Acta Oceanol. Sin., 2014, Vol. 33, No. 12, P. 163–169
DOI: 10.1007/s13131-014-0534-2
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Purification and biochemical characterization of manganese-containing superoxide dismutase from deep-sea thermophile *Geobacillus* sp. EPT3

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Received 15 March 2013; accepted 13 September 2013

Abstract

Thermostable SOD is a promising enzyme in biotechnological applications. In the present study, thermophile *Geobacillus* sp. EPT3 was isolated from a deep-sea hydrothermal field in the East Pacific. A thermostable superoxide dismutase (SOD) from this strain was purified to homogeneity by steps of fractional ammonium sulfate precipitation, DEAE-Sepharose chromatography, and Phenyl-Sepharose chromatography. SOD was purified 13.4 fold to homogeneity with a specific activity of 3354 U/mg and 11.1% recovery. SOD from *Geobacillus* sp. EPT3 was of the Mn-SOD type, judged by the insensitivity of the enzyme to both KCN and H_2O_2. SOD was determined to be a homodimer with monomeric molecular mass of 26.0 kDa. It had high thermostability at 50°C and 60°C. At tested conditions, SOD was relatively stable in the presence of some inhibitors and denaturants, such as β-mercaptoethanol (β-ME), dithiothreitol (DTT), phenylmethylsulfonyl fluoride (PMSF), urea, and guanidine hydrochloride. *Geobacillus* sp. EPT3 SOD showed striking stability across a wide pH range from 5.0 to 11.0. It could withstand denaturants of extremely acidic and alkaline conditions, which makes it useful in the industrial applications.

Key words: *Geobacillus* sp., superoxide dismutase, thermostability, purification

Citation: Zhu Yanbing, Li Hebin, Ni Hui, Liu Jingwen, Xiao Anfeng, Cai Huinong. 2014. Purification and biochemical characterization of manganese-containing superoxide dismutase from deep-sea thermophile *Geobacillus* sp. EPT3. Acta Oceanologica Sinica, 33(12): 163–169, doi: 10.1007/s13131-014-0534-2

1 Introduction

Superoxide dismutases (SODs, EC 1.15.1.1) which are widely distributed among organisms catalyze the dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen (Fridovich, 1975; McCord and Fridovich, 1988). To date, four distinct types of SOD have been found, differing in the identity of the functional metal copper-zinc, iron, manganese, or nickel (McCord and Fridovich, 1969; Weisiger and Fridovich, 1973; Yost and Fridovich, 1973; Youn et al., 1996). Fe-SOD and Mn-SOD are predominantly found in prokaryotes and mitochondria, and share both amino acid sequence and three-dimensional structural homology (McCord, 1976; Parker and Blake, 1988), while the other SODs are structurally unrelated.

Considerable experimental evidences have been accumulated on the therapeutical applications of SODs. They are reported to prevent oncogenesis, tumor promotion and invasiveness, and reduce the cytotoxic and cardiotoxic effects of anticancer drugs (Angelova et al., 2001; Cullen et al., 2003; Nishikawa et al., 2001; Zhong et al., 1997). SODs were also found to be effective in the treatment of inflammatory diseases, and arthritis (Luisa Corvo et al., 2002; Zhang et al., 2002), of infections (Emerit et al., 2006), and of ischemic or burn injuries for tissue protection (Vorauer-Uhl et al., 2001; Yabe et al., 2001; Yunoki et al., 2003). In China, SODs have been used in skin protection products by the cosmetic industry.

