

Young Sun Yun · Young Nam Lee

Purification and some properties of superoxide dismutase from *Deinococcus radiophilus*, the UV-resistant bacterium

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Abstract The superoxide dismutase (SOD, EC 1.15.1.1) of *Deinococcus radiophilus*, a bacterium extraordinarily resistant to UV, ionizing radiations, and oxidative stress, was purified 1,920-fold with a 58% recovery yield from the cell-free extract of stationary cells by steps of ammonium sulfate fractionation and Superdex G-75 gel-filtration chromatography. A specific activity of the purified enzyme preparation was ca. 31,300 U mg⁻¹ protein. *D. radiophilus* SOD is Mn/FeSOD, judging by metal analysis and its insensitivity to cyanide and a partial sensitivity to H₂O₂. The molecular weights of the purified enzyme estimated by gel chromatography and polyacrylamide gel electrophoresis are 51.5 ± 1 and 47.1 ± 5 kDa, respectively. The SOD seems to be a homodimeric protein with a molecular mass of 26 ± 0.5 kDa per monomer. The purified native SOD showed very acidic pI of ca. 3.8. The enzyme was stable at pH 5.0–11.0, but quite unstable below pH 5.0. SOD was thermostable up to 40°C, but a linear reduction in activity above 50°C. Inhibition of the purified SOD activity by β-naphthoquinone-4-sulfonic acid, ρ-diazobenzene sulfonic acid, and iodine suggests that lysine, histidine, and tyrosine residues are important for the enzyme activity. The N-terminal peptide sequence of *D. radiophilus* Mn/FeSOD (MAFELPQLPYAYDALEPHIDA(>D) is strikingly similar to those of *D. radiodurans* MnSOD and *Aerobacter aerogenes* FeSOD.

Keywords *Deinococcus radiophilus* · pI · Mn/Fe superoxide dismutase · N-terminal peptide sequence · UV resistance

Introduction

The genus *Deinococcus*, an obligate aerobic bacterium, has some peculiar features of thick cell wall-contained L-ornithine, an outer membrane-like structure, and a membrane-bound carotenoid pigment (Murray 1986; Müller and Engel 1996). The major peculiarity of *Deinococcus* is its extreme resistance to UV, ionizing radiation, and oxidative stress (Murray 1986; Carroll et al. 1996; Battista et al. 1999; Yun and Lee 2003). Although the unusual radioresistance of *Deinococcus* could be somewhat related with its morphological characteristics, one easily assumes that the extreme resistance of the *Deinococcus* against UV and strong oxygen stress would be associated with efficient reactive oxygen species scavenging systems, along with the repairing of damaged cellular components mediated by toxic oxidants. The repairing genes for damaged DNA were extensively studied (Evans and Moseley 1983; Gutman et al. 1994; Agostini et al. 1996; Minton 1996; Bauche and Laval 1999; Kim et al. 2002), and the entire genome of *D. radiodurans* was analyzed (Makarova et al. 2001), but the radioresistant nature of *Deinococcus* is still ambiguous. Despite the fact that antioxidant scavenging systems, including superoxide dismutase, catalase (hydroperoxidase), peroxidase, thioredoxin reductase, etc., are crucial for protecting cells from the toxic oxidants, their roles in deinococcal resistance toward radiation as well as oxidative stress were less investigated. SOD dismutates superoxide anions into molecular O₂ and H₂O₂. All SODs are metalloenzymes with a redox metal such as Cu²⁺, Zn²⁺, Mn²⁺, and Fe²⁺ at the active site. In addition, an occurrence of NiSOD in *Streptomyces* spp. and *Prochlorococcus marinus* is also known (Youn et al. 1996; <http://www.kazusa.or.jp/cyano/SS120>). Types of SODs can be distinguished based upon differential inactivation of activity by selective chemicals, including cyanide, H₂O₂, and azide (Misra and Fridovich 1978; Halliwell and Gutteridge 1999; Valderas and Hart 2001), as well as by metal analysis. A

