SUPEROXIDE DISMUTASE IN BAKER'S YEAST

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1. Introduction

The copper and zinc containing protein frequently called haemocuprein, hepatocuprein, cerebrocuprein, erythrocuprein or erythrocupro–zinc protein has been found widely distributed not only in mammalian organisms [1, 2] but also in plants [3] and recently in *Neurospora crassa* [4]. This bimetallic protein seems to play a most important rôle in aerobic organisms, namely it disproportionates superoxide anions [5] and/or scavenges singlet oxygen [6–8].

In the present study the successful isolation of this metalloprotein from *Saccharomyces cerevisiae* is described. The UV spectrum of this microbial superoxide dismutase was slightly different from that of the bovine species. Only 3–4 half cystine residues (compared to the 6 half cystines of the mammalian or plant type protein) were detectable. The average molecular weight was 31 200 ± 200; ϵ_{259} = 9800 and ϵ_{680} = 156. 16 000 Mol.wt. subunits appeared already after treatment with urea and sodium dodecyl sulphate and in the absence of mercaptoethanol or $NaBH_4$. The preparation was homogeneous as shown by gel filtration with and without an applied electric field as well as during ultracentrifugation. Further physicochemical and enzymic properties were in accordance with the mammalian or plant type Cu–Zn-protein.

2. Experimental

A homogeneous strain of *Saccharomyces cerevisiae* was purchased from Fa. Lindenmeyer & Co., Heilbronn. The cells of 1 kg yeast were broken by grinding aliquots together with reagent grade quartz sand. The homogenate was suspended in 4.5 l ice cold 5 mM phosphate buffer, pH 7.5 and centrifuged at 13 000 *g* for 1 hr. Further preparation was performed using the modified procedure given in [9]. Amono acid analysis was performed after hydrolysis with 6 N HCl for 36 hr using a Beckman Unichrom amono acid analyser. Copper and zinc were assayed by atomic absorption spectroscopy [9]. Ultraviolet and visible spectra were recorded in a Unicam SP 1800. Disc-polyacrylamide gel electrophoresis was carried out according to Maurer [10]. Superoxide dismutase activity was measured using both the diminished cytochrome *c* reductase activity [11, 12] or the scintillation technique [8].

3. Results

Using the present preparation procedure approx. 11 mg of purified bluish green superoxide dismutase were obtained. The metal content was 1.8 g atoms of copper and 2.0 g atoms of zinc per mole of protein. Storage under liquid nitrogen proved most appropriate. No change in the physicochemical and enzymic properties were observed even after a period of 6 months. The disc electrophoretic behaviour was slightly different from bovine erythrocuprein (fig. 1).

As in the case of erythrocuprein a second weak band is visible which is not detectable using G-75 Sephadex gel filtration or DEAE-23 chromatography. Sodium dodecyl sulphate polyacrylamide gel electrophoresis in the presence of 4 M urea revealed 16 000 mol.wt. subunits. From sedimentation velocity measurements a $S_{20\,w}$-value of 3.1×10^{-13} was determined. The UV absorption (ϵ_{259} = 9800) was rather similar as reported for bovine erythrocuprein (ϵ_{259} = 9840) (fig. 2). However, the absorption profile was somewhat different. It had many features in common with the

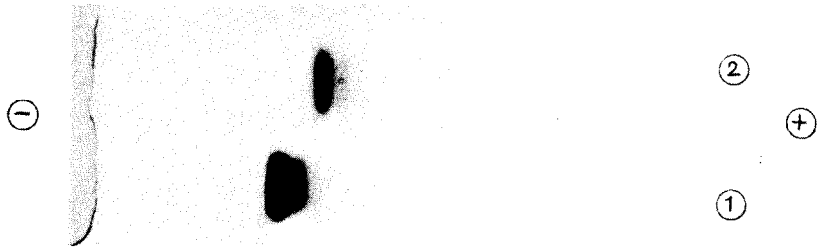

Fig. 1. Disc-polyacrylamide electrophoresis of superoxide dismutase isolated from bovine erythrocytes (1) and *Saccharomyces cerevisiae* (2). The acrylamide concentration was 4% in the concentrating gel and 7% in the separating gel. Electrophoresis was performed at 200 V, 30 mA for 2 hr. The enzyme concentrations were: (1) 50 μg; (2) 18 μg.

absorption profile of apoerythrocuprein. The absorption in the visible region was very low. A maximum at 680 nm (ϵ_{680} = 156) and a shoulder at 430 nm were detectable (fig. 3).

