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Cloning, purification and enzymatic characterization of recombinant human superoxide dismutase 1 (hSOD1) expressed in *Escherichia coli*

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Superoxide dismutase 1 (SOD1) is a metalloenzyme that catalyzes the disproportionation of superoxide into molecular oxygen and hydrogen peroxide. In this study, the human SOD1 (hSOD1) gene was cloned, expressed and purified. The hSOD1 gene was amplified from a pool of Bxpc3 cell cDNAs by PCR and cloned into expression vector pET-28a (+). The recombinant soluble hSOD1 was expressed in *E. coli* BL21 (DE3) at 37 °C and purified using nickel column affinity chromatography. Soluble hSOD1 was produced with a yield of 5.9 μg/mL medium. As metal ions can have a certain influence on protein structure and activity, we researched the influences of different concentrations of Cu^{2+} and Zn^{2+} on hSOD1 activity at induction and the time of activity detection. The results implied that Cu^{2+} and Zn^{2+} do not enhance SOD1 expression and solubility; they can, however, improve the catalytic activity at induction. Meanwhile, Cu^{2+} and Zn^{2+} also enhanced the enzyme activity at the time of detection. Furthermore, most other bivalent cations had the potential to replace Zn^{2+} and Cu^{2+}, and also improved enzyme activity at the time of detection.

Key words: superoxide dismutase 1, *Escherichia coli*, soluble expression, metal ions, catalytic activity

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Abbreviations: hSOD1, human superoxide dismutase 1; GSH-Px, glutathione peroxidase; IPTG, isopropyl-β-D-thiogalactoside

INTRODUCTION

The antioxidant defense system is extremely important as it enables living organisms to scavenge free radicals produced during normal metabolism. Several antioxidative enzymes are known to reduce oxidative damage, such as superoxide dismutase (SOD), glutathione reductase, catalase (CAT) and glutathione peroxidase (GSH-Px) (Shih *et al.*, 2006; Geraghty *et al.*, 2016). Although all of them exhibit definite antioxidative action, SOD is considered the first enzyme in the defense against oxidative stress produced during normal metabolism (Johnson, 2002).

SODs are a major antioxidant enzyme family whose members can convert superoxide radicals to hydrogen peroxide which is further catalyzed to form H_2O and O_2 (Kilic *et al.*, 2014; Vats *et al.*, 2015). Three forms of SOD are present in humans, in all other mammals, and most chordates. SOD1 is expressed in the intermembrane space of mitochondria, the nucleus, and the cytosol. SOD2 is located in the mitochondrial matrix, while SOD3 is secreted into the extracellular space (Hole *et al.*, 2011). SOD1 is a homodimer consisting of two 16-kDa subunits found in the cytoplasm and nucleus of the cell. SOD2 is mitochondrial and the human enzyme has manganese (Mn) in its reactive center and active site, which functions as a metal cofactor (Ghneim, 2016).

Heterologous expression of SOD1 has been conducted in many expression systems including *E. coli* (Hartman *et al.*, 1986; Zhang *et al.*, 2017), yeast (Yoo *et al.*, 1999; Wu *et al.*, 2009), baculovirus systems (Fujii *et al.*, 1995; Hayward *et al.*, 2002) and plant cells (Park *et al.*, 2002). The recombinant hSOD1 proteins are in all cases expressed in the cytosol, and in *E. coli* yields are equivalent to at least 10% of the total bacterial protein, and in many cases much more (Ahl *et al.*, 2004). However, the most common problem has been that the protein produced is Cu^{2+} and Zn^{2+}-deficient at active site resulting in low solubility and enzyme activity. Metal reconstitution *in vitro* is a method of incorporating Cu^{2+} into the apoenzyme (apo-hSOD1). The addition of Cu^{2+} into the *E. coli* culture was reported to improve Cu^{2+} incorporation; however, the production of SOD1 with a full Cu^{2+} complement was still a complication.