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Y. S. Yun · Y. N. Lee (✉)
Division of Life Sciences and Research Institute for Biotechnology,
Chungbuk National University,
Cheongju, Chungbuk, 361-763, Korea
E-mail: ynlee@chungbuk.ac.kr
Tel.: +82-43-2612301
Fax: +82-43-2649600

report showed that the SOD profiles in the mesophilic *Deinococcus* were quite different from each species with respect to the number of SOD possessed, metal cofactors, and their molecular sizes (Yun and Lee 2001). Despite the immense roles of SOD and catalase in the detoxification of toxic oxidants, only catalases of *D. radiophilus* were well studied (see references in Soung and Lee 2000; Yun and Lee 2000). Here, we report some properties of *D. radiophilus* SOD purified to electrophoretic homogeneity.

Materials and methods

Reagents

Pyrogallol, cacodylic acid, ammonium sulfate, Coomassie Brilliant Blue G-250, and materials for gel electrophoresis and isoelectric focusing were purchased from either Sigma Chemical (St. Louis, Mo., USA) or Junsei Chemical (Tokyo, Japan). Most of the medium constituents were bought from Difco Laboratories (Detroit, Mich., USA). Chromatography materials, including Superdex G-75, were from Pharmacia Biotech (Uppsala, Sweden).

Bacterial strain and growth

Deinococcus radiophilus ATCC 27603 (American Type Culture Collection, Rockville, Md., USA) was cultured in a modified TYGM medium (1% tryptone, 0.5% yeast extract, 0.2% glucose, and 0.2% methionine) at 30°C for a few days to reach the stationary phase with continuous shaking (150 rpm) (Yun and Lee 2001). Bacterial growth was monitored by recording an optical density at 600 nm (OD_{600}) (DU-65 Spectrophotometer; Beckman, Fullerton, Calif., USA).

Preparation of cell-free extracts

The cells harvested by centrifugation at 4,500 *g* for 20 min (Spra22K; Hanil, Seoul, Korea) were resuspended in 50 mM potassium phosphate buffer (pH 7.0) and washed three times with the same buffer. The cell-free extracts were obtained from the sonicated preparation of cells at 4°C—20 s pulse on and 40 s pulse off for a total of 20 min (Fisher sonic dismembrator; Fisher Scientific, Hampton, N.H., USA)—by centrifugation at 12,000 *g* for 20 min (Yun and Lee 2003).

Assay of SOD activity

SOD activity was assayed by the method of Marklund and Marklund (1974). One milliliter of 50 mM Tris-Cl buffer (pH 8.2) containing 50 mM cacodylic acid and 1 mM diethylenediamine-pentaacetic acid was placed in a cuvette. Then, either 10 μ l 50 mM potassium phosphate buffer (pH 7.0) (E_{std}) or 10 μ l cell-free extract (E_{test}) was added to the cuvette. Subsequently, 20 μ l of the substrate solution containing 10 mM pyrogallol and 0.05 mM hydrochloric acid was added to the enzyme assay mixture. After a brief shaking of the reaction mixture, its absorbance at 420 nm was recorded for 210 s at ambient temperature. The absorbance difference (ΔE) that occurred between two readings was employed for the SOD activity calculation as follows: $(\Delta E_{std} - \Delta E_{test}) \times 2.06 \times 100 / \Delta E_{std}$. One unit of SOD was defined as the amount of enzyme causing 50% inhibition of the pyrogallol autooxidation rate at 420 nm. Protein concentration was measured by the method of Lowry et al. (1951), using BSA as a standard.

Resolution and activity staining of SOD on polyacrylamide gel

Proteins were resolved by polyacrylamide gel electrophoresis (PAGE) (Gersten 1996). Visualization of SOD bands resolved on gel was made with the activity staining method (Beauchamp and Fridovich 1971) that was modified by Chou and Tan (1990). The gels were soaked in solution of 490 μ M nitro blue tetrazolium for 20 min, then in a solution containing 14 mM tetramethylene diamine, 14 μ M riboflavin, and 36 mM potassium phosphate (pH 7.8) for 15 min. Then, the gel was illuminated with a fluorescent lamp for 5–15 min to visualize the white achromatic zone of SOD activity on the blue background. For protein staining, gels were stained with 0.1% Coomassie Brilliant Blue R-250 and destained with 50% methanol/10% acetic acid, followed by 10% methanol/10% acetic acid.

