기 생 충 학 잡 지 제29권 제3호
The Korean Journal of Parasitology
Vol. 29, No. 3, 259-266, September 1991

Purification and Characterization of a Cu, Zn-Superoxide Dismutase from Adult *Paragonimus westermani*

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Abstract: In cytosolic fraction of adult *Paragonimus westermani*, superoxide dismutase activity was identified (4.3 units/mg of specific activity) using a xanthine-xanthine oxidase system. The enzyme was purified 150 fold in its activity using the ammonium sulfate precipitation, DEAE-Trisacryl M anion-exchange chromatography and Sephadex G-100 molecular sieve chromatography. The enzyme exhibited the enhanced activity at pH 10.0. The enzyme activity totally disappeared in 1.0mM cyanide while it remained 77.8% even in 10 mM azide. These findings indicated that the enzyme was Cu, Zn-SOD type. Molecular mass of the enzyme was estimated to be 34 kDa by gel filtration and 17 kDa on reducing SDS-polyacrylamide gel electrophoresis which indicated a dimer protein.

Key words: *Paragonimus westermani*, cytosol, oxygen toxicity, Cu, Zn-superoxide dismutase

INTRODUCTION

Paragonimus westermani is a trematode parasite that normally thrives in the lung of carnivorous mammals and man. Its enzootic and human infections distribute widely in East and Southeast Asia (Yokogawa, 1982). When infective metacercariae are eaten by the final hosts, the larvae actively penetrate the intestinal wall to peritoneal cavity where they migrate for 2~4 weeks. Then, the worms arrive the lung in which they become adults and are surrounded by granulomatous lesions (Choi *et al.*, 1979). In experimental infections to cats or dogs, over 90% of challenged metacercariae can be recovered as adults. During the migratory stages of the juvenile worms in abdominal cavity and granulomatous lesions in the lung, extensive inflammatory reactions are elicited. In spite of this unfavorable environment of the host, adult *Paragonimus* can survive about 5 years.

To explain the pathophysiologic events in paragonimiasis and long survival of the adults, parasite mechanisms, which enable them to evade from the hostile host defences, should be elucidated. Of the possible mechanisms of parasite evasion, helminthes proteases (Mckerrow, 1989) and anti-oxidant enzymes (Callahan *et al.*, 1988) are increasingly recognized to be important nowadays. In the lesions surrounding the invading parasite, free oxygen radicals such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and hydroxyl radical (•OH) are released from inflammatory cells and platelets. These radicals are known to damage the membranes, proteins and nucleic acids which sometimes sufficient to kill the cells and/or organisms. Therefore, several antioxidant enzymes of parasites such as superoxide dismutase, catalase,

glutathione peroxidase and glutathione S-transferase are important as a protective mechanism against host oxidants (Callahan *et al.*, 1988).

Superoxide dismutase (SOD) converts the superoxide radicals to oxygen and hydrogen peroxide ($2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$) (McCord and Fridovich, 1969). Superoxide radicals can be produced by a variety of biological processes including xanthine oxidations, NADPH-dependent oxidations, activation of macrophage and cytolysis of natural killer cells (Salin and McCord, 1974; Roder *et al.*, 1982; Bromberg and Pick, 1985). The toxic effects of superoxide radicals are, therefore, catalyzed by this scavenger enzyme as soon as it produced in the inflamed tissue (Fridovich, 1975; Halliwell, 1978).

As reviewed by Callahan *et al.* (1988), many studies have been done on the activities of antioxidant enzymes in parasites. SOD were recognized so far in all examined protozoan or helminthes parasites except *Plasmodium* sp. in which host SOD is incorporated and utilized (Fairfield *et al.*, 1983).

In the present study, we identified, purified and characterized a Cu, Zn-SOD which is present in cytosolic fraction of adult *P. westermani*.

MATERIALS AND METHODS

1. Chemicals and reagents

Cytochrome *c*, xanthine, xanthine oxidase, nitroblue tetrazolium (NBT), riboflavin and Sephadex G-100 were purchased from Sigma Chemical Co. (USA). Potassium cyanide, ethylenediamine tetraacetic acid (EDTA) and ammonium sulfate were from E. Merck Co. (FRG). DEAE-Trisacryl M were from LKB Co. (Sweden). Other chemicals used in the study were the reagent grade.

