Superoxide dismutase activity decreases during erythrocyte aging

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Summary. Superoxide dismutase activity was determined by the adrenalin method in bovine erythrocytes separated according to age. Progressive loss of the enzyme activity was found, down to ca 65% of that of the youngest cells.

Numerous evidence has been accumulated in favour of the view that the superoxide radical anion is deleterious to cell constituents, and that superoxide dismutase, ubiquitously present in aerobic cells, is of primary importance in the defence against this dangerous intermediate of oxygen metabolism[3,4]. A hypothesis has been put forward[5] that the primary cause of erythrocyte aging consists in the damage to the red cell membrane and to other red cell constituents by the superoxide radical anion released during autoxidation of hemoglobin[6,7]. It therefore seemed interesting to check whether the activity of superoxide dismutase (SOD) changes during aging of erythrocytes, like activities of other especially -SH-containing enzymes[8], since a possible decrease in the SOD activity could accelerate the red cell aging itself and could constitute an element of this mechanism.

Material and methods. Erythrocytes were obtained from citrated bovine blood (1 vol. 3% sodium citrate: 9 vol. blood) and separated by age according to the method of Murphy[9], employing 2-h centrifugation at 39,600×g at 37 °C in a VAC 601 ultracentrifuge (8×11 ml angular rotor, angle of 34°). 7 equal erythrocyte fractions (1.3 ml) were collected by careful aspiration with a syringe. The cells were washed 4 times with phosphate-buffered saline, hematocrit of the resultant suspension was determined in a TH1 Janetzki centrifuge and the erythrocyte suspension was lysed by a freezing-thawing procedure. Hemoglobin concentration in the hemolysates was estimated according to Drabkin[10]. The hemolysates were diluted to a hemoglobin concentration of 10% and SOD was extracted with a chloroform-ethanol mixture. 100 µl of hemolysate was diluted with 100 µl of distilled water, and with 500 µl of ethanol and 300 µl of chloroform (all reagents chilled). The mixture was shaken for 1 min at 4 °C and centrifuged. SOD activity in the supernatants was estimated by the adrenalin method[11] and converted into equivalent amounts of fully active enzyme by means of calibration with a standard SOD preparation (Sigma).

Results and discussion. The mean cell hemoglobin concentration (MCHC) obtained as a quotient of the hemoglobin content and the packed cell volume increased in density-separated erythrocyte fractions from top to bottom of the centrifuge tubes. The ratio of MCHC in the bottom to that in the top fractions amounted to 1.10±0.03 (mean±SD) in separate experiments from 7 different animals. This result shows a successful separation of the red cells[12]. In the case of the present experiments, blood was not defibrinated. We suspect that the dilution of blood with citrate compensated the effect of higher blood viscosity than in the experiments of Murphy[9], due to the presence of fibrinogen. The mean SOD content of the red cells (averaged values from all age fractions) amounted to 0.66±0.25 µg/g Hb. This value is higher than that reported by Concetti et al.[11] for human erythrocytes, but it is known from a comparative study[13] that the SOD activity is higher in ox than in human erythrocytes.

The relative equivalent amounts of active SOD in individual density fractions of erythrocytes are presented in the table. The SOD activity decreased in a moderate but significant way on erythrocyte aging, the cause of which is not clear. SOD is not inactivated by its substrate[14]. It can be inactivated by its product, H_2O_2[14]. SOD is a cytosolic enzyme, not attached to the erythrocyte membrane[4] and its selective loss during microvesiculation proceeding on erythrocyte aging[15] cannot be postulated.

The question remains whether the loss of SOD activity on red cell aging is of biological importance. Lynch et al.[16] concluded that the amount of SOD present in erythrocytes is more than 10fold higher than that sufficient to prevent completely hemoglobin oxidation. However, other kinds of damage to the erythrocyte can proceed more easily than hemoglobin oxidation, and, moreover, the above-mentioned conclusion was based on studies of diluted hemolysates. The reaction of SOD is diffusion-limited and is significantly slower in a medium of higher viscosity[17]. The microviscosity of red cell interior is about 4 times higher than that of water[18], and can be expected to be higher in older cells, of higher density. Both these aspects may give the decrease in SOD activity in aging erythrocytes a significance for the deterioration of this cell.

SOD in different age fractions of bovine erythrocytes

Fraction No.	Relative SOD activity (%) Mean	SD
1	100	
2	94.6	10.5
3	91.7	16.9
4	84.0	14.8
5	87.8	6.6
6	71.8	12.5
7	64.6	8.8

Activities expressed as percent of activities found in the youngest cell fraction. Combined data from 7 duplicate experiments on blood from individual animals (mean values from duplicates taken for calculations).

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