Purification procedures of SOD

Ammonium sulfate was added to the sonic supernatant to reach 70% saturation, and the mixture was stirred for 2–3 h on ice. After removal of the precipitate by centrifugation at 12,000 *g* for 30 min, the supernatant was concentrated with an Amicon membrane filter (PM-10; Millipore, Bedford, Mass., USA) and dialyzed against 50 mM potassium phosphate buffer (pH 7.0). The dialyzed enzyme preparation was subjected to fast protein liquid chromatography (FPLC), employing a Superdex G-75 column (Φ 1 \times 30 cm, AKTA Explorer 100) equilibrated with the same buffer (Bollag and Edelstein 1996). The fractions containing SOD were pooled and concentrated with the Amicon membrane filter.

SOD typing by chemical treatment

Distinction of the metalloform of SODs was made by soaking the gels in 50 mM potassium phosphate (pH 7.8) containing either 20 mM H_2O_2 or 10 mM KCN for 60 min at room temperature before activity staining for SOD. Such treatments cause the differential inhibition of FeSOD and CuZnSOD (Valderas and Hart 2001). Densitometry of the SOD activity bands on the gel was achieved with a Kodak electrophoresis documentation and analysis system (KODAK ID Image Analysis, Tokyo, Japan).

Metal analysis

Metals of the purified enzyme dissolved in glass distilled, deionized water were analyzed using inductively coupled plasma spectrophotometer (JY 38 Plus, France). This work was carried out at the Center for Research Instruments and Experimental Facilities, Chungbuk National University.

Determination of the native and subunit molecular mass of the SOD

The molecular weight of the purified SOD was determined by both PAGE and FPLC in a Superdex G-75 column. (Φ 1 \times 30 cm) pre-equilibrated with 50 mM potassium phosphate buffer (pH 7.0) (Bollag and Edelstein 1996; Gersten 1996). Molecular mass of the denatured enzyme was measured by 0.1% SDS/12% polyacrylamide gel.

Isoelectric focusing of the purified SOD

Isoelectric point (pI) was determined by isoelectric focusing on 4% polyacrylamide gels containing 2.5% ampholytes. Ampholytes spanning a pH range of 3–10 were used. SOD bands were visualized

by protein staining with Coomassie Blue and by SOD activity staining (Righetti and Drysdale 1971). Amyloglucosidase (pI 3.6), trypsin inhibitor (pI 4.2), myoglobin (pIs 6.8, 7.2), and *Lens culinaris* lectin (pIs 8.2, 8.6, 8.8) (Sigma Chemical) were used as standard markers.

Effect of group-specific reagents on SOD activity

For the information of amino acid residue at active sites of the enzyme, enzyme activity was assayed in the presence of different concentrations of group specific reagents, i.e., iodine, glyoxal, N-ethylmaleimide, *p*-diazobenzene sulfonic acid, and β -naphthoquinone-4-sulfonic acid (Sigma) (Price and Stevens 1988).

Effect of pH and temperature on SOD

The SOD activity was assayed in the standard assay condition after incubation at various temperatures for 70 min and in a pH range of 3.0–11.0 at 25°C for 20 min. The storage stability of the enzyme kept at 10°C was checked at 5-day intervals. The buffer systems used contained 50 mM acetate-HCl, 50 mM KH_2PO_4 - Na_2HPO_4 , 50 mM Tris-Cl, 50 mM glycine-NaOH, and 50 mM carbonate-HCl.

Amino acid composition analysis and N-terminal sequencing

Amino acid composition analysis of the purified SOD was performed with phenylisothiocyanate derivatives of amino acids obtained after HCl hydrolysis of the protein at 110°C for 24 h, and the residues were determined by HPLC (Phenomemex luna C-18 column, Hewlett Packard 100 Series). Sequence determination was performed on an Applied Biosystems model 491 Precise Sequencer. Amino acid composition analysis and N-terminal sequencing were carried out at Life Science (Seoul) and in the proteome analysis laboratory of the Korea Basic Science Institute (Daejeon), respectively.

