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THE COMPLETE AMINO ACID SEQUENCE OF MANGANESE-SUPEROXIDE DISMUTASE FROM SACCHAROMYCES CEREVISIAE

by

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The complete amino acid sequence of manganese superoxide dismutase isolated from Saccharomyces cerevisiae has been determined by automated Edman degradation. Peptides for sequence analysis were produced by cleavage with cyanogen bromide, hydroxylamine and S. aureus protease V8. The native enzyme consists of four identical polypeptide chains 203 residues long each containing a single sulfhydryl group and no disulfide bridges. A subunit molecular weight of 22,690 was calculated from the complete sequence. The sequence exhibits a significant degree of homology with the manganese superoxide dismutase from E. coli and B. stearothermophilus.

Abbreviations: ANS = 2-amino-1,5-naphthalene disulfonic acid; CNBr = cyanogen bromide; GuHCl = guanidinium hydrochloride; HFBA = heptafluorobutyric acid; PMSF = phenylmethylsulfonylfluoride; Polybrene = 1,5-dimethyl-1,5-diazaundecamethylene polymethobromide; PTH = phenylthiohydantoin; SOD = superoxide dismutase; THEED = N,N,N'N'-tetrakis(2-hydroxyethyl)ethylene diamine; Tris = 2-amino-2-hydroxymethyl-1,3-propanediol.

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1. INTRODUCTION

Superoxide dismutases are metalloenzymes whose function is to scavenge the superoxide radical anion O_2^- by catalyzing its dismutation into hydrogen peroxide and dioxygen (11, 12). The enzymes fall into three classes according to their metal content. The Cu,Zn-enzymes appear to be restricted to cytoplasma of eukaryotes and are unrelated in amino acid sequences to the two other classes, the Mn-SOD's and the Fe-SOD's. These two classes are found in prokaryotes and eukaryotes and have homologous amino acid sequences as judged from sequence studies restricted to the N-terminal part of a variety of enzymes. The complete amino acid sequence of Mn-SOD isolated from E. coli (26) and B. stearothermophilus (5) have been published. The present report presents the complete amino acid sequence of Mn-SOD isolated from S. cerevisiae and compares it with that of the two bacterial enzymes.

2. MATERIALS AND METHODS

2.1. Materials

Yeast Mn-superoxide dismutase was isolated as described in the preceding paper (7). S. aureus (strain V8) protease was a product of Miles Laboratories, England. Sephadex G-100 superfine, G-50 superfine and G-15 were obtained form Pharmacia Fine Chemicals, Sweden. CM-52 cellulose was a product from Whatman, England. 2-Amino-1,5-naphthalene-disulphonic acid was a laboratory reagent which was twice recrystallized prior to use. Reagents and solvents used with the sequencer were as described in (18). All other reagents were analytical grade products. Distilled water was used throughout.

2.2. Amino acid analysis

The amino acid composition of the native Mn-SOD was determined as described previously (7). Isolated peptides were hydrolyzed for 24 hours at 110 °C in constant boiling 5.7 M-HCl. Hydrolyzates were analysed on a Durrum 500 automatic amino acid analyzer. Half cystine was determined as cysteic acid after performic acid oxidation.

In one case the amino acid composition of a peptide was determined by digestion with Pronase (23). The lyophilized peptide, 25 nmol, was redissolved in 50 µl 0.020 M-Tris-Cl, 0.020 M-$CaCl_2$, pH 7.6 containing 25 µg Pronase and the solution incubated at 37 °C for 24 hours. The digestion was stopped by the addition of 10 µl PMSF (2 mg · ml^{-1} in ethanol) and the reaction mixture lyophylized. The residue was redissolved in sodium citrate buffer and analyzed on the Durrum 500 automatic amino acid analyser.

2.3. Amino acid sequence determination

Automated sequence determination was performed using a Beckman 890C sequencer by the method of EDMAN and BEGG (9). The use of THEED as coupling buffer (2) and other modifications have been described (18). Samples with succinylated lysine residues were applied to the sequencer cup in 0.25% NH_4OH, instead of in 30% acetic acid. Polybrene was added to the cup with the sample in several instances as suggested by TARR et al. (28) with the omission of the dummy step. In one case, a peptide was coupled to ANS according to the procedure described by FOSTER et al. (10) and the reaction mixture applied directly to the cup. Conversion to the PTH-amino acids was performed as described earlier (18), and identification of the PTH-amino acids was made by HPLC as described by SVENDSEN et al. (27). Additional information was obtained by TLC (20) or by back-hydrolysis to the parent amino acid (22).