At present, SODs are produced by extraction from animal tissues, mostly bovine liver or erythrocytes. A microbial production process could represent a significant improvement in terms of yields, costs, and product safety. Many attempts to produce SOD from microorganisms have been performed (Raimondi et al., 2008; Yoo et al., 1999; Yu, 2007; Zhang et al., 2011). Thermophiles are a group of microorganisms inhabiting geothermal and hydrothermal environments. They are adapted to survive at high temperature. Enzymes from thermophilic organisms have high thermostability. Their structural characteristics also lead high resistance against organic solvents as well as resistance to many denaturing conditions (Vieille and Zeikus, 2001). Thermostable enzymes have high potential of utilization in industrial process. SODs from thermophilic organisms have stability at high temperatures and denaturing conditions, and have become objects of special interest for the related industrial applications. Furthermore SODs from thermophiles are suitable models for studies of enzyme structure and mechanism. During the past few years, many SODs have been characterized from thermophiles (Guo et al., 2008; He et al., 2007; Li et al., 2005; Zhang et al., 2011; Zhu et al., 2011). Most thermostable

Foundation item: The National Natural Science Foundation of China under contract No. 31371751; the Science and Technology Program of Xiamen, China under contract No. 201303120001; the Foundation for Innovative Research Team of Jimei University, China under contract No. 2010A006.
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Fe/Mn-SODs have been identified in thermophilic archaea (Dello Russo et al., 1997; Kardinahl et al., 2000; Klenk et al., 1993; Knapp et al., 1999; Lee et al., 2010; Ursby et al., 1999; Whittaker and Whittaker, 2000; Yamano et al., 1999), and a few thermostable Fe/Mn-SODs have been identified in thermophilic bacteria (Amo et al., 2003; Boyadzhieva et al., 2010; Lancaster et al., 2004; Lim et al., 1997; Liu et al., 2011; Wang et al., 2008; Yu et al., 2004; Zhu et al., 2011; Zou et al., 2011).

Thermophilic microbial communities can inhabit deep-sea hydrothermal environments (Miroshnichenko and Bonch-Osmolovskaya, 2006). A limited number of thermostable enzymes have been identified from thermophiles of these sources (Liu et al., 2006; Wu et al., 2006). However, until now, characterization of thermostable SOD from these environments has not been reported to our knowledge. In this study, a native Mn-SOD was purified from a thermophilic bacterium *Geobacillus* sp. EPT3, isolated from a deep-sea hydrothermal field sediment sample. The enzyme was then biochemically characterized. We found this thermostable Mn-SOD showed a high stability over a wide pH range.

2 Materials and methods

2.1 *Bacterial strain identification and growth conditions*

Strain EPT3 was isolated from samples collected from a deep-sea hydrothermal field in the East Pacific (12°36′12″N, 104°19′28″W) at a depth of 3 191 m using ZoBell 2216E (Oppenheimer and ZoBell, 1952) agar plates at 65°C. The 16S rRNA gene was amplified from the genomic DNA of strain EPT3 by PCR using primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). The PCR product was cloned into pMD-18T vector (TaKaRa) and sequenced by Shanghai Sangon Biological Engineering Technology and Service in China. Homology search of the 16S rRNA gene sequence was performed against GenBank database with BLAST (Altschul et al., 1997).

Strain EPT3 was cultivated aerobically with vigorous shaking (200 r/min) at 65°C in ten 500-mL flasks containing 100 mL ZoBell 2216E medium (Oppenheimer and ZoBell, 1952). After incubation for 3 d, cells were harvested by centrifugation (5 000 *g*, 10 min) for the subsequent SOD purification.

2.2 *Purification of SOD*

The cells from the strain EPT3 culture were resuspended in buffer A (50 mmol/L Tris-HCl, pH 7.5). They were disrupted by ultrasonication on ice for 5 min, and the supernatant was obtained by centrifugation (12 000 *g*, 20 min, 4°C). Afterwards, solid ammonium sulfate was slowly added to the supernatant with constant stirring to 40% saturation. After 12 h at 4°C, the precipitate was centrifuged at 12 000 *g* for 20 min, and ammonium sulfate was added to the supernatant to 90% saturation. After 12 h at 4°C, the precipitate from subsequent centrifugation (12 000 *g*, 20 min, 4°C) was dissolved in buffer A and dialyzed at 4°C for 24 h against three changes of the same buffer. Chromatographic purification procedures were performed at room temperature. The sample was loaded on a DEAE-Sepharose Fast Flow (GE Healthcare) column (1.0 cm×15 cm) equilibrated with buffer A. After the column was washed with five column volumes of buffer A, a 200 mL linear gradient of 0 to 0.3 mol/L NaCl in buffer A was applied. Fractions with SOD activity were pooled. Then the sample with 50% saturation ammonium sulfate added was applied to a Phenyl-Sepharose CL-4B (GE Healthcare) column (1.0 cm×20 cm) previously equilibrated with buffer A containing 50% saturation ammonium sulfate. After the column was washed with five column volumes of buffer A containing 50% saturation ammonium sulfate, the sample was eluted with a 100 mL decreasing linear gradient of 50% to 0% saturation ammonium sulfate in buffer A. Fractions containing SOD activity were pooled and dialyzed at 4°C for 24 h against three changes of buffer A for further experiments. The protein concentration was determined by Bradford method (Bradford, 1976) with bovine serum albumin (BSA) as the standard.

