ENZYME ACTIVITY OF SUPEROXIDE DISMUTASE PROTOMERS

F. MARMOCCHI, G. VENARDI, G. CAULINI and G. ROTILIO
Institute of Biological Chemistry, University of Camerino, and CNR Centre for Molecular Biology, Rome, Italy

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1. Introduction

Superoxide dismutase from eucaryotic cells are dimeric enzymes of 33 000 mol. wt. containing 1 cupric copper and 1 zinc per protomer [1]. The copper site is the active site, while the zinc has a structural role [2,3]. The enzymes purified from mammalian cells are considerably resistant to denaturating agents such as 4% sodium dodecyl sulfate (SDS) and 10 M urea [3]. In particular SDS is not able to dissociate the mammalian enzymes into protomers of 16 000 mol. wt. in the absence of 2-mercaptoethanol, although it has been shown that no covalent linkages exist between the two subunits [4]. Recently it has been reported [4] that wheat germ contains two cupro-zinc isozymes, which differ from each other in so far that one of them dissociates into protomers in SDS even in the absence of 2-mercaptoethanol.

As the activity of the dimer is unaffected by SDS while treatment with SDS and 2-mercaptoethanol produces inactive protomers [4] it seemed worthwhile to test the activity of the protomers obtained by the action of SDS alone. Recent studies on the mechanism of superoxide dismutase [5] suggested that only half the active sites of the bovine enzyme function in catalysis, even though the two copper centers are indistinguishable in terms of spectral features [5] and anion reactivity [6]. This may indicate a flip-flop type of mechanism [7], in which the reaction of one copper with a superoxide ion renders the other unreactive.

In the light of these considerations, we now report on the enzyme activity of wheat germ superoxide dismutase as affected by SDS and subsequent removal of the denaturating agent.

2. Materials and methods

The cupro–zinc isozymes from wheat germ were purified according to Beauchamp and Fridovich [4], with minor modifications of the procedure. Polyacrylamide gel electrophoresis was carried out according to Davis [8]. The gels were stained for activity according to Beauchamp and Fridovich [9]. Polyacrylamide gel electrophoresis in SDS and localization of superoxide dismutase activity on gels were performed as described in ref. [4]. Assays of the enzyme activity of the samples incubated in SDS were performed according to the method of the inhibition of epinephrine autoxidation at high pH [10], as autoxidation of epinephrine was found to be unaffected by the presence of 1% SDS. SDS was removed from the protein by the method of Weber and Kuter [11], with the exception that no 2-mercaptoethanol was added to the solutions.

3. Results and discussion

Fig. 1 shows the results of polyacrylamide gel electrophoresis of the preparations of two cupro–zinc isozymes from wheat germ used in this work. The more basic isozyme is referred to as isozyme I, and the more acid as isozyme II [4]. Although the isozyme I was not completely homogeneous there is no cross-contamination between the two isozymes.

Fig. 2 shows the results of a SDS polyacrylamide electrophoresis of isozyme I after incubation in 1% SDS in phosphate buffer, pH 7.5, for 24 hr at 38°C. According to the earlier report [4], the major portion of isozyme I had a mobility commensurate with a mol. wt. of

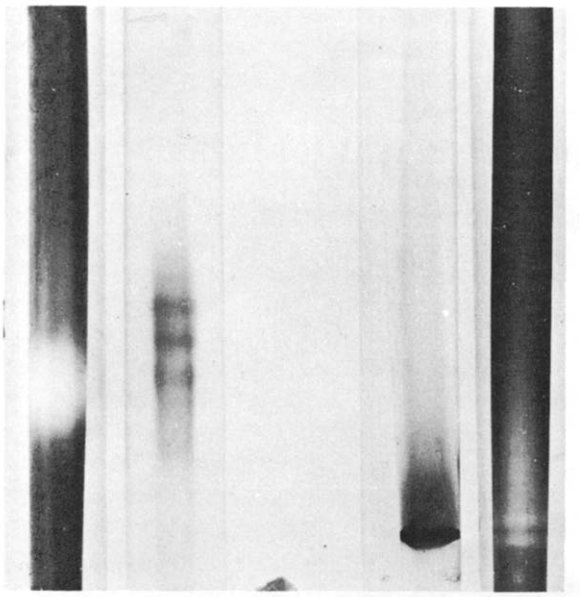

Fig. 1. Polyacrylamide gel electrophoresis of the two isozymes from wheat germ. From the left: isozyme I, stained for activity (20 μg of protein); isozyme I, stained for protein (50 μg) isozyme II, stained for protein (50 μg); isozyme II, stained for activity (20 μg).

approximately 16 000. In the same conditions isozyme II retained the mobility of the dimeric protein. If incubation was omitted before the run in SDS-polyacrylamide gel both isozymes moved as the dimer. Staining for activity clearly shows (fig. 2, right) that after incubation in SDS isozyme I gives two bands of activity, corresponding to molecular weights of ~ 32 000 and 16 000. Therefore both the dimeric and monomeric forms display dismutase activity. After removal of SDS (fig. 3) isozyme I partially recovers the mobility in SDS-polyacrylamide electrophoresis typical of the dimer.