The amino acid analysis revealed some interesting results (table 1). The content of half cystine, glycine and isoleucine, respectively, was considerably low, while arginine, glutamate, tyrosine and phenylalanine were present in much higher concentrations compared to bovine erythrocuprein.

Further physicochemical studies employing CD and EPR-spectroscopy revealed great similarities with native bovine erythrocuprein. The specific enzymic activity was exactly the same as found for mammalian superoxide dismutase.

4. Discussion

With the present isolation and characterisation of another microbial superoxide dismutase the ubiquity of this Cu–Zn-protein in all aerobic cells appears to become progressively established. The differences in the amino acid composition can be attributed to evolutionary reasons. An interesting phenomenon was the splitting of the native protein into 16 000 mol.wt. subunits in the presence of protein unfolding agents, and in the absence of disulphide reducing agents. In contrast to the bovine erythrocuprein, the microbial enzyme probably has an activated disulphide bonding, or, disulphide bridges may even be completely absent.

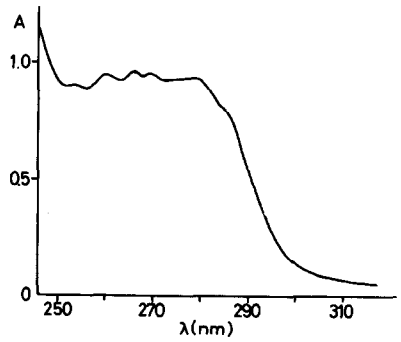

Fig. 2. UV spectrum of superoxide dismutase from *Saccharomyces cerevisiae*. The protein concentration was 3 mg/ml in 50 mM phosphate buffer, pH 7.2. One cm light path cells were employed.

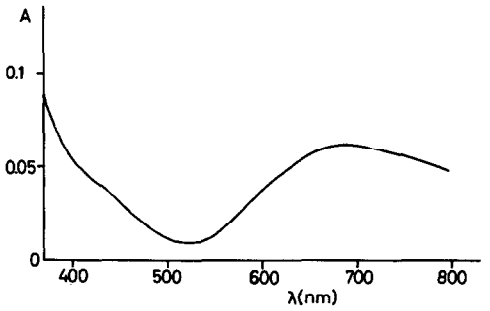

Fig. 3. Absorption of superoxide dismutase from *Saccharomyces cerevisiae* in the visible region. Protein concentration 3 mg/ml dissolved in 50 mM phosphate buffer, pH 7.2. 4 cm light path cells.

Table 1
Amino acid analysis of superoxide dismutase isolated from bovine erythrocytes and *Saccharomyces cerevisiae*.

	Superoxide dismutase isolated from	
	Erythrocytes [13]	*S. cerevisiae*
Lysine	22.8	27.4
Histidine	16.8	14.8
Arginine	8.7	13.6
Aspartic acid	34.1	39.1
Threonine	22.4	19.2
Serine	15.4	18.7
Glutamic acid	22.5	35.5
Proline	14.6	15.2
Glycine	50.4	23.0
Alanine	19.6	19.7
Valine	28.4	27.2
Methionine	2.1	0
Isoleucine	18.1	13.0
Leucine	17.5	16.4
Tyrosine	2.3	4.0
Phenylalanine	8.5	19.1
Tryptophan	2	
Half cystine	5.9	3.5

The values given in the table represent the number of amino acid residues per mole of protein.

The higher concentrations of phenylalanine and tyrosine can be made responsible for the higher absorption in the 280 nm region. Bovine erythrocuprein contains only half of these amino acids with the consequence of an unusual low absorption at 280 nm.

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