Communications to the Editor

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A Superoxide Dismutase-like Substance in Rabbit Plasma

Superoxide dismutase(SOD)-like activity was found in rabbit plasma and the active substance was separated. Its properties were similar to those of copper-zinc SOD in cyanide and azide sensitivity and heat-stability, but its molecular weight was presumed to be 140000 by Sephadex G-150 column.

Keywords——superoxide dismutase; rabbit plasma; xanthine oxidase method; superoxide dismutase-like substance; plasma superoxide dismutase

Three types of superoxide dismutase (SOD), *i.e.* copper-zinc, mitochondrial and bacterial SOD, have been separated from animals, plants and microorganisms.[1] In animal red cells, high activity of copper-zinc SOD was found by many workers. Crapo and Tierney[2] showed very low activity of SOD in rat plasma, but the active substance was not studied further.

In this work, we observed the presence of an SOD-like substance in rabbit plasma and examined its properties.

Plasma was obtained from heparinized rabbit blood by centrifugation and diluted with saline (0.9% NaCl) to measure the SOD activity. The rabbit plasma contained 40—50 units/ml of SOD when assayed by xanthine oxidase method modified by Imanari *et al.*[3] When the plasma was subjected directly to gel filtration on a column of Sephadex G-150, the SOD activity was eluted at the fractions which corresponded to molecular weight 140000 (Fig. 1. A). This active fractions (Fraction 60 to 65 in Fig. 1. A) were combined, applied to a DE-52 column (0.9×20 cm) and eluted with a linear NaCl gradient (Fig. 1. B). The elution pattern of SOD-like substances showed also only one activity peak and the corresponding fraction (called p-SOD by us) was used for studying its properties. As shown in Table I,

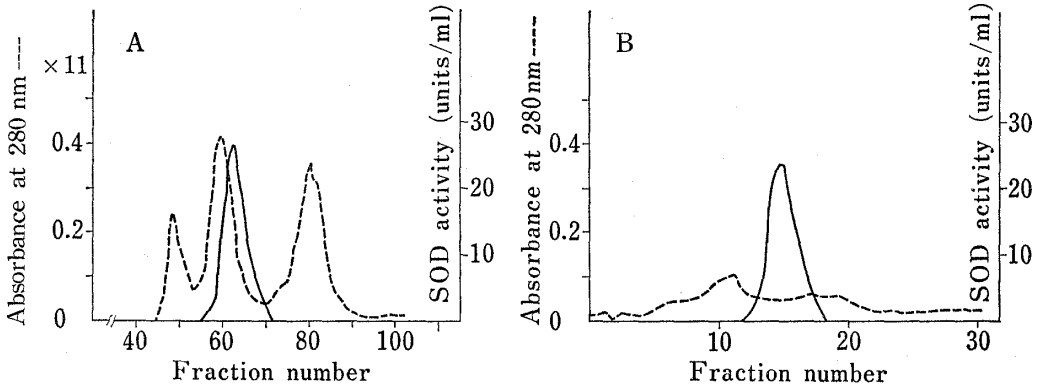

Fig. 1. Elution Patterns of SOD-like Substances in Rabbit Plasma from Sephadex G-150 Column (A) and DE-52 Column (B)

Condition of Sephadex G-150 Column (A)
gel bed; 95 cm×2.6 cm i. d.
flow rate; 10.5 ml/hr
eluate; 0.01M Tris-HCl buffer (pH 7.8)-1.0M NaCl
sample; 3 ml of rabbit plasma
fraction vol.; 2.8 ml.

Condition of DE-52 Column (B)
gel bed; 20.0×0.9 cm i. d.
flow rate; 10 ml/hr
eluate; NaCl gradient (0.01M sodium phosphate buffer (pH 7.8), 100 ml and the buffer-0.5M NaCl, 100 ml)
sample; see the text
fraction vol.; 2.0 ml.

1) I. Fridovich, "Advances in Enzymology," Vol. 41, ed. by Alton Meister, John Wiley and Sons, New York, London, Sidney, Tronto, 1974, p. 35.
2) J.D. Crapo and D.F. Tierney, *Amer. J. Physiol.*, **226**, 1401 (1974).
3) T. Imanari, M. Hirota, M. Miyazaki, K. Hayakawa, and Z. Tamura, *Igaku no Ayumi*, **101**, 496 (1977).

Table I. Effects of NaCN and NaN_3 on p-SOD and Cu,Zn–SOD

Additions	Residual activiy (%)	
	p-SOD	Cu,Zn-SOD
None	100	100
1 mM NaCN	0	0
1 mM NaN_3	89	92
5 mM NaN_3	57	87

Sodium cyanide and azide were added to the assay mixture containing 1 to 1.5 units of the enzyme, and then the enzymatic activity was determined.

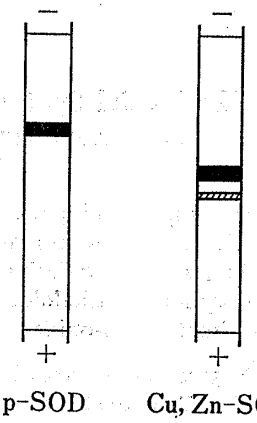

Fig. 2. Polyacrylamide Gel Electrophoretic Patterns of p-SOD and Cu,Zn–SOD

To each gel, 4 units of p-SOD and Cu, Zn-SOD were applied and electrophoresis was performed in 7.5% acrylamide gels with glycine buffer, pH 8.3 and a constant current of 2 mA per gel. Gels were stained for SOD activity using riboflavin and NBT as described by Beauchamp and Fridovich.[5] Dark bands diagramatically show SOD activity.

both p-SOD and copper-zinc SOD (prepared from rabbit red cells by the method of McCord and Fridovich[4]) exhibited almost similar sensitivity to cyanide and azide (Table I). Moreover, activities of both enzymes were not destroyed by treatment with chloroform plus ethanol and also by heating the enzymes in saline (10 units or 0.2 mg protein/ml for p-SOD) at 60° for 20 min. On disc-electrophoresis according to the method of Beauchamp and Fridovich,[5] both enzymes showed CN-sensitive activity bands, but, the mobility of p-SOD was different from that of copper-zinc SOD (Fig. 2). The results above-mentioned suggest the presence of an SOD-like substance in rabbit plasma.

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5) C. Beauchamp and I. Fridovich, *Anal. Biochem.*, **44**, 276 (1971).