Results and discussion

Purification of SOD

As summarized in Table 1, *Deinococcus radiophilus* SOD was purified through the steps of sonic disruption of cells, ammonium sulfate fractionation, and repeated (first and second) Superdex G-75 FPLC. During ammonium sulfate fractionation, SOD remained in the

supernatant fraction (AS 70% supernatant) of ammonium sulfate fractionation. The first FPLC of the concentrate of the AS 70% supernatant allowed a separation of SOD from the majority of foreign proteins, but the concentrate of SOD pooled after FPLC was judged slightly impure because of a few faint protein bands beside SOD resolved by PAGE (Fig. 1A, lane 3). However, the second consecutive FPLC with the pooled SOD concentrate yielded two separated protein peaks (data not shown), and this SOD preparation showed a single protein band (Fig. 1A, lane 4) possessing SOD activity (Fig. 1B). The SOD was simply purified 1,919-fold in four steps to homogeneity with a specific activity of ca. 31,300 U mg^{-1} protein and 58% recovery. The figures of purification fold and yield are higher compared with other SODs purified from various sources (Jung et al. 1993; Pagani et al. 1995; Öztürk-Ürek et al. 1999; Öztürk-Ürek and Tarhan 2001). The poor precipitative property of *D. radiophilus* SOD even at 70% saturation concentration of ammonium sulfate is rather peculiar, since most of bacterial SOD was precipitated between 35 and 75% saturation of ammonium sulfate (Jung et al. 1993; Pagani et al. 1995; Öztürk-Ürek et al. 1999; Öztürk-Ürek and Tarhan 2001). However, there is a report that *Bacillus subtilis* MnSOD was precipitable at 70–90% saturation of ammonium sulfate, as was the *D. radiophilus* SOD (Inaoka et al. 1998). A precipitability of both SODs at high concentrations of ammonium sulfate suggests a sharing of common feature—perhaps a similar amino acid composition between these proteins.

SOD type

It is known that CuZnSOD is sensitive to H_2O_2 and cyanide, whereas FeSOD is sensitive to H_2O_2 , but not to cyanide. In contrast, MnSOD is insensitive to both cyanide and H_2O_2 (Halliwell and Gutteridge 1999; Valderas and Hart 2001). KCN caused no inhibition of *D. radiophilus* SOD activity, whereas a partial inactivation (ca. 38% reduction of activity) was caused by H_2O_2 (Fig. 2). Insensitivity of the purified SOD to cyanide and a partial inhibition by H_2O_2 suggest that *D. radiophilus*

Table 1 Purification of superoxide dismutase (SOD) from *Deinococcus radiophilus*

| Step | Total activity ^a (U) | Total protein (mg) | Total activity (U/mg) | Yield ^b (%) | Fold purification |
|---|---------------------------------|--------------------|-----------------------|------------------------|-------------------|
| Sonic extract | 8,053 | 495 | 16.3 | 100 | 1 |
| Concentrate of the 70% AS supernatant | 7,596 | 6.53 | 1,163 | 94.3 | 71.3 |
| 1st Superdex G-75 chromatography ^c | 6,670 | 1.80 | 3,705 | 82.8 | 227 |
| 2nd Superdex G-75 chromatography ^c | 4,694 | 0.15 | 31,293 | 58.3 | 1,919 |

^aOne unit of SOD was defined as the amount of enzyme causing 50% inhibition of the pyrogallol autooxidation rate at 420 nm

^bTotal activity of each step was compared with the total activity of sonic cell-free extract

^cConcentrated by Amicon ultrafiltration (PM 10)