In the course of this study, we observed unacceptably large decreases in the repetitive yield when serine or threonine were encountered in a sequence. This appeared to be caused by esterification of these amino acids by HFBA-anhydride followed by an N→O acyl shift of the HFBA group when serine or threonine became the N-terminal residue. This drop in yield was alleviated by addition of 1% (v/v) distilled water to the HFBA prior to use in the sequencer. The addition of water did not cause any appreciable increase in non-specific cleavage of peptides during sequencing.

2.4. Succinylation of Mn-SOD

One µmole, 22 mg, of the native enzyme was dissolved in 1.5 ml 5 M-guanidine hydrochloride and the pH of the solution adjusted to 8.5 with

NaOH. Solid succinic anhydride, 100 mg, 70 fold excess over total lysines, was added in small portions over a period of 45 min at room temperature. The pH of the solution was maintained at 8.5 with 6 M-NaOH in a pH-stat. The yellow brown solution was stirred for 1 hour after the final anhydride addition. In order to remove any succinyl groups present on threonine or serine hydroxylamine hydrochloride was added to a final concentration of 0.1 M and the resulting pale yellow solution stirred for an additional hour at pH 8.5 (17). The succinylated protein was separated from reactants and salts by dialysis against 2000 volumes 0.010 M-NH_4HCO_3, pH 8 for 24 hours at room temperature and finally lyophilized.

2.5. Pyridylethylation of cysteine residues

The lyophilized cysteine containing peptide, 600 nmoles, was dissolved in 1 ml 0.1 M-Tris-Cl, 5 M-GuHCl, pH 7.8. Five µl 2-mercaptoethanol, (120 fold excess over cysteine), were added and the solution stirred under N_2 for 16 hours at room temperature. 4-Vinylpyridine, 8.5 µl (1.1:1 molar ratio over total sulfhydryls) was added and the solution stirred for 4 hours under N_2 at room temperature (13). The reaction mixture was acidified with 0.3 ml glacial acetic acid. The modified peptide was desalted by gel filtration on Sephadex G-15 in 30% acetic acid and lyophilized.

2.6. Cyanogen bromide cleavage

The native enzyme, 22 mg, 1 µmole, was dissolved in 2 ml 70% formic acid and 50 mg CNBr were added. This solution was stirred for 24 hours in the dark under N_2 at room temperature (19). Excess CNBr was removed by aeration of the solution with N_2 and the solution was then diluted with 20 ml distilled water and lyophilized.

The freeze-dried material was suspended in 3 ml of 0.1 M-NH_4HCO_3 and the pH adjusted to 9.5 with NH_4OH. After centrifugation the supernatant was applied to a column of Sephadex G-100 superfine (1.5 × 90 cm) eluted with 0.01 M-NH_4HCO_3. The precipitate was dissolved in 1 ml 30% acetic acid and chromatographed on Sephadex G-100 superfine as for the supernatant but using 30% acetic acid as eluant. The resulting peptide peak was recovered after lyophilization.

2.7. Hydroxylamine cleavage

Hydroxylamine cleavage was performed using a modification of the method described by BORNSTEIN (3), omitting GuHCl from the reagent solution. One µmole, 22 mg, succinylated Mn-SOD was dissolved in 3 ml ice cold 0.2 M-K_2CO_3, containing 2 M-$NH_2OH \cdot HCl$, the pH adjusted to 9.0 with NH_4OH, and the solution incubated at 45 °C for 4 hours. A white precipitate, formed during the digestion, was removed by centrifugation and the resulting supernatant desalted by gel filtration on Sephadex G-15 in 10 mM-NH_4HCO_3. The precipitate was redissolved in 2 ml 0.1 M-NH_4HCO_3 and likewise desalted on Sephadex G-15. The desalted peptide solutions were lyophilized prior to solubilization of each in 1 ml of 0.01 M-NH_4HCO_3 and chromatographed on a column of G-50 superfine (1.5 × 90 cm) using 0.01 M-NH_4HCO_3 as eluant.