2.3 *Assay of SOD activity*

SOD activity was measured using the nitro blue tetrazolium (NBT) photochemical reduction assay (Stewart and Bewley, 1980) at 25°C. One unit of superoxide dismutase activity was defined as the amount of enzyme which inhibited the rate of nitro blue tetrazolium photochemical reduction by 50%. Native polyacrylamide gel electrophoresis (PAGE) was stained for SOD activity by the NBT reaction according to the method described previously (Beauchamp and Fridovich, 1971).

2.4 *Determination of molecular mass of purified SOD*

The homogeneity of SOD and the molecular mass of its subunit were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis performed according to standard procedures (Laemmli, 1970). Size exclusion chromatography was used to determine the native molecular mass of SOD. Experiments were performed on a Sephacryl S-200 (Amersham Pharmacia Biotech) column (1.0 cm× 100 cm) developed in 50 mmol/L Tris-HCl (pH 7.5). The column was calibrated by chromatographic protein standards (cytochrome *c*, 12.4 kDa; carbonic anhydrase, 29 kDa; albumin, 66 kDa; alcohol dehydrogenase, 150 kDa; β-amylase, 200 kDa).

2.5 *SDS typing by chemical treatment*

The SOD inhibitors KCN and H_2O_2 were used to determine their effects on SOD activity. The enzyme was preincubated with 1 mmol/L KCN or 1 mmol/L H_2O_2 at 25°C for 1 h. The specific activities of the samples were determined under assay conditions. The enzyme with distilled water was treated in the same way as the control.

2.6 *Thermal stability assay*

The thermostability of SOD at different conditions was estimated by measuring the residual activities after heat treatments. The thermostability of SOD was examined by incubating the enzyme in 50 mmol/L Tris-HCl (pH 7.5) at 50°C, 60°C, 70°C, 80°C and 90°C for 1 h, respectively. The residual activities were measured by the standard method as described above. SOD activity before incubation was defined as 100%.

2.7 *pH stability assay*

The pH tolerance profile of SOD was determined by keeping the enzyme in buffers with different pH values (ranging from 3.0 to 12.0) at 25°C for 1 h. The buffer systems used were 50 mmol/L acetate buffer (pH 3.0–6.0), potassium phosphate buffer (pH 6.0–8.0), Tris-HCl buffer (pH 8.0–9.0), glycine-NaOH buffer (pH 9.0–11.0), and Na_2HPO_4-NaOH buffer (pH 11.0–12.0). After the enzymes were dialyzed against 50 mmol/L Tris-HCl (pH 7.5), the residual activities were determined by the standard method as described above. The SOD activity of the enzyme without the

treatment was defined as 100%.

2.8 *Effects of divalent metal ions on SOD activity*

The effects of metal ions on SOD activity were determined by adding various divalent metal salts ($MgCl_2$, $ZnSO_4$, $BaCl_2$, $CaCl_2$, $CuCl_2$, $NiCl_2$, $CoCl_2$, and $MnCl_2$) at final concentrations of 0.1 mmol/L or 1 mmol/L. The enzyme was incubated with each ion at 25°C for 30 min in 50 mmol/L of Tris-HCl buffer (pH 7.5), individually. Residual activities were measured by the standard assay as described above. The SOD activity of the enzyme without addition of metal ions was defined as 100%.