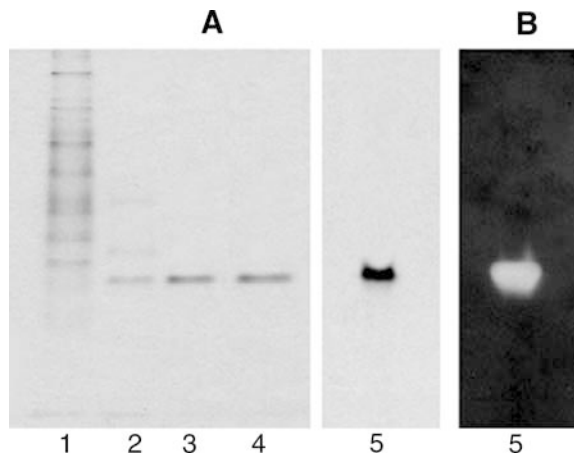


Fig. 1A, B Protein patterns in each step of superoxide dismutase (SOD) purification on polyacrylamide gel. **A** Proteins visualized by Coomassie Brilliant Blue staining on 10% gel. *Lane 1* Sonic cell-free extract (10 μ g protein), *lane 2* concentration of 70% AS supernatant (1 μ g protein), *lane 3* first Superdex G-75 (1 μ g protein), *lanes 4 and 5* second Superdex G-75 (1 and 5 μ g of protein, respectively). **B** Activity band of the purified SOD (5 μ g protein), corresponding to *lane 5* of **A**. See details in Materials and methods

SOD is not CuZnSOD, but probably an SOD containing both Mn and Fe. This was confirmed by an observation of the presence of both Mn (0.53 μ g/mg of protein, 0.98 mol/mol enzyme) and Fe (0.21 μ g/mg of protein, 0.39 mol/mol enzyme) in the purified enzyme. The incidence of SOD activation with both Mn and Fe does not seem to be rare, since SODs from several bacteria, such *Propionibacterium shermanii* and *Streptococcus mutans*, were known as the cambialistic Mn/FeSODs (Meier et al. 1997; Halliwell and Gutteridge 1999).

pI of SOD

Isoelectric focusing of the purified SOD revealed that *D. radiophilus* SOD has rather low pI (ca. 3.8), whereas the putative pI of *D. radiodurans* MnSOD is reported as 5.55 [The Institute for Genomic Research (TIGR) database *DR1279* locus information]. The pI value of *D. radiophilus* MnSOD seems to be the lowest pI of SODs reported in a number of prokaryotes, ranging from 4.5–7.1 (Pagani et al. 1995; An and Kim 1997; Hakamada et al. 1997).

The molecular mass of the SOD

Molecular weight of the purified SOD estimated by PAGE was 47.1 ± 5 kDa (data not shown), whereas that estimated by gel filtration using a Superdex G-75 column was 51.5 ± 1 kDa (data not shown). Discrepancy of SOD molecular weights estimated by nondenaturing PAGE and gel filtration seems to be attributed to the

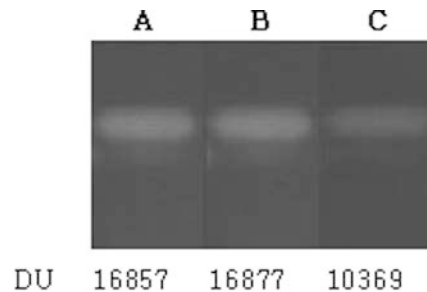


Fig. 2A–C SOD sensitivity to cyanide and H_2O_2 . An aliquot of purified SOD (3 μ g of protein) was resolved by nondenaturing polyacrylamide gel electrophoresis (PAGE). **A** Activity band of SOD on 10% gel (untreated control). **B** Activity band of SOD on 10% gel after KCN treatment, which causes inhibition of CuZnSOD activity. **C** Activity band of SOD on 10% gel after H_2O_2 treatment, which causes inhibition of FeSOD activity. *DU* Densitometer unit. See details in Material and methods