2. Measurement of the enzyme activity

SOD activity was assayed by the inhibition of cytochrome *c* reduction using a xanthine-xanthine oxidase system as described by McCord and Fridovich (1968) and Crapo *et al.* (1978). The final volume of the reaction mixture was composed of 2.3 ml of 50 mM potassium phosphate buffer (pH 7.8) containing 0.1 mM EDTA, 0.3 ml of 0.1 mM cytochrome *c*, 0.3 ml of 0.5 mM xanthine and 0.1 ml of 0.05 mM potassium cyanide. The enzyme activity was measured and recorded using a spectrophotometer at 550 nm (Beckman M-35). One unit of SOD was defined as the amount of enzyme that causes 50% inhibition of cytochrome *c* reduction. Specific activity was defined as the units of activity per milligram of protein.

3. Preparation of the crude enzyme

Thirteen-week after the experimental infection of the metacercariae, adult *Paragonimus westermani* were harvested from a dog lung. After washing in physiologic saline, a total of 80 worms was homogenized with Potter-Elvejem homogenizer with 50mM potassium phosphate buffer (pH 7.8) containing 0.1 mM EDTA. The homogenate was centrifuged twice at 700*g* for 5 minutes. Supernatant was obtained by recentrifugation at 20,000*g* for an hour. Resulting supernatant was regarded as the crude enzyme. Protein content was measured by Lowry *et al.* (1951). All procedures for enzyme preparation were done at 4°C, unless otherwise specified.

4. Enzyme purification

(1) Ammonium sulfate precipitation

The crude enzyme was saturated at a final concentration of 30% ammonium sulfate and let it stand for an hour with stirring. The suspension was centrifuged at 12,000*g* for 30 minutes. Ammonium sulfate was added to the supernatant to make 85% saturation. This solution was stirred for 4 hours and centrifuged at 12,000*g* for 30 minutes. The pellet was dissolved in 20 mM Tris-HCl buffer (pH 7.2) and dialyzed against the same buffer overnight. This solution was centrifuged at 1,500*g* for 5 minutes to remove insoluble materials.

(2) DEAE-Trisacryl M anion-exchange chromatography

At the first step, a total of 14.2 ml (52 mg of protein) of ammonium sulfate treated crude enzyme was applied on 2.6(ϕ)×15 cm sized

DEAE-Trisacryl M column and eluted through 20 mM Tris-HCl buffer (pH 7.2) by 0～0.5 M linear gradient salt concentration. The flow rate was 50 ml/hour. Eluent was allocated in 3.5 ml. The active fractions were pooled, dialyzed and lyophilized.

For the next purification, 2.6(ϕ)×7 cm sized DEAE-Trisacryl M column was equilibrated with 20 mM Tris-HCl buffer (pH 8.0). Then, 9.4 ml of clarified solution was eluted by 0～0.5 M linear gradient salt solution and the enzyme activity was assayed. The active fractions were dialyzed against 20 mM acetate buffer (pH 6.2) containing 0.1 mM EDTA and concentrated.

DEAE-Trisacryl M column chromatography of 1.6(ϕ)×7 cm long was used for the third purification. After equilibration with 20 mM acetate buffer (pH 6.2), 5.2 ml of the pooled enzyme was eluted by a salt gradient from 0 to 0.3 M and assayed. The active fractions were dialyzed against 50 mM potassium phosphate buffer (pH 7.8) containing 0.2 M KCl and lyophilized.

(3) Sephadex G-100 molecular sieve chromatography

For further purification and estimation of molecular mass of the enzyme, the partially purified enzyme was brought to Sephadex G-100 gel filtration of 1.6(ϕ)×40 cm long. The flow rate was 20 ml/hour. Eluent was allocated in 1.4 ml. Standard proteins of bovine serum albumin (68 kDa), ovalbumin (43 kDa), chymotrypsinogen A (25 kDa) and cytochrome *c* (12.4 kDa) were filtrated concomitantly.

5. Characterization of the enzyme

(1) Inhibitor effects

To observe the inhibitory effect of cyanide to cytosolic SOD, the methods of Rotilio *et al.* (1972) and Dryer *et al.* (1980) were adopted. In case of azide, the method of Misra and Fridovich (1978) was followed. The enzyme activity was measured in the presence of cyanide (0.1 mM～3.0 mM) and azide (1 mM～20 mM).

(2) Effect of pH

The effect of pH on activity of SOD was measured according to Salin and McCord(1974). The reaction mixture was incubated in 50 mM sodium carbonate buffer containing 0.1 mM xanthine. Other procedures were done as described above.

(3) SDS-PAGE

To observe the purity and subunits composition of the enzyme, SDS-PAGE method of Laemmli (1970) was adopted. Stacking gel of 2.5% and separating gel of 12.5% were used for protein separation in reducing condition. Constant current of 25 mA was supplied. After the electrophoresis, the gel was stained with Commassie briliant blue R-250.