2.9 *Effects of inhibitors, detergents, and denaturants on SOD activity*

The effects of inhibitors on SOD activity were determined by using ethylenediaminetetraacetic acid (EDTA), β-mercaptoethanol (β-ME), dithiothreitol (DTT), and phenylmethylsulfonyl fluoride (PMSF) at final concentrations of 1 mmol/L or 10 mmol/L. The enzyme was incubated with each inhibitor at 25°C for 30 min in 50 mmol/L of Tris-HCl buffer (pH 7.5), individually. Residual activities were measured by the standard assay as described above. The effects of detergents on SOD activity were investigated by using sodium dodecyl sulfate (SDS), 3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfonate (Chaps), Tween 20, and Triton X-100 at final concentrations of 0.1% (w/v or v/v) or 1% (w/v or v/v). The effects of denaturants on SOD activity were examined by using urea and guanidine hydrochloride at final concentrations of 1 mol/L. They were also monitored by the same procedure as described above. Reaction mixture without additives was used as a reference.

3 Results

3.1 *Purification of SOD from Geobacillus sp. EPT3*

After the sequence alignment in Genbank, the 16S rDNA from strain EPT3 shared high identities of 99.9%, 99.9%, and 99.8% with the 16S rDNA from *Geobacillus kaustophilus* HTA426, *Geobacillus thermoleovorans* CCB_US3_UF5, *Geobacillus kaustophilus* G2, respectively. So this strain was assigned to *Geobacillus* sp. EPT3. As summarized in Table 1, SOD from *Geobacillus* sp. EPT3 was purified through the steps of ultrasonic disruption of cells, ammonium sulfate fractionation, anion-exchange chromatography and hydrophobic interaction chromatography. SOD was purified 13.4 fold to homogeneity with a specific activity of 3354 U/mg and 11.1% recovery. A single band of 26.0 kDa was observed after SDS-PAGE of the purified SOD preparation (Fig. 1a, Lane 2). On the native polyacrylamide gel, the mobility of the protein band stained with Coomassie Brilliant Blue coincided with that of the SOD activity band by NBT staining (Fig. 1b). The molecular mass of the native protein was determined to be 53.1 kDa by size exclusion chromatography using a Sephacryl S-200 column (data not shown). These results suggest that SOD from *Geobacillus* sp. EPT3 was a dimeric protein comprising two 26.0 kDa subunits.

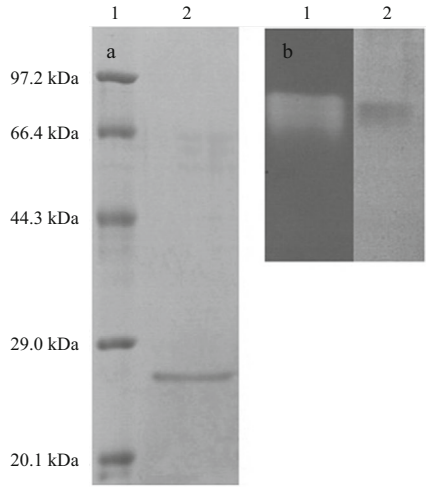

Fig.1. The purified SDS on polyacrylamide gel. a. SDS-PAGE anaylsis of SOD. Lane 1 represents molecular weight protein marker and Lane 2 the purified SOD. b. Native PAGE analysis of SOD. Lane 1 represents SOD stained with NBT and Lane 2 SOD stained with Coomassie Brilliant Blue.

3.2 *SOD type*

KCN and H_2O_2 were used as enzyme inhibitors to determine the SOD type. After treatment the enzyme using 1 mmol/L KCN and 1 mmol/L H_2O_2 at 25°C for 1 h, the inhibition rates of the SOD activity by KCN and H_2O_2 were 1.5% and 2.3%, respectively. These results indicated that SOD from *Geobacillus* sp. EPT3 was of the Mn-SOD type.

