DISSOCIATION OF Cu–Zn SUPEROXIDE DISMUTASE INTO MONOMERS BY UREA

Evidence from gel filtration and molecular hybridization

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1. Introduction

Copper–zinc superoxide dismutases are dimeric enzymes with mol. wt ~33 000, which contain 1 functional copper and 1 auxiliary zinc, located close to each other on identical subunits [1]. In spite of spectroscopic and structural equivalence of the copper binding sites, the copper ion on one subunit influences equilibrium and kinetic properties of the copper [2]. Intersubunit interaction appear to be also relevant to the catalytic mechanism [3]. Thus, obtaining active monomers of copper–zinc superoxide dismutase is vital to the understanding of the enzyme mechanism, especially considering that monomeric superoxide dismutases have never been discovered. Sodium dodecyl sulfate (SDS) has been used to attempt dissociation of these enzymes and has been found to have different effects on various superoxide dismutases. While the bovine enzyme, which is the most extensively studied, has been reported to be resistant to dissociation in SDS [4] partially active monomers ($\leqslant$ 50% native enzyme activity) have been obtained by SDS treatment of one isoenzyme from wheat germ [5]. Evidence for dissociation in 8 M urea of a fish superoxide dismutase has been presented on the basis of a gel filtration experiment [6]. In this report we show that dissociation into monomers of copper–zinc superoxide dismutases by 8 M urea seems to be a general property of this class of enzymes and indicates a peculiar sensitivity of their quaternary structure to this denaturing agent.

2. Materials and methods

Superoxide dismutase was purified from bovine erythrocytes as in [7], from yeast as in [8], and from wheat germ (isozyme II) as in [9].

Urea solutions were deionized with AG 501–X8D mixed bed resin (Bio Rad) immediately before use.

Gel filtration chromatography was carried out in 0.1 M phosphate buffer, pH 7.4, on an Ultrogel Ac 54 (LKB) column (82 × 1.5 cm), with elution rate of 14 ml/h and 1.8 ml fraction volume. Polyacrylamide gel electrophoresis and activity staining of gels were carried out as in [10].

3. Results

3.1. *Gel filtration experiments*

Figure 1 reports elution volumes of bovine and yeast superoxide dismutases in various conditions with reference to protein protomers of known molecular weight. In 8 M urea, either with or without β-mercaptoethanol, both superoxide dismutases elute with cytochrome *c*. This result is independent of incubation time in urea prior to chromatography. Elution volumes of superoxide dismutase samples eluted from columns in 8 M urea and then rechromatographed in the absence of urea were the same as that of native protein. Moreover, the mobility of the same samples in polyacrylamide gel electrophoresis in 1% SDS was that of dimeric molecules.

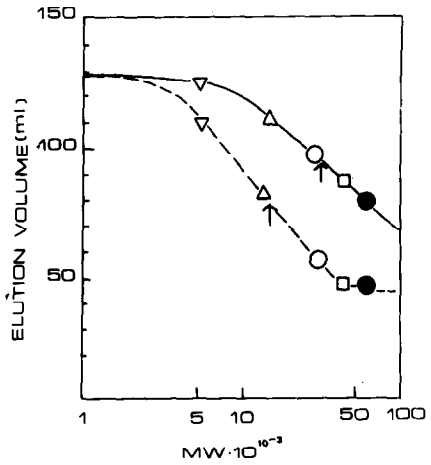

Fig.1. Gel filtration chromatography of bovine and yeast superoxide dismutases (↑) in the presence (– – – –) and absence (————) of urea (8 M). 1 ml protein samples (3–10 mg/ml) were applied to the columns. (▽) Insulin; (△) cytochrome *c*; (○) carbonic anhydrase; (□) ovalbumin; (●) serum albumin. The columns were also calibrated with pyridoxal-5-phosphate and blue dextran in the low and high mol. wt ranges, respectively. All solutions were in 0.1 M phosphate buffer, pH 7.4.

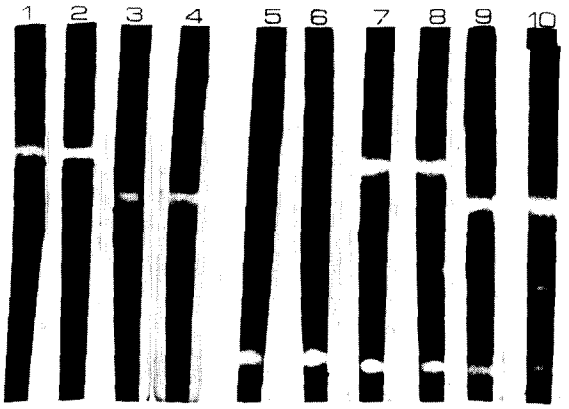

Fig.2. Polyacrylamide gel electrophoresis of copper–zinc superoxide dismutases in various conditions. (1–2) bovine enzyme; (3–4) yeast enzyme; (5–6) WG II enzyme; (7–8) bovine plus WG II enzymes; (9–10) yeast plus WG II enzymes. Samples 2,4,6,8 and 10 were incubated in 8 M urea and then dialyzed against buffer before electrophoresis. Protein solutions, 3 μl (1 mg/ml in 0.1 M phosphate buffer, pH 7.4) were applied to each gel.

3.2. *Formation of hybrid superoxide dismutases after urea treatment*

Bovine and yeast enzyme were separately incubated in 8 M urea with another copper–zinc superoxide dismutase known to have a very distinct mobility in gel polyacrylamide electrophoresis, i.e., the electrophoretically-faster wheat germ isozyme, referred to as WG II [9]. After overnight incubation at 4°C, urea was dialysed out, and the mixture subjected to electrophoresis. Figure 2 reports activity stained electropherograms of the two separate experiments with the proper controls. It is apparent that active hybrid bands are formed in both cases after urea treatment. No hybrid was detected if the mixture in urea was applied to the gels without prior dialysis against buffer.

4. Discussion

Sword fish copper–zinc superoxide dismutase has been suggested [6] to dissociate into monomers in 8 M urea on the basis of chromatographic evidence. Our gel filtration experiments show that the same chromatographic behaviour as the sword fish enzyme is observed with the bovine and yeast superoxide dismutases, that is two molecules structurally most distant from each other within the same class of homologous proteins [11]. Thus this appears to be a general property of copper–zinc superoxide dismutases. The question arises if transport methods in the presence of denaturants can give an unequivocal answer as far as molecular weight is concerned. In fact such methods rely on the assumption of complete denaturation of the molecule under study. Therefore conclusions are uncertain especially when a molecule that does not apparently change any typical property in 8 M urea such as bovine superoxide dismutase [12] is compared to protein molecules routinely used as molecular weight standards in denaturing conditions.

While preparing a detailed study of the effect of urea on copper–zinc superoxide dismutases, we now present evidence that monomers are actually formed in urea by these enzymes. Formation of hybrids after incubation in 8 M urea of either bovine or yeast superoxide dismutase with WG II is an unequivocal proof of that. We did not attempt to quantify the

extent of hybrid formation, since the process is obviously affected by the degree of homology between the hybridizing species. Work is in progress along this line, with special aim to the isolation of these hybrids on a preparative scale.

Some conclusive points are however clear even at this stage:

1. The effect of urea seems to be limited to destabilization of the dimeric structure, as other properties of these enzymes appear to be almost unaffected [6,12,13].
2. Urea at 8 M, at variance with SDS, leads to monomer formation independently of the enzyme source and other complementary treatments, such as the presence of β-mercaptoethanol or the removal of metals [4,14].

Thus urea appears to be a very specific destabilizing agent for the quaternary structure of this class of enzymes.

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