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ACTIVE SUBUNITS FROM SUPEROXIDE DISMUTASE

by

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Summary: When copper-zinc superoxide dismutase from swordfish liver is dialyzed against 8 M urea, the enzyme dissociates into monomers which are catalytically active.

Introduction: Copper-zinc superoxide dismutase (E.C. 1.15.1.1) is a dimeric enzyme with a molecular weight of approximately 33,000 and contains 2 atoms of copper and 2 atoms of zinc. The copper is the functional metal in the enzyme. The enzyme from bovine erythrocytes has been extensively studied and it has been found to be resistant to dissociation. Monomers of about 16,000 molecular weight could only be obtained in the presence of 2-mercaptoethanol (1) even though from the sequence of the protein, it has been found that no interchain disulphide bridges are present (2). Beauchamp and Fridovich (3) have, however, reported that one of the isozymes from wheat germ dissociates into monomers in the presence of sodium lauryl sulphate alone, whilst we have observed that during detergent-gel electrophoresis, the enzyme from swordfish liver dissociates into monomers in the presence of urea (4). Since Forman and Fridovich had reported that the bovine erythrocyte enzyme is active in the presence of 10 M urea (5), we decided to investigate the effect of urea on the enzyme from swordfish liver.

Materials and Methods: Copper-zinc superoxide dismutase from the liver of the swordfish (Xiphias gladius) was prepared as previously described (4). Solutions of the enzyme (30 mg/ml) were dialyzed

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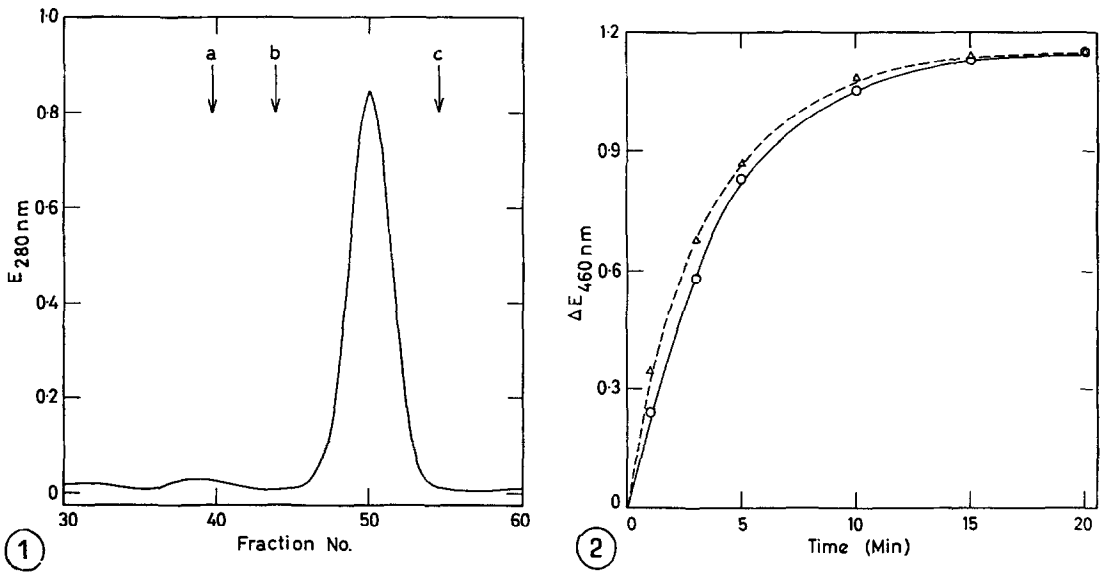

Fig. 1. Gel filtration of 30 mg superoxide dismutase in 8 M urea containing 0.1 M phosphate buffer, pH 7.4. Fraction volume = 1.8 ml. Arrows indicate elution peaks of (a) ovalbumin, (b) chymotrypsinogen A, and (c) cytochrome c.

Fig. 2. Time course of the assay reaction. (0-0) native enzyme (Δ-Δ) monomeric enzyme. Final protein concentration in the cuvette was 0.027 mg/ml.

against 0.1 M phosphate buffer pH 7.4 containing 8 M urea at 4°C. The urea was de-ionized with Amberlite Monobed MB-3 Resin (B.D. H. Ltd., Poole, England). Chromatography was carried out on a column 90 x 1.5 cm containing Ultrogel Ac 54 (LKB Produkter AB, Bromma, Sweden) equilibrated with the dialysis buffer. Enzyme activity was measured by a photochemical augmentation assay recently described by Misra and Fridovich (6) which takes advantage of the increased rate of aerobic photooxidation of dianisidine sensitized by riboflavin. The reaction is monitored at 460 nm and was found to be unaffected by 8 M urea.

Results and Discussion: In the native state swordfish copper-zinc superoxide dismutase behaves as a dimer of 32,500 molecular weight with the

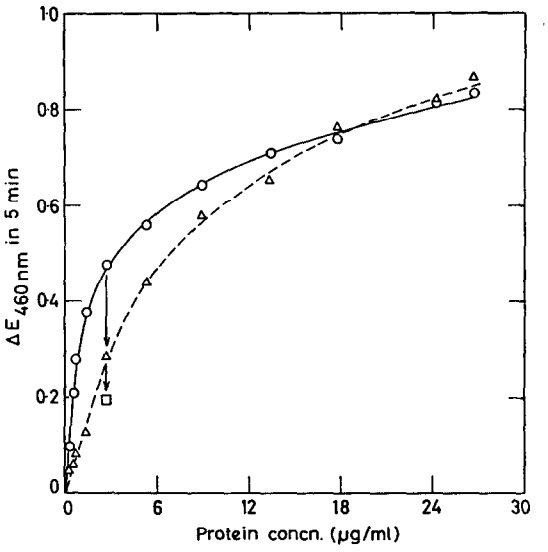

Fig. 3. Reaction rate as a function of protein concentration. (0-0) native enzyme, (Δ-Δ) monomeric enzyme. Arrows indicate lowered activity in 8 M urea and 50% glycerol at low protein concentration.

same activity as bovine enzyme (4). Gel filtration on Ultrogel Ac 54 of native enzyme dialyzed against 8 M urea indicates that the enzyme readily dissociates into monomers (Fig.1). The elution patter indicated a molecular weight of 16,300. The activity of the monomer (in 8 M urea) was compared with the activity of native dimer. Both the dimer and the monomer tended to show the same activity as a function of both time and protein concentration (Figs. 2 and 3). At low protein concentration the monomer had a slightly lower activity than the dimer (Fig. 3). This lower activity is due to the viscosity of the medium and the fact that the reaction is diffusion-limited (7) as similarly low activity was present in 50% glycerol (Fig. 3).

These preliminary investigations indicate that the two active sites in the enzyme might be able to act independently of each other when catalyzing the dismutation of superoxide radicals. The results would also seem to show that redox cycling of the copper in dimeric enzyme, as proposed by Fielden et al. (8) is not an absolute necessity for the dismutase activity. A detailed kinetic study of monomeric and dimeric swordfish liver superoxide dismutase is in preparation.

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