2.8. S. aureus protease cleavage

One µmole, 22 mg, succinylated Mn-SOD was dissolved in 2 ml 0.05 M-NH_4HCO_3, pH 8.0, and 0.7 mg S. aureus protease added (8). The solution was incubated for 16 hours at 25 °C. The reaction was stopped with an excess of PMSF and the mixture chromatographed on Sephadex G-50 superfine as described in 2.7. The short peptides were further purified on CM-52 cellulose after lyophilization. The peptides were dissolved in 2 ml of 0.025 M-sodium acetate buffer, pH 4.5 and applied to a column of CM-52 cellulose (1.5 × 2 cm) equilibrated in this buffer. The column was washed with 35 ml of equilibration buffer and eluted with a linear gradient to 0.2 M-NaCl. The relevant peptide was located by the phenanthroquinone spot test (16) and subsequently desalted on Sephadex G-15 and lyophilized.

2.9. Peptide nomenclature

The two CNBr fragments are referred to as CN-1 and CN-2 corresponding to the N-terminal

and C-terminal fragment, respectively. Hydroxylamine peptides are numbered HA-1 to HA-3 according to the order in which they occur in the native protein starting from the N-terminal. The two S. aureus protease peptides of interest were designated SP-10 and SP-12 in accordance with their location in the complete sequence.

3. RESULTS

3.1. Amino acid sequencing of the holoenzyme

Amino terminal sequence analysis of the holoenzyme was performed twice using 100 nmoles each time and allowed the unambiguous identification of the first 46 amino acid residues; the repetitive yield being 95% in each case (Figure 5).

3.2. Cyanogen bromide peptides

Since the native Mn-SOD contained a single methionine residue, two peptides were expected from cyanogen bromide cleavage. The lyophilized CNBr fragments were suspended in 0.1 M-NH_4HCO_3, centrifuged and the supernatant was chromatographed on Sephadex G-100 superfine in 0.010 M-NH_4HCO_3, pH 8.0, resulting in the chromatogram shown in Figure 1A. The precipitate was dissolved in 1 ml of 30% acetic acid and gel filtered on Sephadex G-100 superfine in this solvent (Figure 1B). The amino acid composition of peak IIIA was identical with that of peak IIIB and both contained homoserine and homoserine lactone, suggesting that they were the N-terminal cyanogen bromide fragment CN-1 which was later confirmed by sequence analysis. Fragment CN-2 eluted as two peaks in both the basic (Figure 1A, IA and IIA) and acidic (Figure 1B, IB and IIB) chromatograms. The amino acid composition of peaks IA, IIA, IB and IIB appeared to be identical and peak I in each chromatogram appeared to be an aggregate of the peptide eluting in peak II. The aggregate may be a dimer of peptide CN-2 formed by two Mn-SOD monomers through the single cysteine which had not been modified prior to cleavage. Therefore, the four peaks (I and II from Figure 1A and IB) were combined and lyophilized, followed by reduction of any disulphide bridges present and pyridylethylation of the cysteine (13)

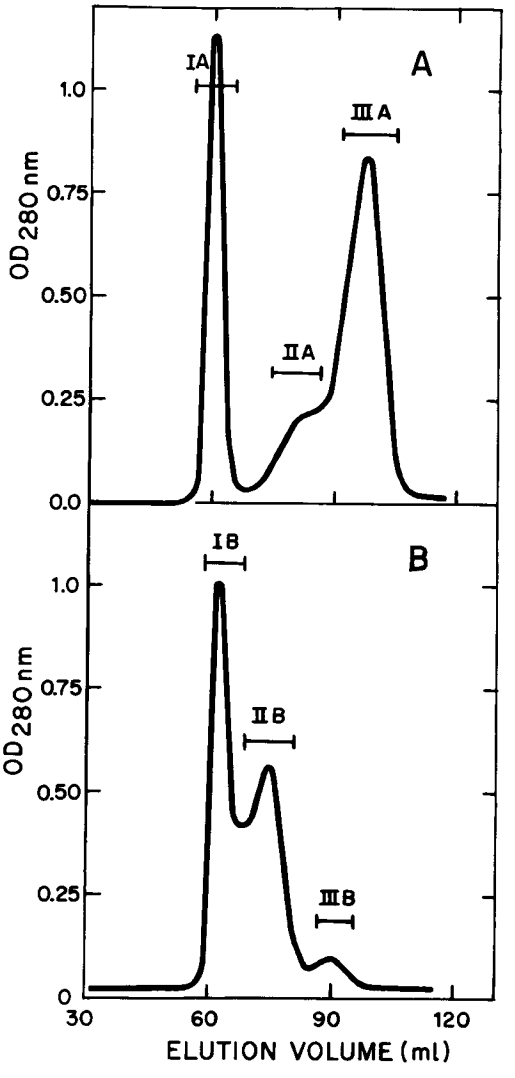

Figure 1. Separation of cyanogen bromide peptides from yeast Mn-SOD on Sephadex G-100 superfine.