3.3 *Effect of temperature on the stability of SOD*

The thermostability of SOD was investigated by pre-incubating the enzyme at different temperatures for 1 h and then measuring the residual activities (Fig. 2). SOD retained over 65% of the initial activity after heat treatment at 50°C and 60°C for 1 h. However, incubation at higher temperatures induced activity loss. The enzyme retained 31% of the initial activity after incubation at 70°C for 1 h. Even after heating the enzyme at 80°C for 1 h, 16% of the initial activity was maintained. These results indicated that SOD from *Geobacillus* sp. EPT3 was thermostable.

3.4 *Effect of pH on the stability of SOD*

When tested in a series of buffers with different pH (3.0–12.0), SOD showed remarkable stability in pH ranging from 5.0 to 11.0, retaining over 60% of the initial activity after incubation at 25°C for 1 h (Fig. 3).

Table 1. Purification of the superoxide dismutase from *Geobacillus* sp. EPT3

Purification step	Total activity/U	Total protein/ mg	Specific activity/U·mg^{-1}	Yield/%	Purification (fold)
Crude extract	48396	193.4	250	100.0	1.0
Ammonium sulfate precipitation	20178	22.0	917	41.7	3.7
DEAE-Sepharose	7218	3.6	2005	14.9	8.0
Phenyl-Sepharose	5366	1.6	3354	11.1	13.4

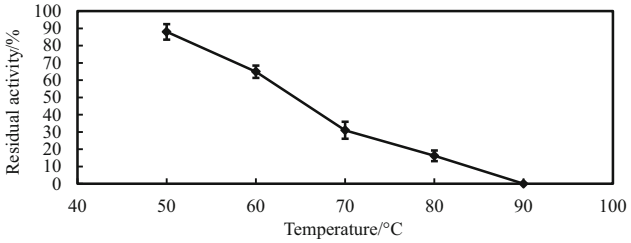

Fig.2. Thermal stability of SOD. SOD was incubated in 50 mmol/L Tris-HCl (pH 7.5) at 50°C, 60°C, 70°C, 80°C and 90°C for 1 h, individually. Residual activities were measured by using the standard assay described in Section 2. Activity before incubation was defined as 100%.

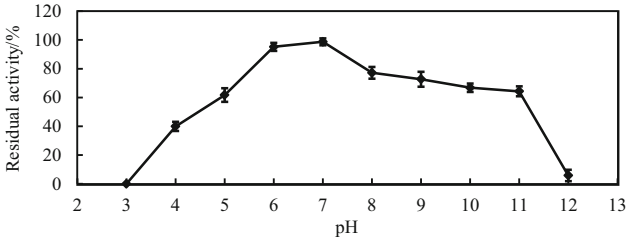

Fig.3. pH stability of SOD. SOD was incubated in buffers with different pH values (ranging from 3.0 to 12.0) at 25°C for 1 h. The buffer systems used were 50 mmol/L of acetate buffer (pH 3.0–6.0), potassium phosphate buffer (pH 6.0–8.0), Tris-HCl buffer (pH 8.0–9.0), glycine-NaOH buffer (pH 9.0–11.0), and Na_2HPO_4-NaOH buffer (pH 11.0–12.0).

3.5 *Effects of divalent metal ions on SOD activity*

The effects of metal ions on SOD activity were determined by using various divalent metal ions: Mg^{2+}, Zn^{2+}, Ba^{2+}, Ca^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+} and Mn^{2+} at final concentrations of 0.1 mmol/L or 1 mmol/L (Table 2). Positive influences on SOD activity were observed upon adding Mn^{2+} at 0.1 mmol/L and 1 mmol/L. Ca^{2+} at 0.1 mmol/L had no influence on SOD activity, although at 1 mmol/L, its effect became positive. At concentrations of 0.1 mmol/L and 1 mmol/L, Cu^{2+} and Co^{2+} inhibited the enzymatic activity. With Mg^{2+}, Zn^{2+}, Ba^{2+} and Ni^{2+}, the enzyme activity was not affected at both concentrations tested.