RESULTS

1. Activity of cytosolic SOD of adult *P. westermani*

The activity of cytosolic SOD was 97.4 units/g and protein content was 22.4 mg/g. Therefore, the specific activity of the enzyme was 4.4 units/mg.

2. Purification of cytosolic SOD

As shown in Table 1, the enzyme activity of the worm was 486.8 units and total protein was 112.2 mg (specific activity: 4.3 units/mg). When 30～85% ammonium sulfate precipitation was done, about 80% (389 units) of the enzyme was recovered. The protein content was 52 mg and specific activity was 7.4. This clarified enzyme was chromatographied onto the first DEAE-Trisacryl M anion-exchange column (Fig. 1). As shown in Table 1, 221.4 units of the enzyme was harvested. Fig. 2 showed the elution profile of the second DEAE-Trisacryl M anion exchange chromatography. Four active fractions were pooled. A total of 137 units of SOD was obtained (protein content: 1.7 mg, specific activity: 80.4) (Table 1). The third purification was continued as illustrated in Fig. 3. By the step, a total of 65 units of SOD was partially purified. The specific activity was 325 (protein content of 0.2 mg). The enzyme was purified 76 folds. This enzyme solution was subjected to Sephadex G-100 gel filtration (Fig. 4). From the final step, 26 units of SOD was purified and the

Table 1. Purification of cytosolic superoxide dismutase from *P. westermani*

	Activity (units)	Protein (mg)	S.A.* (units/mg)	Purification (fold)	Recovery (%)
Cytosol	486.8	112.2	4.3	1.0	100
30%~85% ammonium sulfate treatment	388.8	52.4	7.4	1.7	79.9
1st DEAE-column	221.4	12.6	17.6	4.1	45.5
2nd DEAE-column	136.6	1.7	80.4	18.7	28.1
3rd DEAE-column	65.0	0.2	325.0	75.6	13.4
Sephadex G-100 column	25.8	0.04	645.0	150.0	5.3

* Specific activity

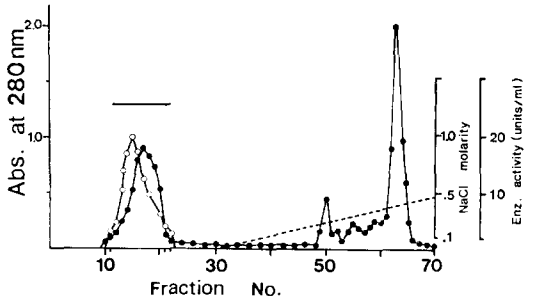

Fig. 1. Elution profile of SOD on the 1st DEAE-Trisacryl M anion-exchange chromatography. An allocation of each fraction was assayed for SOD activity (○) and protein (●). A linear gradient was indicated by dotted line. Pooled fractions containing SOD activity was shown by a bar (—).

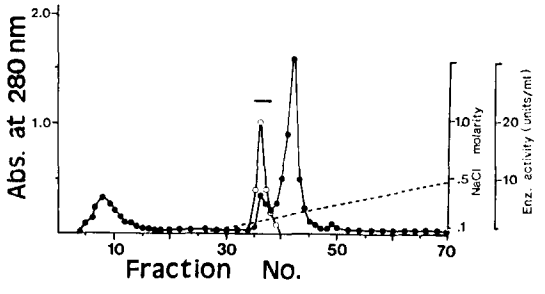

Fig. 2. Elution profile of SOD on the 2nd DEAE-Trisacryl M anion-exchange chromatography. Markings are the same as described in Fig. 1.

specific activity was calculated as 645 (protein content of 0.04 mg). The enzyme was purified 150 folds. Various yields and clarified enzyme during purification processes were summarized in Table 1 and illustrated in Fig. 5.

3. Biochemical properties of SOD

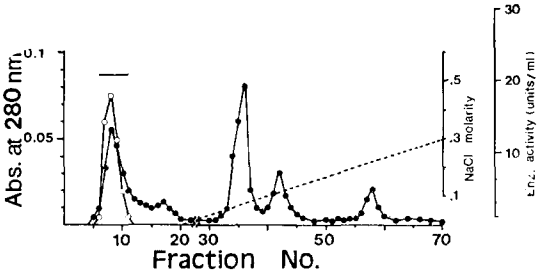

Fig. 3. Elution profile of SOD on the 3rd DEAE-Trisacryl M anion-exchange chromatography. Markings are the same as described in Fig. 1.