A. NH_4HCO_3 soluble fraction, 1.5 ml, applied to a 1.5×90 cm column in 10 mM-NH_4HCO_3. B. Acid soluble fraction, 1 ml, applied to a 1.5×90 cm column in 30% acetic acid. Flow rate 7.5 ml·hour^{-1}, fractions of 1 ml were collected. Fractions were pooled as indicated by the horizontal bars.

prior to sequence analysis. The amino acid composition of CN-1 and CN-2 are presented in Table I. As expected, the sum of the amino acid composition of CN-1 and CN-2 closely resembles the composition of the native protein with the exception of valine, which apparently was

Table I

Amino acid composition of Mn-SOD and its two cyanogen bromide peptides.

Amino acid	Composition (residues/molecule)								
	CN-1			CN-2			CN-1 + CN-2	Mn-SOD	
Asx	7.7	(8)[d]		15.9	(17)		23.6	24.0	(25)
Thr	3.6	(4)		6.6	(7)		10.2	10.7	(11)
Ser	3.2	(3)		5.0	(5)		8.2	8.1	(8)
Glx	7.7	(8)		15.7	(16)		23.4	23.7	(24)
Pro	3.8	(4)		4.6	(5)		8.4	9.0	(9)
Gly	4.5	(3)		13.8	(14)		18.3	17.4	(17)
Ala	5.3	(5)		13.2	(13)		18.5	18.4	(18)
Val	2.7	(3)		5.6	(9)		8.3	11.1	(12)
Met	0.6[a]	(1)		0.0	(0)		0.6	1.1	(1)
Ile	2.4	(2)		6.7	(8)		9.1	9.9	(10)
Leu	6.6	(7)		12.0	(12)		18.6	19.0	(19)
Tyr	2.8	(3)		4.9	(5)		7.7	7.7	(8)
Phe	3.1	(3)		6.5	(7)		9.6	9.9	(10)
His	2.3	(3)		3.0	(3)		5.3	5.7	(6)
Lys	4.6	(5)		9.9	(10)		14.5	15.0	(15)
Arg	0.9	(1)		1.6	(2)		2.5	2.8	(3)
Cys	0.0	(0)		0.9[b]	(1)		0.9	1.0	(1)
Trp	N.D.	(1)		N.D.	(5)		N.D.	6.0	(6)
Total residues		64			139				203
Yield[c]	95%			61%					

[a] As homoserine and homoserine lactone.
[b] As cysteic acid.
[c] On the basis of 100% prior to CNBr-cleavage.
[d] Residues in parentheses are obtained from sequence studies.

not completely liberated during hydrolysis of CN-2.

No attempt to extend the sequence of CN-1, already determined from the holoenzyme (section 3.1) was made. Two sequence runs on pyridylethylated CN-2 provided identification of the first 56 residues out of the total 139 present. In the first experiment 150 nmoles were used and in the second 100 nmoles with the repetitive yield 96% and 95%, respectively.

3.2.1. Peptides from hydroxylamine cleavage

Sequence analysis of a preliminary hydroxylamine digest revealed that yeast Mn-SOD contained two asparagine-glycine bonds. One of these, located between residues 36 and 37, was known from the sequence analysis of the holoprotein, section 3.1. Comparison with the published sequences of Mn-SOD from E. coli (26) and B. stearothermophilus (5) indicated that the second asparagine-glycine bond was probably located near residue 140 in the yeast Mn-SOD polypeptide chain. Cleavage yields with hydroxylamine are generally 50–60% (3). Thus, a mixture of three hydroxylamine peptides, HA-1 to HA-3, two partially cleaved fragments, HA-1,2 and HA-3,2, and some uncleaved material could be expected from the digestion of yeast Mn-SOD with hydroxylamine. In order to simplify the peptide purification procedure, a strategy was adopted which required only the isolation of the approximately 60 residue long C-terminal peptide HA-3.