3.6 *Effects of inhibitors, detergents, and denaturants on SOD activity*

The effects of various inhibitors on SOD activity were examined by using EDTA, β-ME, DTT, and PMSF (Table 3). At both concentrations tested, negative influences on the SOD activity were determined upon adding EDTA and DTT, with EDTA having the strongest negative effect at 10 mmol/L. With PMSF and β-ME at 1 mmol/L, the enzyme activity was not influenced, although at 10 mmol/L, the effects became negative. At both concentrations tested, more than 60% of relative activities were retained after treatment with β-ME, DTT, and PMSF. The influences of some detergents on the enzymatic activity were determined by using SDS, Chaps, Tween 20, and Triton X-100 (Table 3). At both concentrations tested, they all inhibited the

Table 2. Effects of divalent metal ions on SOD activity

Divalent metal ions	Concentration/mmol·L^{-1}	Relative activity/%
Control	—	100.0±2.9
Mg^{2+}	0.1	101.2±1.9
	1	98.8±4.4
Zn^{2+}	0.1	98.3±2.7
	1	100.2±3.6
Ba^{2+}	0.1	102.1±1.7
	1	99.7±1.9
Ca^{2+}	0.1	102.6±3.1
	1	113.6±4.8
Cu^{2+}	0.1	88.6±2.3
	1	76.1±5.1
Ni^{2+}	0.1	102.0±2.1
	1	99.2±3.7
Co^{2+}	0.1	91.8±4.1
	1	81.6±2.4
Mn^{2+}	0.1	109.7±3.3
	1	134.3±4.1

Notes: The enzyme was incubated with each divalent metal ion with final concentrations of 0.1 mmol/L or 1 mmol/L in 50 mmol/L of Tris-HCl buffer (pH 7.5) at 25°C for 30 min, individually. Residual activities were measured by using the standard assay described in Section 2. Reaction mixture without metal ion was used as a control.

Table 3. Effects of inhibitors, detergents and denaturants on the activity of superoxide dismutase from *Geobacillus* sp. EPT3

Inhibitors, detergents and denaturants	Concentration	Relative activity/%
Control	—	100.0±2.7
EDTA	1 mmol/L	84.4±3.4
	10 mmol/L	38.9±2.1
β-ME	1 mmol/L	96.4±4.6
	10 mmol/L	60.6±2.2
DTT	1 mmol/L	89.0±1.8
	10 mmol/L	66.0±4.3
PMSF	1 mmol/L	99.0±2.5
	10 mmol/L	67.8±3.6
SDS	0.1%	68.0±2.9
	1%	NA
Chaps	0.1%	66.6±3.1
	1%	57.7±1.6
Tween 20	0.1%	76.6±5.4
	1%	25.0±3.2
Triton X-100	0.1%	77.5±2.4
	1%	20.2±2.0
Urea	1 mol/L	74.0±3.9
Guanidine hydrochloride	1 mol/L	76.5±4.8

Notes: The enzyme was incubated with each inhibitor, detergent and denaturant with different final concentrations in 50 mmol/L of Tris-HCl buffer (pH 7.5) at 25°C for 30 min, individually. Residual activities were measured by using the standard assay described in Section 2. Reaction mixture without inhibitor, detergent and denaturant was used as a control. NA stands for no activity.

SOD activity. The negative effects were strong at a concentration of 1%. With SDS, no enzymatic activity was measured at this concentration. With denaturants urea and guanidine hydrochloride at a final concentration of 1 mol/L, more than 70% of relative activities were maintained (Table 3).

4 Discussion

Mn-SODs have been characterized from a wide range of organisms including bacteria, fungi, plants, and animals (Bannister et al., 1987). In recent years, the biological functions of Mn-SOD have attracted increasing attention among researchers. It has been shown that Mn-SOD is involved in senescence, cell impairment, and carcinogenesis (Bostwick et al., 2000; Melov et al., 2000). In this study, we purified and characterized a Mn-SOD from deep-sea thermophilic bacterium *Geobacillus* sp. EPT3, laying a good foundation for its structural characterization and application.

