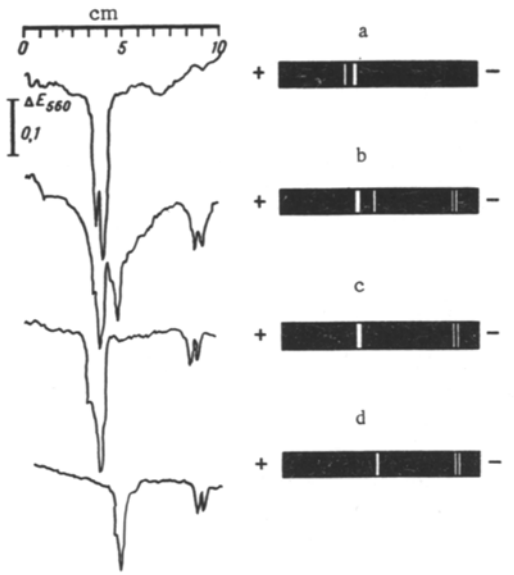

Fig. 1

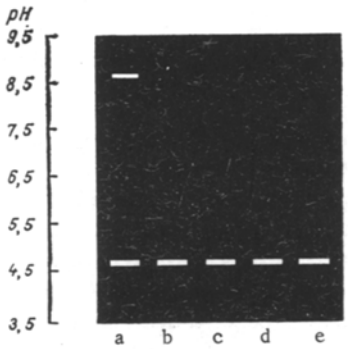

Fig. 2

Fig. 1. Densitograms of SOD in erythrocytes and platelets after fractionation in polyacrylamide gel. a) Lysate of erythrocytes; b) homogenate of platelets; c) homogenate of platelets treated with mixture of chloroform and ethanol; d) homogenate of platelets incubated with KCN at 22°C for 5 min.

Fig. 2. Isoelectric focusing of SOD from various blood cells in polyacrylamide gel. a) Hemolysate; b) hemolysate after treatment with mixture of chloroform and ethanol; c) homogenate of platelets; d) homogenate of lymphocytes; e) homogenate of granulocytes.

with pH range 3.5-9.5. The gels were scanned on an attachment to the Gilford model 240 spectrophotometer (USA).

EXPERIMENTAL RESULTS

The level of SOD activity in the various blood cells was found to decrease in the following order: platelet > erythrocyte > lymphocyte > granulocyte (Table 1). Low SOD activity in the granulocytes was rather unexpected, considering that phagocytic cells can generate the superoxide anion, by means of which they evidently perform their bactericidal function. High SOD activity would prevent their function, but the mechanism of protection of the leukocytes themselves against O_2^- under these conditions remains unexplained. SOD inhibits the bactericidal activity of leukocytes, but in a model myeloperoxidase system containing xanthine oxidase as the source of O_2^-, SOD weakly inhibited the bactericidal action of myeloperoxidase [9]. It has been suggested that SOD regulates the pathway of the superoxide anion during phagocytosis, directing it to the cytochrome c acceptor or converting it into H_2O_2, the substrate for myeloperoxidase. The high level of SOD activity

in the platelets is difficult to explain at present on the grounds of functional necessity, for the metabolic role of free-radical reactions in these cells has not been investigated. Nevertheless it should be noted that agglutinating agents (thrombin and adrenalin) stimulate the free-radical oxidation of platelets [8, 10]. It seems probable that SOD participates in the mechanism of platelet function in blood clotting. SOD of erythrocytes has received much more detailed study than in other cells and its protective, antioxidative function, produced by different mechanisms, has been demonstrated [1, 7, 11]. Allowing for the relative numbers of the different blood cells, the results indicate that changes in the level of enzyme activity discovered in whole blood in pathological processes [3, 14] reflect chiefly the state of SOD in the erythrocytes.

The results of electrophoretic fractionation and isoelectric focusing of SOD from various blood cells in PAG are given in Figs. 1 and 2. After electrophoresis of the lysate of erythrocytes two bands of SOD activity can be seen, the slowly migrating fraction being much stronger. Treatment of the hemolysate with organic solvents [12] to remove hemoglobin did not change the electrophoretic spectrum of the SOD from the erythrocytes. Meanwhile the addition of 3 mM KCN led to disappearance of both bands of enzyme. The electrophoretic spectra of SOD from platelets, lymphocytes, and granulocytes were identical. SOD of these cells formed three achromatic zones, that at the cathode end of the gel sometimes consisting of two bands. The achromatic zone at the cathode was absent on electrophoresis of erythrocyte lysate and did not disappear following treatment of the extracts with organic solvents and cyanide. Sinet et al. [13] regarded this fraction of SOD as being mitochondrial and concluded that SOD in the mitochondria of human tissues differs from the enzyme in other sources in its resistance to treatment with a mixture of chloroform and ethanol. However, one of the bands of SOD from platelets, lymphocytes, and granulocytes located in the middle part of the gel disappeared after such treatment (Fig. 1c), so that the middle fraction of SOD can be regarded as mitochondrial and the more rapidly migrating fraction as belonging to the cytosol, for it is inhibited by cyanide (Fig. 1d). This conclusion is supported by the relative width of the achromatic zones, for the proportion of the cytosol enzyme in the cells is much larger [4, 13]. The nature of the cathodic achromatic zone requires special study.

Under the conditions of electrophoresis used, the erythrocytic SOD consisted of two components, both of which were inhibited by cyanide. The mechanism of the heterogeneity of erythrocytic SOD is unknown. The erythrocytes are known to contain one SOD isozyme [6]. In fact, during isoelectric focusing of SOD from erythrocytes in polyacrylamide gel one fraction with an isoelectric point of 4.65 was discovered (Fig. 2). In this respect SOD from platelets, lymphocytes, and granulocytes does not differ from the erythrocytic enzyme. It can be concluded from the identity of the isoelectric point that the electrophoretic multiplicity is due to differences in the size and shape of the molecules of the SOD fractions. SOD from the mitochondria and cytosol of chicken liver are known to differ in molecular weight [15].

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