The soluble fraction of the hydroxylamine digest (section 2.7) produced three peaks when separated on Sephadex G-50 superfine (Figure 2A). The redissolved digest-precipitate produced 2 peaks and a small shoulder when applied to the same column (Figure 2B). The amino acid

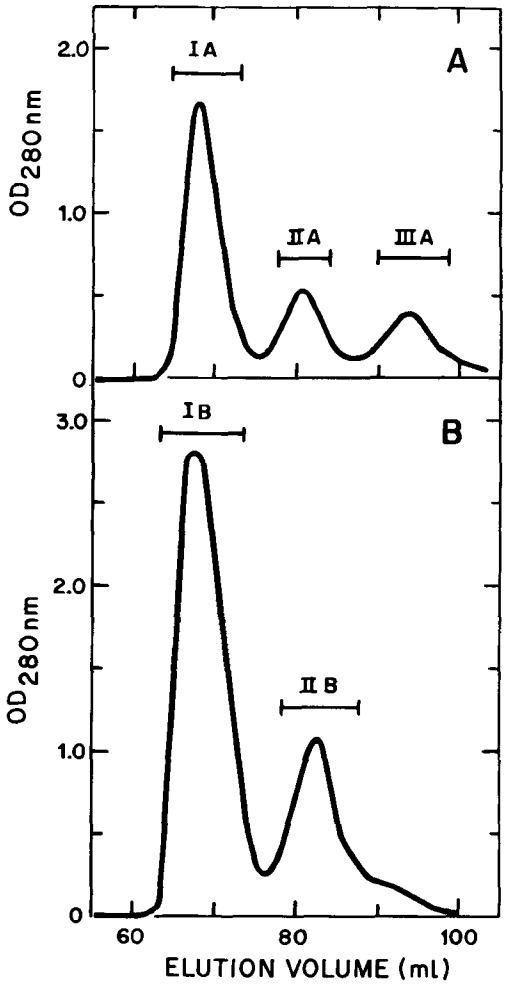

Figure 2. Fractionation of hydroxylamine peptides from yeast Mn-SOD on Sephadex G-50 superfine.

A. The desalted and lyophilized soluble fraction of the reaction mixture (A) and the reaction precipitate (B) were redissolved in 1 ml 10 mM-NH_4HCO_3. Each sample was applied to a 1.5 × 90 cm Sephadex G-50 superfine column and eluted with 10mM-NH_4HCO_3 pH 8.0 at a flow rate of 9 ml·hour^{-1}. One ml fractions were collected. Fractions were pooled as indicated by the horizontal bars.

composition of peak III was similar to the composition of HA-1 calculated from the known N-terminal sequence (Table II). This peak was not analysed further. The amino acid composition of peaks IIA and IIB were identical, consisting of about 60 amino acids including two arginines, suggesting that it was the C-terminal peptide. Consequently, these peaks were pooled and lyophilized. Sequence analysis permitted the identification of this peptide as HA-3 on the basis of an overlap with a peptide from the S. aureus protease digest, (see below), and homology with portions of the sequence of E. coli (26) and B. stearothermophilus (5) Mn-SOD. HA-2 was eluted in the void volume (peaks IA and IB) along with fragments HA-1,2 and HA-2,3, and uncleaved Mn-SOD. In this mixture, only the de novo N-terminal at HA-2 was susceptible to Edman degradation since the native N-terminal (HA-1) was succinylated prior to hydroxylamine digestion. Accordingly, peaks IA and IB were pooled, lyophilized, and subjected to sequence analysis without further purification. The amino acid compositions of hydroxylamine peptides HA-1 and HA-3 are seen in Table II. Sequence analysis of HA-2 and HA-3 provided 33 and 58 amino acid residues, respectively, with approximately 100 nmoles sequenced twice for each

Table II

Amino acid composition of isolated hydroxylamine peptides from yeast Mn-SOD.

Amino acid	Composition (residues/molecule) HA-1	HA-3
Asx	4.1 (4)[a]	7.6 (9)
Thr	2.5 (3)	2.7 (3)
Ser	1.1 (1)	1.0 (1)
Glx	4.3 (4)	5.9 (6)
Pro	1.6 (2)	1.8 (2)
Gly	2.3 (2)	3.9 (3)
Ala	1.5 (1)	6.1 (6)
Val	1.7 (2)	4.8 (7)
Met	0.0 (0)	0.0 (0)
Ile	2.0 (2)	2.7 (2)
Leu	4.0 (4)	4.1 (4)
Tyr	2.4 (3)	4.1 (5)
Phe	1.1 (1)	2.0 (2)
His	2.9 (3)	0.9 (1)
Lys	3.2 (3)	4.9 (5)
Arg	0.0 (0)	1.7 (2)
Cys	N.D. (0)	N.D. (0)
Trp	N.D. (1)	N.D. (3)
Total residues	36	61
Yield	45%	52%

[a] Residues in parentheses are obtained from sequence studies.