Table 2. Effects of inhibitors on activity of cytosolic SOD of *P. westermani*

Inhibitor	Concentration (mM)	Activity (units/ml enzyme)	Inhibition (%)
Cyanide	Control	20.7	0
	0.1	16.7	22.2
	0.3	9.6	53.6
	0.5	5.1	75.4
	1.0	0	100
	2.0	0	100
Azide	Control	20.7	0
	1.0	20.7	0
	5.0	18.7	11.1
	10.0	16.7	22.2

The inhibitory effects of cyanide and azide to SOD activity were presented in Table 2. As the cyanide molarity increased up to 1.0 mM, the enzymatic activity was totally droppedout. On the contrary, when an azide was added to 5 mM and 10 mM concentration, 88.9% and 77.8% of the enzyme activity were remained respectively. Table 3 showed the effect of pH

Table 3. Effect of pH on activity of cytosolic SOD of *P. westermani*

	Activity (units/ml enzyme)	Activation (fold)
Control	20.7	1
pH 10.0*	110.7	5.4

* Same as in the standard assay, except for containing 50 mM sodium carbonate and 0.1 mM of xanthine in the buffer.

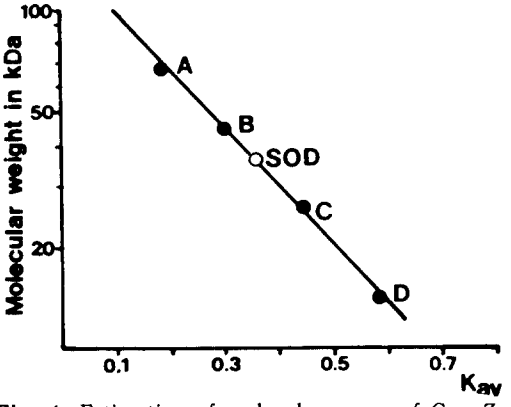

Fig. 4. Estimation of molecular mass of Cu, Zn-SOD by Sephadex G-100 gel filtration.
A : Bovine serum albumin (68 kDa)
B : Ovalbumin (43 kDa)
C : Chymotrypsinogen A (25 kDa)
D : Cytochrome *c* (12.4 kDa)

on SOD. At pH 10.0 of the buffer, the enzyme showed the enhanced activity of 5.4 times. Molecular mass of SOD was estimated to be 34 kDa utilizing k_{av} calibration (Fig. 4). It was composed of an equal sized subunit of 17 kDa. Lane 4 of Fig. 5 exhibited the SDS-PAGE findings of Sephadex G-100 purified SOD of adult *P. westermani*.

DISCUSSION

According to their metal binding capacity, 3 SODs are now recognized, *i.e.*, Fe-, Mn- and Cu, Zn-SOD (Yost and Fridovich, 1973; Dryer *et al.*, 1980; Geller and Winge, 1982). Of them, Cu, Zn-SOD distributed widely in multicellular plants and animals including parasites. The enzyme is susceptible to inhibitory effect of cyanide while resistant to azide (McCord and Fridovich, 1969). In this study, we could isolate

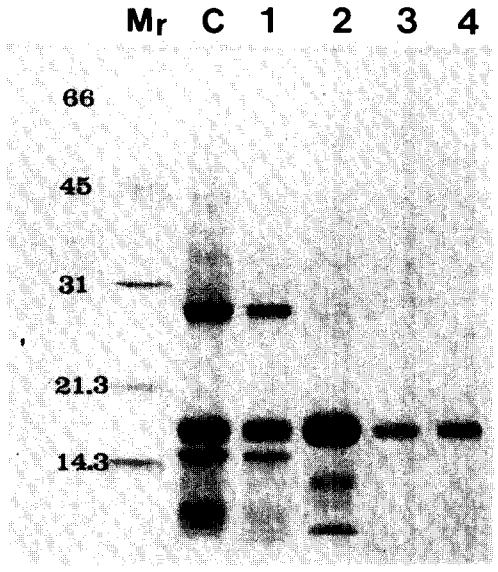

Fig. 5. Findings on SDS-PAGE of Cu, Zn-SOD of *P. westermani*. The samples were electrophoresed at 12.5% gel in reducing condition.
Mr: Molecular mass in kDa
C: Crude extract
1: 1st DEAE-Trisacryl M anion-exchange chromatography
2: 2nd DEAE-Trisacryl M anion-exchange chromatography
3: 3rd DEAE-Trisacryl M anion-exchange chromatography
4: Sephadex G-100 column chromatography

the Cu, Zn-SOD in cytosol of adult *P. westermani* using xanthine-xanthine oxidase system. It was vulnerable to cyanide as shown in Table 2. The enzyme activity totally disappeared when cyanide molarity increased up to 1.0 mM. The inhibition test by azide showed that the enzyme was resistant even in 10 mM concentration. Enhancement of the enzyme activity in pH 10.0 also provide the evidence of a Cu, Zn-SOD.