SOD1 is a metalloenzyme containing one copper ions and one zinc ion per molecule. Many early investigations of SOD1 focused on the metal-binding properties of the enzyme. The structural integrity of SOD1 depends critically on the correct coordination of zinc and copper (Nordlund *et al.*, 2009). Banci *et al.* found by in-cell NMR that hSOD1 needs to bind one Zn^{2+} ion and one catalytic Cu^{2+} ion per molecule and to form an intramolecular disulfide bridge before it exerts its catalytic function (Banci *et al.*, 2011). Defective metal binding or decreased affinity for zinc and copper is a feature of many SOD1 mutants and has been suggested to play a role in the pathogenic mechanism of amyotrophic lateral sclerosis (ALS) (Sangwan *et al.*, 2017). However, excess cofactors can create toxicity, i.e. zinc toxicity may be due to the binding of zinc to inappropriate sites that inhibit enzyme function or to the displacement of other metal ions from the active sites of enzymes (Wu *et al.*, 2009).

In this research, the open reading frame (ORF) of hSOD1 was cloned and the recombinant enzyme was expressed in *E. coli* BL21 (DE3). The antioxidative activity of the recombinant hSOD1 protein was detected. Because the zinc and copper cofactors are involved in the stable structure and high activity of eukaryotic SOD1 (Leitch *et al.*, 2009; Girotto *et al.*, 2014; Lin *et al.*, 2015), we investigated the influences of Cu^{2+} and Zn^{2+} on

hSOD1 activity at induction. Meanwhile, we also studied the effects of Cu^{2+} and Zn^{2+} on hSOD1 enzymatic activity. Furthermore, we examined the effects of substitutions of metal ions on hSOD1 activity at the time of detection.

MATERIALS AND METHODS

Materials. TRIzol (Sangon Biotech, China), random hexamers (50 ng/mL), dNTP Mix (10 mM each), 0.1 M DTT, 5×first strand buffer, reverse transcriptase M-MLV (Rnase H-), rTaq polymerase, restriction enzymes, T4 DNA ligase, DNA markers and protein markers were purchased from TaKaRa (Dalian, China). The expression vector pET-28a (+) and *E. coli* strain DH5α, BL21 were obtained in our lab. All chemicals were all from Sigma (St. Louis, MO, USA) or a domestic provider in China if not stated otherwise.

Construction of an expression vector containing the hSOD1 gene. The hSOD1 specific primers, forward primer (5' CCAAGCTTGGATGGCGACGAAGGCCGTG 3') with a HindIII site (underlined), and reverse primer (5' CCCTCGAGGGTTATTGGGCGATCCCAAT 3') with an XhoI site (underlined) were designed to amplify the hSOD1 gene (GenBank accession number CR541742.1). cDNA of Bxpc3 cell lines served as a template for amplifying the hSOD1 gene with PCR. Following this, the PCR product was digested using two kinds of restriction endonucleases (HindIII and XhoI). Finally, the digested product was cloned into expression vector pET-28a (+) between the HindIII and XhoI restriction sites. The recombinant plasmid was verified by DNA sequencing.

Expression and purification of the recombinant protein. The constructed recombinant expression plasmids were transformed into *E. coli* BL21 (DE3) for protein production. The freshly transformed colony was cultured in LB medium supplemented with kanamycin (50 μg/mL). Expression of the recombinant protein was induced by 1 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG) for 6 h at 37°C. Cells were harvested by centrifugation (4 500 × *g*, 4°C, 15 min). The cell pellet was resuspended in Tris-HCl (50 mM, pH 8.0), and then incubated with 1 mg/mL lysozyme on ice for 30 min. Finally, the suspension was lysed by sonication, and the precipitate and supernatant were separated by centrifugation twice at 8 000 × *g* and 4°C for 20 min.

The supernatant was loaded onto a Ni-NTA affinity column (GenScript, Nanjing, China). Purification conditions followed the manufacturer's instructions. After washing the column with 50 mM and 100 mM imidazole, the fusion protein was eluted with 400 mM imidazole. The eluted protein was dialyzed against dialysis buffer (50 mM Tris-HCl, pH 8.0) at 4°C to remove imidazole. The protein concentration was detected by the BCA Protein Assay Kit (Pierce, Bonn, Germany).