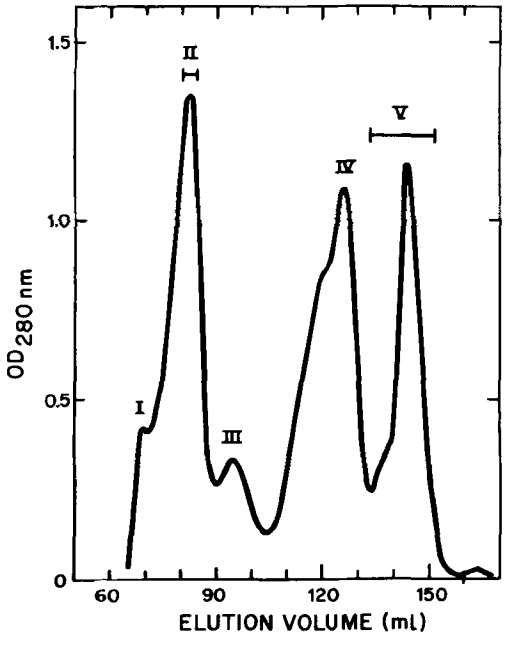

Figure 3. Fractionation of S.aureus protease peptides from yeast Mn-SOD on Sephadex G-50 superfine.

The digest, 2 ml, was applied to a 1.5 × 90 cm column in 10 mM-NH_4HCO_3 and eluted at a flow rate of 9 ml·hour^{-1}, fractions of 1 ml were collected. Fractions were pooled as indicated by horizontal bars.

peptide. The repetitive yields were 95% for HA-2 and 96% for HA-3 which was sequenced in the presence of Polybrene.

3.2.2. Peptides from digestion with S. aureus protease

The S. aureus protease digest was fractionated on Sephadex G-50 superfine (Figure 3). Peak II, which eluted shortly after the void volume, contained a single peptide, later identified as SP-10, composed of about 50 amino acids (Table III). The first 31 amino acid residues were identified by sequence analysis of 100 nmoles in separate experiments, the repetitive yield was 95% in both.

Sequence analysis of the C-terminal hydroxylamine peptide, HA-3, indicated that the C-terminal S. aureus protease peptide was composed of 6 or 7 residues, 2 of which were arginine.

Table III

Amino acid composition of selected S. aureus peptides.

Amino acid	Composition (residues/molecule) SP-10	SP-12
Asx	6.8 (7)[a)]	1.1 (1)
Thr	4.5 (5)	– –
Ser	1.9 (2)	1.1 (1)
Glx	4.4 (4)	0.4 –
Pro	2.1 (2)	– –
Gly	5.8 (6)	0.5 –
Ala	4.0 (4)	1.0 (1)
Val	5.4 (7)	– –
Met	0.0 (0)	– –
Ile	2.5 (3)	– –
Leu	7.0 (7)	– –
Tyr	0.9 (1)	– –
Phe	1.1 (1)	1.0 (1)
His	– –	– –
Lys	2.9 (4)	0.4 –
Arg	– –	1.8 (2)
Cys	N.D. –	N.D. –
Trp	N.D. (2)	N.D. –
Yield	62%	34%

[a)] Residues in parentheses are obtained from sequence studies.

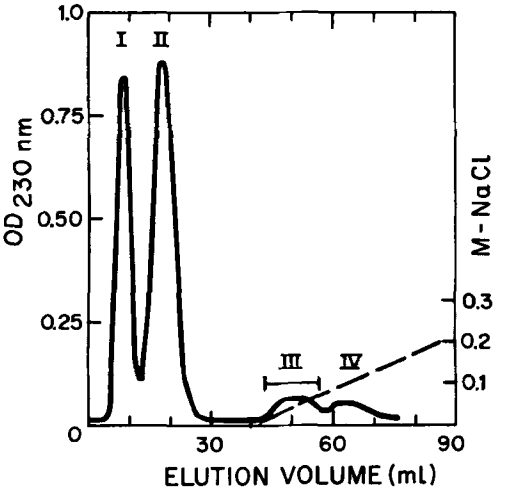

Figure 4. Rechromatography of peak V, Figure 3, on a 1.5 × 2 cm CM-52 cellulose column equilibrated in 25 mM-sodium acetate pH 4.5.