A more quantitative evaluation of these observations was attempted by activity measurements in solution (fig. 4). The activity of isozyme II was not affected by incubation in 1% SDS, while the activity of isozyme I decreased to less than 10% of the original value after incubation. This value certainly includes the activity of the dimer, as demonstrated by the two bands of activity on the gels (fig. 2), and can be taken as an upper limit for the activity of the protomer in 1% SDS with respect to the activity of the dimer. Removal of SDS led to a partial recovery of the activity

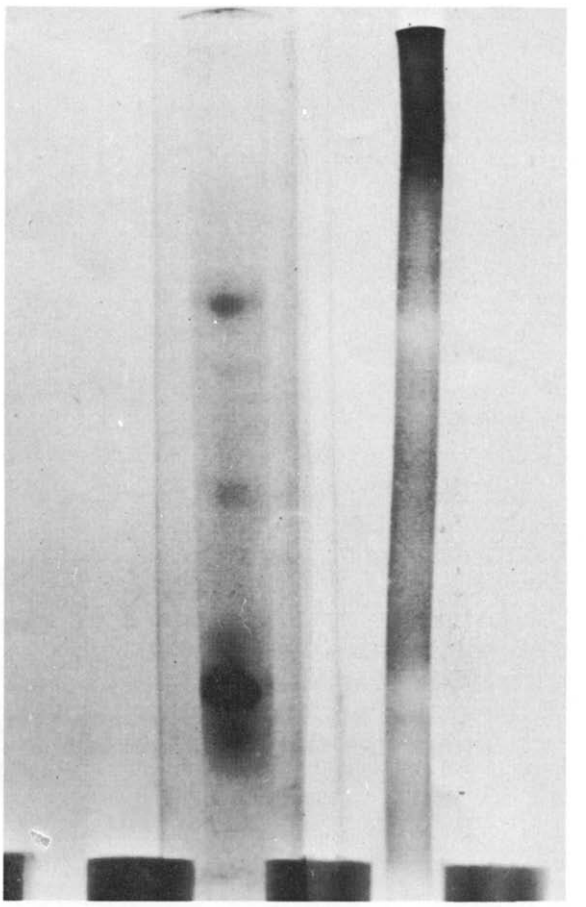

Fig. 2. SDS-polyacrylamide gel electrophoresis of isozyme I. A sample of the isozyme (10.1 mg/ml) was incubated for 24 hr at 38°C in 1% SDS before application to the gels. Left: run for protein staining (80 μg of protein); right: run for activity staining (40 μg).

which amounted to about 30%, after 2 days standing at 4°C.

It can be concluded that there is good evidence that the dimeric structure itself renders superoxide dismutase a more efficient catalyst. In fact the extent of renaturation of isozyme I after removal of SDS indicates that the detergent does not bring about an irreversible alteration of the active site of the enzyme, and that the change of activity parallels the change protomer–dimer in a way suggesting reversible effects on the active site by the quaternary assembly of the molecule. However direct contribution by SDS binding in determining the low activity of the protomer can not be ruled out.

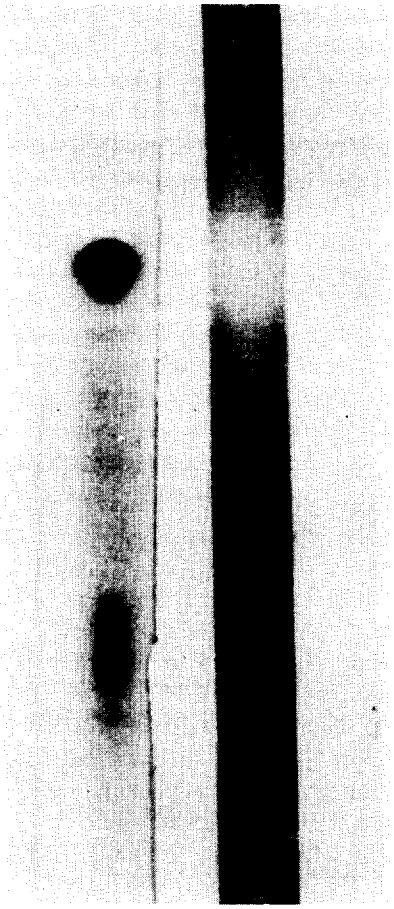

Fig. 3. SDS-polyacrylamide gel electrophoresis of isozyme I. Before application to the gels, the sample was incubated as described under fig. 2, treated to remove SDS (see text) and then left 48 hr at 4°C. Left: run for protein staining (80 μg of protein); right: run for activity staining (40 μg).

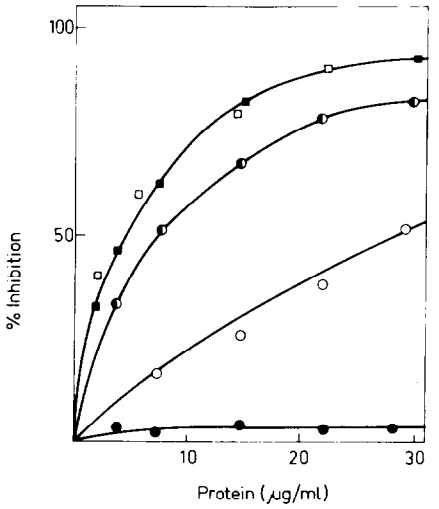

Fig. 4. Assay for superoxide dismutase activity of wheat germ isozymes (■) isozyme II; (□) the same after incubation for 24 hr at 38°C in 1% SDS; (◐) isozyme I; (●) the same after incubation for 24 hr at 38°C in 1% SDS; (○) the same, after incubation in SDS, removal of SDS (see text), and 2 days standing at 4°C.

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