Western blotting. Western blotting was performed according to the method described by Zhou and coworkers (Zhou *et al.*, 2017). Proteins were resolved on a 15% SDS-PAGE gel and electro-transferred onto a PVDF membrane. The membrane was subsequently blocked and incubated with mouse anti His-tag antibody (1:1 000 dilution) at 4°C overnight, followed by goat anti-mouse IgG (1:2 000 dilution) for 2 h. Lastly, the bands were visualized with ECL Western Blotting Substrate (Vazyme, Nanjing, China).

Enzymatic activity assay. The catalyzing activity of the recombinant hSOD1 was measured using CuZn-SOD and Mn-SOD Assay Kits with WST-8 (Beyotime Biotechnology, Shanghai, China). The assay was based on measuring the color of a formazan dye. According to the manufacturers' instructions, the WST-8/enzyme working solution and reaction starting solution were prepared beforehand. A preliminary experiment was conducted to find the optimal amount of hSOD1, so that the inhibition percentage of hSOD1 lay between 30% and 70%. A certain amount of hSOD1 (final volume was 20 μL), WST-8/enzyme working solution (160 μL) and reaction starting solution (20 μL) were incubated at 37 °C for 30 min. The absorbance at 450 nm was detected. Crude cell lysate (only containing pET-28a (+)) was regarded as a negative control. The activity of hSOD1 was calculated according to the formula in the manufacturers' instructions.

The influence of Cu^{2+} and Zn^{2+} on hSOD1 activity at induction. Overnight cultured bacteria were inoculated into fresh LB medium and shaken at 37°C until the OD_{600}=0.4, and the IPTG was added (to a final concentration of 1 mM). Meanwhile, different concentrations of Cu^{2+} (30–1 000 μM) and Zn^{2+} (10–100 μM) were also individually injected into the bacterial solution. Bacteria samples with no Cu^{2+} or Zn^{2+} added were seen as the control. After the bacteria were exposed to individual Cu^{2+} (30–1 000 μM) or Zn^{2+} (10–100 μM) at induction, we selected the concentrations at which the hSOD1 activity was highest. Then three Cu^{2+} + Zn^{2+} combinations (750 μM Cu^{2+}/15 μM Zn^{2+}; 1,500 μM Cu^{2+}/15 μM Zn^{2+}; and 750 μM Cu^{2+}/30 μM Zn^{2+}) were chosen. The method of detecting hSOD1 activity was mentioned previously.

Effect of Cu^{2+}, Zn^{2+}, other metal ions and a denaturant on hSOD1 activity at the time of detection. To examine the effects of Cu^{2+} and Zn^{2+} on hSOD1 activity, various concentrations of Cu^{2+} (30, 100, 500, 750, 1000, 2000, 5000, 6000 μM) and Zn^{2+} (10, 20, 30, 100, 500, 1000, 2000, 5000, 6000 μM) were added to the purified hSOD1. Meanwhile, a stock solution containing $CaCl_2$, $MgCl_2$, $MnCl_2$, $FeCl_3$, $CdCl_2$, $NiSO_4$ and $CoCl_2$ was added to the purified hSOD1 to achieve 5 mM. Here, the purified hSOD1 was obtained from standard LB medium without supplementation Cu^{2+} and/or Zn^{2+}. As a detergent, the effects of 1%, 5%, 10% SDS on the enzyme were also examined according to the aforementioned methods. The activity of the control enzyme with no metals or detergent was taken as 100%. The results presented are the average of three independent experiments.

RESULTS AND DISCUSSION

Expression and purification of recombinant protein

The 465-bp DNA fragment of hSOD1 encoding the mature protein was cloned from the Bxpc3 cell line cDNA. DNA sequencing confirmed hSOD1 was correctly fused to the N-terminal His-tag, and it also revealed the cloned hSOD1 gene was the same as the published hSOD1 (GenBank accession number CR541742.1). In general, the exogenous recombinant plasmid that was expressed in E. *coli* BL21 easily formed inclusion bodies at 37°C. Usually, lower temperatures were selected to induce the expression of infusion protein (Vasina & Baneyx, 1997; Swalley *et al.*, 2006; Huo *et al.*, 2010). hSOD1 was largely expressed as an insoluble form when the expression of pET-28a (+)-hSOD1 was induced at 37°C with 1 mM IPTG (Fig. 1A). The