The lyophilized pool was redissolved in 2 ml equilibration buffer, applied to the column and eluted at a flow rate of 15 ml·hour^{-1}, fractions of 1 ml were collected. The arginine containing peptide in peak III was pooled as indicated, desalted on Sephadex G-15, and lyophilized.

Figure 5. Complete amino acid sequence of Mn-superoxide dismutase from S. cerevisiae.

⟶ Indicates amino acids sequenced in the following peptides: INT: intact protein; HA-2 and HA-3: hydroxylamine peptides 2 (peak IA and IB in Figure 2) and 3 (peak IIA and IIB in Figure 2); CN-2: cyanogenbromide peptide 2 (peak IIA and IIB in Figure 1); SP-10 and SP-12: staphylococcal protease peptides 10 (peak II, Figure 3) and 12 (peak III in Figure 4).

This peptide, SP-12, was isolated by ion exchange chromatography on CM-52 and identified on the basis of its arginine content. Of the 4 peaks in Figure 4, only peak III gave a positive reaction for arginine when treated with phenanthroquinone at alkaline pH (16). This peak was desalted on Sephadex G-15 in 50 mM-NH_4HCO_3 and lyophilized. The amino acid composition of

SP-12 is seen in Table III. Digestion with Pronase (23) indicated that SP-12 contained aspartic acid but not asparagine. Attempts to digest the peptide from its C-terminal end with carboxypeptidase Y (21) were unsuccessful. The C-terminal of SP-12 was coupled to ANS (10) prior to sequence analysis in order to minimize the loss of peptide material from the sequencer cup. Polybrene was also added to the cup with the ANS-treated material. The repetitive yield was low in this case but allowed the identification of all six residues starting with 200 nmoles (Figure 5).

3.3. Reconstruction of complete sequence

The complete amino acid sequence of Mn-SOD from S. cerevisiae is seen in Figure 5. This sequence has been reconstructed from the partial sequences determined for the holoprotein and 5 isolated peptides. Residues 1–46 were determined by sequence analysis of the Mn-SOD. Sequence analysis of fragment HA-2, beginning at glycine-37, gave a 10 residue overlap with the N-terminal segment and extended the known sequence to residue 69. Fifty-six Edman degradation cycles were performed on peptide CN-2, giving a 5 residue overlap with HA-2 and the sequence of residues 65–120. Sequence analysis of SP-10 gave a 4 residue overlap with CN-2 and established the sequence of residues 121 to 147. Of the 61 residues in HA-3, 58 were identified by sequence analysis, beginning with a 5 residue overlap with SP-10, and extending the known sequence to residue 200 of the native chain. Coupling of ANS to the C-terminal of SP-12 permitted the sequencing of all 6 residues of this peptide, which gave a 3 residue overlap with HA-3 and established the sequence of the remaining 3 C-terminal residues.

