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 Tierney2) 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 x 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,

![Absorbance at 280 nm vs Fraction number](image)

![SOD activity (units/ml) vs Fraction number](image)

**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 x 2.6 cm i. d.
  - Flow rate: 0.5 ml/hr
  - Eluate: 0.01M Tris-HCl buffer (pH 7.8)-1.0% NaCl
  - Sample: 3 ml of rabbit plasma
  - Fraction vol.: 2.8 ml

- **Condition of DE-52 Column (B)**
  - Gel bed: 20.0 x 0.9 cm i. d.
  - Flow rate: 10 ml/hr
  - Eluate: NaCl gradient (0.01% sodium phosphate buffer (pH 7.5), 100 ml and the buffer-
  0.5% NaCl, 100 ml)
  - Sample: see the text
  - Fraction vol.: 2.0 ml

Table I. Effects of NaCN and NaN₃ on p-SOD and Cu₂Zn-SOD

<table>
<thead>
<tr>
<th>Additions</th>
<th>Residual activity (%)</th>
<th>p-SOD</th>
<th>Cu₂Zn-SOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>1 mm NaCN</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1 mm NaN₃</td>
<td>89</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>5 mm NaN₃</td>
<td>57</td>
<td>87</td>
<td></td>
</tr>
</tbody>
</table>

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.

Both p-SOD and copper-zinc SOD (prepared from rabbit red cells by the method of McCord and Fridovich) 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°C for 20 min. On disc-electrophoresis according to the method of Beauchamp and Fridovich, 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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