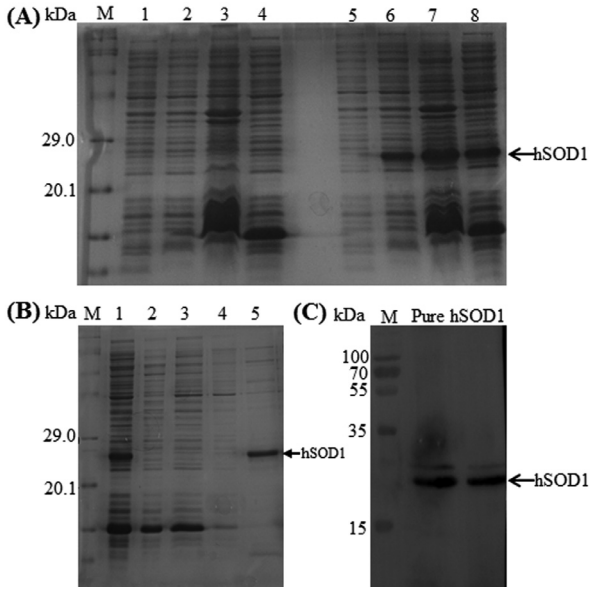

Figure 1. SDS-PAGE stained with Commassie blue and Western blotting analysis of recombinant hSOD1.
(**A**) Lane 1, 2: the total proteins of BL21 (DE3) harboring empty plasmid induced with 1 mM IPTG at 0 and 5 h; lane 3, 4: sediments and supernatants of BL21 (DE3) harboring empty plasmid with 1 mM IPTG at 5 h. lane 5, 6: the total proteins of BL21 (DE3) harboring the pET-28a (+)-hSOD1 induced with 1 mM IPTG at 0 and 5 h lane 7, 8: sediments and supernatants of BL21 (DE3) harboring the pET-28a (+)-hSOD1 at 5 h. (**B**) lane 1: crude cell lysate of hSOD1; lane 2: surplus crude cell lysate of hSOD1 after incubating with Ni-charged resin; lane 3: the flowing solution that Ni-charged resin was washed using solution I (50 mM Tris, pH8.0, 300 mM NaCl, 50 mM imidazole); lane 4: the flowing solution that Ni-charged resin was washed using solution II (50 mM Tris, pH8.0, 300 mM NaCl, 100 mM imidazole); lane 5: purified hSOD1 by dialysis. (**C**) Western blotting analyses of pure hSOD1. M: PageRuler Plus Prestained Protein Ladder.

theoretical molecular mass of hSOD1 is 15.9 kDa (Ahl *et al.*, 2004). The apparent molecular mass of hSOD1 expressed in *E. coli* was 20.1 kDa as determined with SDS-PAGE, and the native enzyme had a size of 32 kDa ((Hartman *et al.*, 1986). In the current study, a five-His-tag with a linker sequence was fused into the N-end hSOD1, so the apparent molecular weight of the recombinant hSOD1 is higher than 20.1 kDa, at 25–26 kDa (Fig. 1). It is unknown what caused the higher apparent molecular weight on SDS-PAGE. The SDS-PAGE method itself could be the reason of the higher apparent molecular weight observed on recombinant hSOD1 or due to some unknown posttranslational modifications.

The recombinant hSOD1 represented approximately 74% of the total bacterial protein, as was determined with densitometric scanning (Fig. 1A, lane 6). Protein expressions in the soluble and insoluble fractions were compared. Soluble and insoluble hSOD1 accounted for approximately 47% and 48% of the soluble and insoluble proteins, respectively (Fig. 1A, lanes 7, 8). Then, the soluble hSOD1 was purified to 90% with high affinity Ni-charged resin (Fig. 1B, lane 5). Further, we used western blotting to verify the purification results. In the Fig. 1C pure hSOD1 displays a major band identified using the anti His-tag antibody, and an extra minor band might represent aggregated hSOD1 or other proteins recognized by the antibody non-specifically. The amount of pure protein product was approximately 0.59 mg per 100 mL *E. coli* culture, estimated using the Bradford method with BSA as the standard (see Table 1).