Types of SOD can be distinguished by selective chemicals such as potassium cyanide and H_2O_2 (Asada et al., 1975; Valderas and Hart, 2001). It is known that Cu, Zn-SOD is sensitive to H_2O_2 and cyanide, whereas Fe-SOD is sensitive to H_2O_2, but not to cyanide. In contrast, Mn-SOD is insensitive to both cyanide and H_2O_2. The insensitivity of the purified SOD to both cyanide and H_2O_2 suggested that SOD from *Geobacillus* sp. EPT3 was of the Mn-SOD type. In general, Mn-containing SODs found principally in prokaryotes are either dimers or tetramers that are composed of identical subunits (An and Kim, 1997; Bannister et al., 1987; Hassan, 1989; Song et al., 2009; Zhu et al., 2011). In this study, the molecular mass of *Geobacillus* sp. EPT3 Mn-SOD was approximately 53.1 kDa with two identical 26.0 kDa subunits, which is similar to the Mn-SODs from *Pseudomonas carboxydohydrogena* (An and Kim, 1997) and a strain of alkaliphilic *Bacillus* (Hakamada et al., 1997). The purified enzyme showed a specific activity of 3 354 U/mg. This value is higher than those of Mn-SODs from *Chaetomium thermophilum* (Guo et al., 2008), *Thermomyces lanuginosus* (Li et al., 2005), and *Thermoascus aurantiacus* var. *levisporus* (Song et al., 2009). The activities of these Mn-SODs were all determined with the method of Stewart and Bewley (1980).

We found that SOD from *Geobacillus* sp. EPT3 was stable against thermal denaturation, as expected from the fact that the protein was derived from a deep-sea hydrothermal field sample. *Geobacillus* sp. EPT3 Mn-SOD was highly stable at a wide pH range as compared to Mn-SODs from some other thermophiles. It retained over 60% of the full activity after incubation at 25°C for 1 h across a pH span from 5.0 to 11.0. When the thermostable recombinant Mn-SOD from *Geobacillus* sp. was incubated for 3 h at various pHs. It retained over 80% of its full activity in the pH range of 8.0–10.0. Its activity disappeared rapidly out of this range. It retained about 15% and 40% of relative activities at pH 7.5 and 11.5, respectively (Zou et al., 2011). The pH stability of a thermostable native Mn-SOD from *Chaetomium thermophilum* was determined after it had been kept for 1 h in various pH conditions. The enzyme was able to retain over 50% of the full activity over a range of pH 6.0 to 9.5. It retained about 20% of relative activity at pH 10.0 (Guo et al., 2008). For the thermostable Mn-SOD from *Thermoascus aurantiacus* var. *levisporus*, the enzyme retained over 40% of relative activities after incubation for 1 h in the pH span of 6.0–9.0 (Song et al., 2009). *Geobacillus* sp. EPT3 Mn-SOD in this study could withstand denaturants of extremely acidic and alkaline conditions.

In this study, 1 mmol/L of Mn^{2+} stimulated the SOD activity, which is similar to the thermostable Mn-SODs from *Thermus thermophilus* wl (Song et al., 2012) and *Thermus thermophilus* HB27 (Liu et al., 2011). *Geobacillus* sp. EPT3 Mn-SOD was inhibited by 1 mmol/L of Cu^{2+} in this study, however *Thermus thermophilus* wl Mn-SOD was simulated by 1 mmol/L of Cu^{2+} (Song et al., 2012), and positive influence on *Thermus thermophilus* HB27 Mn-SOD activity was observed upon adding 10 mmol/L of Cu^{2+} (Liu et al., 2011). At tested conditions, *Geobacillus* sp. EPT3 SOD showed a relatively good tolerance to some inhibitors and denaturants, such as β-ME, DTT, PMSF, urea, and guanidine hydrochloride.

The potential therapeutic effect of *Geobacillus* sp. EPT3 Mn-SOD is not clear, so further studies on it are necessary.

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