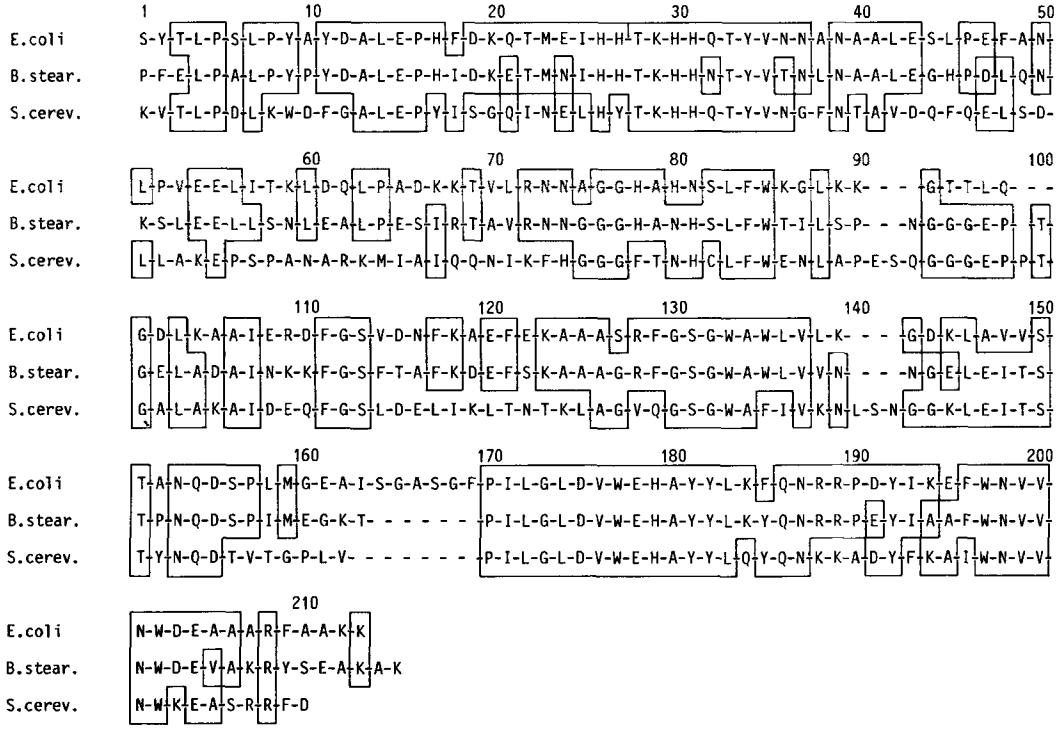

Figure 6. Comparison of amino acid sequences of Mn-superoxide dismutases from E. coli, B. stearothermophilus, and S. cereviciae.

------ Indicates gaps introduced to obtain maximal homology. Identical amino acids are boxed.

4. DISCUSSION

Yeast Mn-superoxide dismutase is an enzyme made up of four identical subunits each comprised of 203 amino acid residues and each having a molecular weight of 22,690. Previously the complete amino acid sequences of two bacterial Mn-SOD's isolated from E. coli (26) and B. stearothermophilus (5) have been published. A comparison of the sequence of yeast Mn-SOD with the sequences of these enzymes is made in Figure 6. The sequences are aligned to provide a maximum degree of homology. A high degree of homology is preserved among these enzymes. Thus, 82 amino acid residues are found in identical positions in the yeast and E. coli enzymes, while 76 residues are found in identical positions in the yeast and B. stearothermophilus enzymes. As might be expected, the homology between the two bacterial enzymes is higher (124 identical positions) than with that of the fungal enzyme. The homology is distributed throughout the molecule and indicates great similarities in the folding of the three enzymes. An attempt has already been made to compare the secondary structures of the two bacterial enzymes from their primary structure. It was shown that these enzymes were very similar in predicted α-helix, β-structure, bends and irregular structure (5). A similar conclusion is reached when all three enzymes are analysed according to the method of CHOU and FASMAN (6). The major differences are found in those parts of the sequence where it has been necessary to make deletions or insertions in order to maximize homology. These are all regions of irregular structure indicating that extra loops have been added (or removed) without impairing the main folding of the molecules. The sections in question are residues 90–100, 140–144, and 165–170.

Of particular interest are the sequences around the histidine residues since these (or some of these) are most probably involved in binding of the metal (5). Yeast Mn-SOD has histidine residues situated in positions 26, 30, 31, 74, 81, and 179 (Figure 6). Of these His 26, 30, 31, and 179 are situated in regions very homologous with the equivalent regions in the two bacterial enzymes while His 74 and 81 are not. The histidine residue in position 74 of the yeast enzyme has no counterpart in the two bacterial enzymes which may indicate that it is not of importance with respect to metal binding. The same argument may apply to His 17, 27, and 78 in both bacterial enzymes, His 45 in B. stearothermophilus and His 80 in E. coli which have no counterparts in the yeast enzyme. Since the three enzymes probably act via the same catalytic mechanism it is reasonable to suggest that identically situated histidine residues Nos. 26, 30, 31, and 179 are involved. No X-ray crystallographic data are yet available to prove or disprove this hypothesis.

No homology has been detected between the Mn-SOD described in the present paper and Cu, Zn-SOD's from various sources (14, 15). The presentation of a third complete amino acid sequence of an Mn-SOD adds further evidence to the postulate that Fe- and Mn-SOD's have developed independently of the Cu,Zn-SOD's and that the similarity in their reaction mechanism is a result of convergent evolution. X-ray crystallographic studies of several different manganese SOD's are reported to be in progress (1, 4, 24, 25). However, even without the results of these studies, it is evident that the tertiary structures of the manganese and copper-zinc SOD's will be markedly different.

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