Influence of Cu^{2+} and Zn^{2+} on hSOD1 activity at induction

The correct coordination of Cu^{2+} and Zn^{2+} can ensure the structural integrity of hSOD1 (Nordlund *et al.*, 2009; Li *et al.*, 2010). Under normal conditions, correctly folded SOD1 catalyzes the degradation of superoxide radicals (Shaw & Valentine, 2007); however, the lack of Cu^{2+} and Zn^{2+} seriously affects the folding of the protein, leading to the loss of protein activity (Wittung-Stafshede, 2004; Rumfeldt *et al.*, 2009). Given this phenomenon, various concentrations of Cu^{2+}, Zn^{2+} and Cu^{2+} plus Zn^{2+} were added to the medium to see whether this altered the specific hSOD1 activity in crude bacterial lysates at induction. Our results showed that supplementation of Cu^{2+} and Zn^{2+} increased the specific activity of hSOD1. The highest enzymatic activity was observed when the individual Cu^{2+} and Zn^{2+} concentrations were 750 μM and 15 μM (Fig. 2), respectively, and the SOD1 activity declined when the concentrations of Cu^{2+} and Zn^{2+} continued to increase which indicated that excess Cu^{2+} and Zn^{2+} may be toxic to the cells at induction (Fig. 2). Furthermore, from Fig. 3, the yield of SOD1 did not significantly increase compared to the control.

The highest activity of hSOD1 produced with 750 μM Cu^{2+} added was 7.7-fold greater than that of the control. This is consistent with a previous observation that supplementation of Cu^{2+} increases the specific activity of hSOD1 in *E. coli* (Hartman *et al.*, 1986) and sf21 cells (Fujii *et al.*, 1995). Our data showed that Cu^{2+} had no obvious effects on improving the solubility (data not shown) or enhancing the yield of SOD1 (Fig. 3A), but

Table 1. Purification of recombinant hSOD1 from a 100 mL *E. coli* culture

Proteins	Total protein (mg)	Specific activity (unit/mg)	Total activity (unit)	Yield (%)	Purification (-fold)
Cell lysate[a] (all proteins of *E. coli* contained EP)	6.0	29.0	174.0		
Cell lysate with expression of hSOD1[b] (all proteins of *E. coli* contained RP)	7.9	154.3	1219		
hSOD1 in cell lysate[c]	7.9	125.3	989.9	100.0	1.0
Purified hSOD1[d]	0.59	1365.3	805.5	81.4	10.9

[a,b]4 mL of supernatant were obtained through ultrasonication after 100 mL *E. coli* culture were collected. [c]the enzyme specific activity of hSOD1 in cell lysate was calculated by subtracting the enzyme specific activity of cell lysate with empty vector from that of cell lysate with hSOD1 expression. [d]3 mL of purified hSOD1 were obtained through nickel column chromatography from 4 mL of supernatant. EP: empty plasmid pET-28a (+). RP: recombinant plasmid containing the hSOD1 gene.