acidic nature of the enzyme. A single band of 26 ± 0.5 kDa was observed after SDS-PAGE of the purified SOD preparation followed protein staining (Fig. 3). These molecular weights of the native and denatured forms of *D. radiophilus* SOD suggest that the enzyme is a dimeric protein comprising two 26 ± 0.5 kDa subunits. In general, MnSODs contain two or four subunits. Most of MnSODs found in mitochondria or chloroplast of eukaryotes comprise four protein subunits (Halliwell and Gutteridge 1999). By contrast, most, but not all, of the bacterial MnSODs have two subunits with a molecular weight of 20–25 kDa (An and Kim 1997; Hakamada et al. 1999). That *D. radiophilus* SOD is composed of two equally sized subunits with an apparent molecular weight of 26 ± 0.5 kDa suggests that *D. radiophilus* SOD resembles other bacterial MnSODs in their molecular weights and structures.

Amino acid at the active site of SOD

As SODs are metalloenzymes, the liganding metal ions of the enzymes of amino acids are crucial for SOD activity. The purified SOD was completely inactivated by 1 mM β -naphthoquinone-4-sulfonic acid, which is known to modify lysine residues. Also, 5 mM ρ -diazobenzene sulfonic acid (histidine residue modifier) and iodine (tyrosine residue modifier) caused 45 and 23% inactivation of activity, respectively. Glyoxal and N-ethylmaleimide showed no effect on the enzyme activity (Table 2). These results suggest that lysine, histidine, and tyrosine residues are probably located at or near active sites of the enzyme, whereas arginine and cysteine residues are not crucial for SOD activity. There were reports that histidine residues are essential for the catalytic mechanism of bovine erythrocyte CuZnSOD (Uchida and Kawakishi 1994) and histidine; methionine also seemed to be important for the structure and enzyme activity of chicken liver CuZnSOD (Öztürk-Ürek and Tarhan 2001). In human mitochondrial MnSOD,

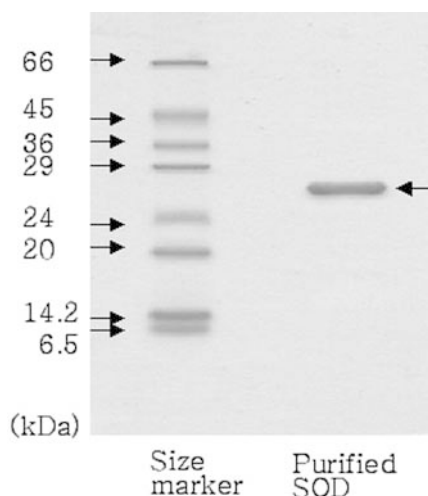


Fig. 3 Sodium dodecyl sulfate (SDS)-PAGE of the purified SOD. Denaturing PAGE (12% polyacrylamide/0.1% SDS) was performed with various size markers: aprotinin (6.5 kDa), bovine α -lactalbumin (14.2 kDa), soybean trypsin inhibitor (20 kDa), bovine trypsinogen (24 kDa), bovine RBC carbonic anhydrase (29 kDa), rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (36 kDa), chicken egg albumin (45 kDa), and bovine serum albumin (66 kDa). See details in Material and methods

the tetramer protein, histidine, and aspartic acid influence enzyme activity (Borgstahl et al. 1992). Interestingly, the strong inhibition of the *D. radiophilus* MnSOD activity by β -naphthoquinone-4-sulfonic acid suggests an important role of lysine in SOD activity. Nevertheless, reduction of *D. radiophilus* SOD activity by ρ -diazobenzene sulfonic acid and by iodine indicates involvement of histidine and tyrosine, respectively, for the enzyme activity.

Optimum temperature for SOD and SOD stability

The SOD showed an optimum temperature between 10 and 30°C, but a linear reduction of enzyme activity between 40 and 60°C (data not shown). SOD was quite thermostable, maintaining full activity even after 70-min incubation at 40°C, but demonstrated a linear decrease in activity above 50°C. The purified SOD maintained nearly 80% of the activity, even after 100-day storage in 50 mM potassium phosphate buffer (pH 7.0) at 10°C, and there was no loss of its activity at -20°C for several months (data not shown). The pH optimum for the SOD activity was not measurable, because autooxidation of pyrogaoll for SOD assay is a pH-dependent reaction. The purified SOD was remarkably stable at pH 5.0–11.0; however, it was highly unstable below pH 5.0. Thermal and pH stabilities of the purified *D. radiophilus* MnSOD are similar to other SODs reported from various sources (Jung et al. 1993; Pagani et al. 1995; An and Kim 1997; Meier et al. 1997; Öztürk-Ürek et al. 1999; Osatomi et al. 2001; Öztürk-Ürek and Tarhan 2001).