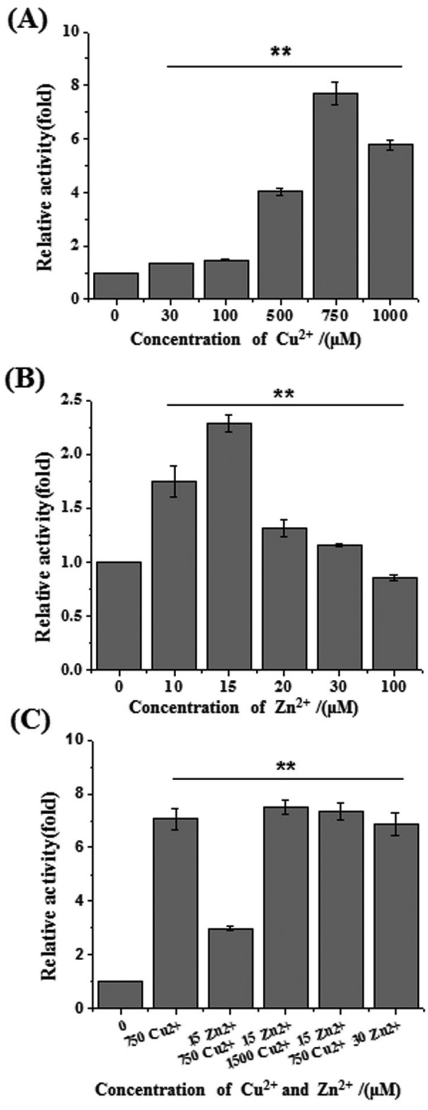

Figure 2. Activity of hSOD1 isolated from *E. coli* that was exposed to different concentrations of Cu^{2+} (A), Zn^{2+} (B), Cu^{2+} and Zn^{2+} (C).
X-axis represents the different concentrations of ions. Y-axis represents the fold of specific activity. The control was set as 1. Data represent mean ± S.D. for three independent experiments. ** indicates $P < 0.01$.

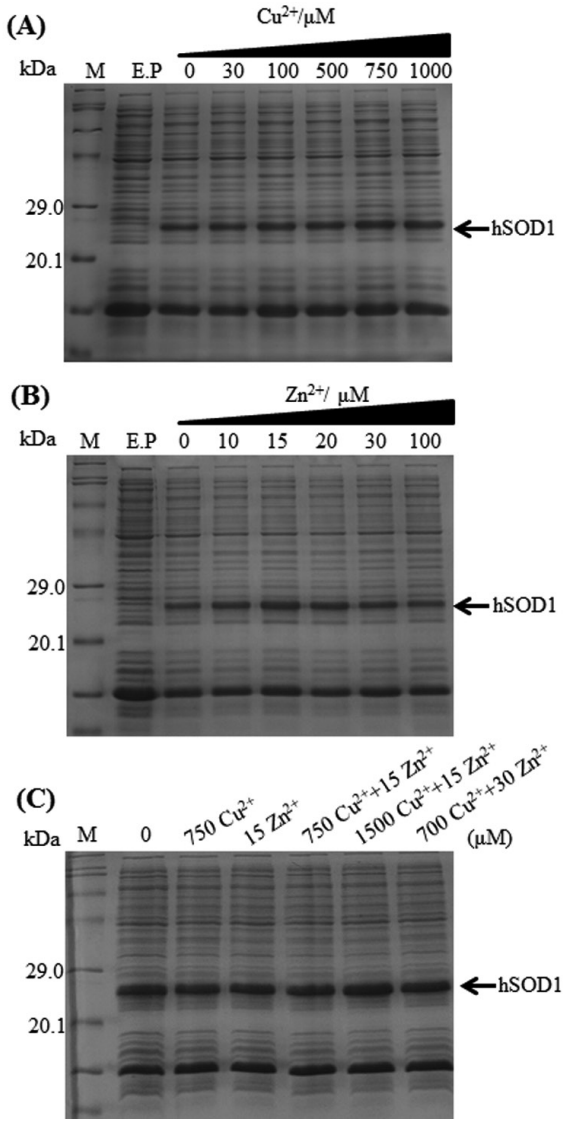

Figure 3. SDS-PAGE analyses of hSOD1 isolated from *E. coli* that was exposed to different concentrations of Cu^{2+} (A), Zn^{2+} (B), Cu^{2+} and Zn^{2+} (C).
M: protein molecular weight marker; E.P: empty plasmid pET-28a (+).

it made a major contribution to the recovery of SOD1 activity (Fig. 2A).

Li and coworkers demonstrated that stoichiometric zinc played an important role in the oxidative refolding of bovine SOD1 by accelerating the oxidative refolding, suppressing aggregation during refolding and helping the protein to form a compact conformation with high protease resistance activity (Li *et al.*, 2010). We expected an increase in the soluble expression levels of SOD1 in *E. coli* upon supplementation with Cu^{2+} and Zn^{2+}; however, no significant improvement in hSOD1 solubility was found by inducing at 37 °C compared with samples without supplementation Cu^{2+} and Zn^{2+} (data not shown). Meanwhile, a 2.3-fold increase in the specific activity of hSOD1 with 15 μM Zn^{2+} supplementation was observed. Fujii *et al.* reported that supplementation with Zn^{2+} alone did not enhance the SOD activity (Fujii *et al.*, 1995), whereas our data showed that Zn^{2+} slightly increased SOD activity. Wu and coworkers noted that SOD1 activity was decreased by about 50% in zinc-limited cells (Wu *et al.*, 2009). Li *et al.* indicated that copper played a dominant role in SOD1 activity and zinc only made a small contribution to SOD1 activity (Li *et al.*, 2010).

The specific activity of hSOD1 upon supplementation with 750 μM Cu^{2+}/15 μM Zn^{2+} (Fig. 2C) was 7.5-fold greater than that of the control, which was approximately equal to 7.7-fold enhancement with supplementation of 750 μM Cu^{2+} alone. This implied that, when the Cu^{2+}-binding sites were fully saturated, it was possible that the metallation of Cu^{2+} site facilitated the metallation of Zn^{2+} sites or the Zn^{2+} could be replaced by Cu^{2+} with full function. Crow and coworkers reported that zinc was more likely to disassociate than copper, because SOD has an approximately a 7000-fold lower affinity for zinc than it does for copper (Crow *et al.*, 1997).

The completely metal-free apo-hSOD1 has no specific activity (Hartman *et al.*, 1986). Hartman and coworkers

Table 2. Effects of metal ions and denaturant on the purified hSOD1

Metals/denaturant	Concentration	Relative activity (-fold)
Control	0	1.0 ± 0.00
$CaCl_2$	5 mM	1.9 ± 0.20
$MgCl_2$	5 mM	1.4 ± 0.31
$MnCl_2$	5 mM	16.9 ± 1.62
$FeCl_3$	5 mM	5.5 ± 0.25
$CdCl_2$	5 mM	3.9 ± 0.38
$NiSO_4$	5 mM	3.4 ± 0.35
$CoCl_2$	5 mM	6.7 ± 0.25
SDS	1, 5, 10%	0.0 ± 0.00

Control activity (1.0) was determined when none of the metal ions were added. Data represent mean ± S.D. for three independent experiments.

suggested that the intracellular concentration of Cu^{2+} in *E. coli* is insufficient to saturate human CuZnSOD, especially at high expression levels (Hartman *et al.*, 1986). Our results showed that, in LB medium, the addition of 750 μM of Cu^{2+} to the medium apparently raised the intracellular Cu^{2+} concentration to levels that are sufficient to saturate the active sites for the overproduced hSOD1. In contrast, adequate Zn^{2+} was incorporated into hSOD1 when 15 μM of Zn^{2+} was present in the medium.

Effects of Cu^{2+}, Zn^{2+}, the other metal ions and a denaturant on hSOD1 activity at the time of detection

To study the effects of Cu^{2+} and Zn^{2+} on hSOD1 enzymatic activity, we added various concentrations of Cu^{2+} and Zn^{2+} to the purified hSOD1. The results showed that specific hSOD1 activity significantly increased at concentration of Cu^{2+} ranging from 30 to 6000 μM (Fig. 4A). The specific hSOD1 activity only slightly increased with 0–2000 μM Zn^{2+}, but it significantly increased at Zn^{2+} concentrations of 5000 and 6000 μM (Fig. 4B). This indicated that Cu^{2+} and Zn^{2+} also enhanced the enzyme activity at the time of detection.

The effects of the other metal ions and a denaturant on the purified recombinant hSOD1 were studied and are shown in Table 2. hSOD1 exhibited high activity at 5000 μM Cu^{2+} or Zn^{2+} (Fig. 4). Then, we selected 5 mM of metal ions ($CaCl_2$, $MgCl_2$, $MnCl_2$, $FeCl_3$, $CdCl_2$, $NiSO_4$ and $CoCl_2$), and individually added them to the purified hSOD1. Compared to the control without metals, the activity of hSOD1 was enhanced to varying degrees. The influences of Co^{2+} and Mn^{2+} on the activity of hSOD1 were very significant, in that the activity increased by 6.7- and 16.9-fold compared to the control. hSOD1 exhibited little effect upon the addition of other metal ions (Ca^{2+}, Mg^{2+}, Fe^{3+}, Cd^{2+}, and Ni^{2+}), whose influence ranged from 1.9- to 5.5-fold. From these results, we determined that all of the divalent metal ions (M^{2+}) have the potential to replace Zn^{2+} and Cu^{2+}. Furthermore, the activity of hSOD1 was totally inhibited by SDS.