Table 2 Effect of group-specific reagents on SOD activity

| Reagents | Relative activity (%) | |
|---|-----------------------|------|
| | 1 mM | 5 mM |
| None | 100 | 100 |
| Iodine (tyrosine modifier) | 102 | 67 |
| Glyoxal (arginine modifier) | 108 | 107 |
| N-ethylmaleimide (cysteine modifier) | 100 | 104 |
| ρ -diazobenzene sulfonic acid (histidine modifier) | 86 | 45 |
| β -naphthoquinone-4-sulfonic acid (lysine modifier) | 0 | 0 |

| | 1 | 5 | 10 | 15 | 20 |
|----------------------------------|--------------------------|---|----|----|----|
| <i>D. radiophilus</i> (Mn/FeSOD) | MAFELPQLPYAYDALEPHIDA... | | | | |
| <i>D. radiodurans</i> (MnSOD) | MAVELPQLPYAYDALEPHIDA... | | | | |
| <i>A. aerogenes</i> (FeSOD) | MAVELPQLPYAYDALEPHIDA... | | | | |
| <i>E. coli</i> (MnSOD) | MSYTLPSLPYAYDALEPHFDK... | | | | |
| <i>E. coli</i> (FeSOD) | MSFELPALPYAKDALAPHISA... | | | | |
| <i>B. subtilis</i> (MnSOD) | MAVELPELPYAYDALEPSIDA... | | | | |

Fig. 4 Alignment of N-terminal amino acid sequences of bacterial SODs. Conserved sequences are *underlined*. The amino acid residues that are not identical among *D. radiophilus*, *D. radiodurans*, and *A. aerogenes* SODs are in *boldface*. The compared sequences are from the GenBank (National Center for Biotechnology Information, USA)

Amino acid composition and N-terminal peptide sequence

A deduced amino acid composition of *D. radiodurans* MnSOD is available from its total genome sequences (TIGR database DR1279 locus information). The comparison of the amino acid compositions of MnSODs from *D. radiodurans*, *D. radiophilus*, and *B. subtilis*—of which the latter two SODs showed similar behavior during ammonium sulfate fractionation as described above—was made. The amino acid compositions of deinococcal SODs are quite similar to each other with minor differences in mol% of serine, glycine, and tyrosine residues. The amino acid composition of SODs from *D. radiophilus* and *B. subtilis* also resemble each other (Inoaka et al. 1998), but mol% of glycine, alanine, and methionine are relatively higher in *D. radiophilus* SOD. The N-terminal peptide sequence of *D. radiophilus* SOD was MAFELPQLPYAYDALEPHIDA (> D). A comparison of the sequence of *D. radiophilus* SOD was made with the deduced amino acid sequences of other bacterial SODs (Fig. 4). Some residues are totally conserved among the SODs compared. A striking similarity was found between the N-terminal sequence of *D. radiophilus* SOD, *D. radiodurans* MnSOD, and *Aerobacter aerogenes* FeSOD, differing by one amino acid residue.

The *D. radiophilus* SOD composed of two equally sized subunits with an apparent molecular weight of 26 ± 0.5 kDa is not dissimilar to other bacterial MnSODs in their molecular mass and structures. However, the SOD

has some peculiar properties, such as an unprecipitable nature at 70% saturation concentration of ammonium sulfate, low pI value, and a requirement of lysine residue for enzyme activity—properties that are not reported in other SODs. Therefore, further detail studies on structural properties by crystallography, etc., are required to unravel the peculiar feature of *D. radiophilus* SOD.

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