Substitutions of the native Cu and Zn ions by non-native metal ions cause minimal structural changes, and result in high enzymatic activity for those derivatives when Cu remains in the Cu site. Studies of the derivatives in which Zn^{2+} was replaced by another divalent metal ion, M^{2+} (i.e., Cu_2M_2SOD1 with M=Co, Ni, Cd, Hg, Cu) found the enzyme to be little changed structurally by the metal ion replacement and to retain full enzymatic activity. Studies of the derivatives in which Cu was replaced by another metal ion (i.e., M_2Zn_2SOD1 with M=Co, Ni, Ag, Cd, Zn) also suggested that non-native metal ion substitutions causes little if any rearrangement of the ligand geometries in the metal binding region of the protein (Ming & Valentine, 2014).

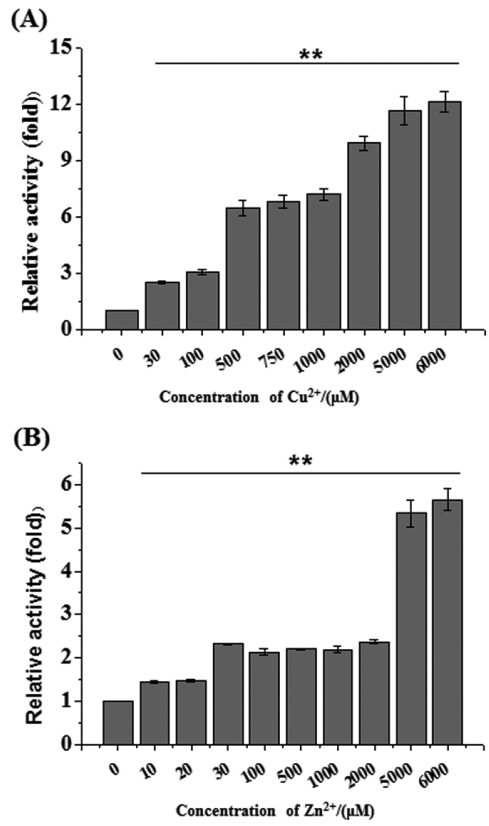

Figure 4. Effects of Cu^{2+} (A) and Zn^{2+} (B) on purified hSOD1 activity.
X-axis represents the different concentrations of ions. Y-axis represents the fold of specific activity. The control was set as 1. Data represent mean ± S.D. for three independent experiments. **indicates $P < 0.01$.

Mn^{2+} is essential for the activation of SOD2 as it acts as a cofactor that coordinates with each of the enzyme's four subunits. Ghneim and coworkers reported there were statistically significant increases in SOD2 activities in senescent fibroblasts incubated with all of the Mn^{2+} supplemented media (Ghneim, 2016). Our results suggested that supplementation of Mn^{2+} could also significantly increase the specific activity of hSOD1. One possibility is that SOD1 and SOD2 have a close evolutionary relationship (Haddad & Yuan, 2005). These studies provide some reference points for improving the catalytic efficiency of the enzyme.

CONCLUSIONS

In this research, we have cloned and expressed a homo Cu/Zn SOD, hSOD1 in *E. coli* BL21 (DE3). The purified recombinant hSOD1 protein was capable of inhibiting the formation of formazan dye suggesting that the hSOD1 gene encodes a functional superoxide dismutase. Considering that metal cofactors are essential to the structure and activity of hSOD1, hSOD1 activity was measured upon the addition of Cu^{2+} and Zn^{2+} at induction compared to no addition. The results implied that

Cu^{2+} and Zn^{2+} do not enhance SOD1 expression and solubility; however, they can improve the catalytic activity at induction. This adequately showed that the recombinant hSOD1 was well folded in the presence of Cu^{2+} and Zn^{2+}, and would be suitable for further functional study. Meanwhile, Cu^{2+} and Zn^{2+} also enhanced the enzyme activity at the time of detection. Furthermore, most other bivalent cations had the potential to replace Zn^{2+} and Cu^{2+}, and also improved enzyme activity at the time of detection.

Acknowledgements

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