It is interesting that Cu, Zn-SOD from various animals have comparable molecular masses disregarding their phylogenetic origins. For example, molecular mass of Cu, Zn-SOD of human term placenta was reported as 33 kDa (Moon *et al.*, 1983), that from *Drosophila melanogaster* was 32 kDa (Lee *et al.*, 1981) and that of *Trichinella spiralis* was 36 kDa (Rhoads, 1983). In addition, they were all dimer proteins

with the same sized subunits. In the present study, native molecular mass of Cu, Zn-SOD of adult *P. westermani* was 34 kDa when observed by gel filtration. On reducing SDS-PAGE of the purified SOD, it composed of the same sized subunit of 17 kDa. These findings indicate that Cu, Zn-SOD of adult *Paragonimus* was also a dimer protein.

New-born larvae of *Trichinella spiralis* have low levels of anti-oxidant enzymes including SOD whereas muscle larvae and adults have them in much larger amount. The difference in SOD between new-born and muscle larvae, is considered to be a reason of different resistance to killing (Callahan *et al.*, 1988). Kazura and Meshnick (1984) showed that co-culturing adult *T. spiralis* with the new-born larvae protected the latter from killing by oxidants generated by acetaldehyde-xanthine oxidase. This indicates that SOD, secreted by adults, may protect the new-born larvae. On the contrary, in the initial stages of *Paragonimus* infection, such parental protection is not exist. To survive in an inflamed tissue where oxidants from macrophages and inflammatory cells prevail, it is assumed that juvenile *Paragonimus* should have considerable anti-oxidant enzymes including SOD. High rates of larval infection to adult in experimental paragonimiasis support the assumption of high levels of anti-oxidant enzymes in early developmental stages. But this should be confirmed in the future.

Two different forms of Cu, Zn-SOD are known to exist; *i.e.*, intracellular form of 16 kDa dimer protein (Cu, Zn-SOD) and extracellular tetramer with 30 kDa subunits (EC-SOD) (Getzoff *et al.*, 1986). Human EC-SOD is synthesized with a putative 18-amino acid signal peptides which cleaved upon secretion (Hjalmarsson *et al.*, 1987). Up to present, both EC-SOD and Cu, Zn-SOD revealed species-specific antigenicity (Callahan *et al.*, 1988). In this respect, what we purified in this study was the intracellular Cu, Zn-SOD of adult *Paragonimus*. It is not known yet whether another EC-SOD is existing in helminth parasites. It seems necessary to study the presence of SOD activity in the secretory-excretory product of adult *Paragonimus*.

As shown in SDS-PAGE (Lane C of Fig. 5), the band of 17 kDa (corresponding to the subunit of SOD) was a major component protein in the cytosol. It is uncertain whether the band of 17 kDa was composed only of SOD or not. But the SOD is evidently a major protein of *Paragonimus* any way. If the purified Cu, Zn-SOD is only localized intracellularly, its contribution to host antibody formation may be negligible. Unlike this speculation, however, Kim *et al.* (1988) showed that 16.5 kDa protein in crude extract of *Paragonimus westermani* had a moderate antigenicity when observed by SDS-PAGE/immunoblot using homologous human infected sera. In this connection, relationship between Cu, Zn-SOD and its antigenicity deserves further studies.

REFERENCES

Bromberg, Y. and Pick, E. (1985) Activation of NADPH-dependent superoxide production in a cell-free system by sodium dodecyl sulfate. *J. Biol. Chem.*, **290**:13539-13545.

Callahan, H.L., Crouch, R.K. and James, E.R. (1988) Helminth anti-oxidant enzymes: A protective mechanism against host oxidants? *Parasitology Today*, **4**:218-225.

Choi, W.Y., Lee, O.R., Jin, Y.K. and Chi, J.G. (1979) Lung findings in experimental paragonimiasis. *Korean J. Parasit.*, **17**:132-146. (in Korean with English abstract)

Crapo, J.D., McCord, J.M. and Fridovich, I. (1978) Preparation and assay of superoxide dismutase. In: *Methods in Enzymology* (Ed: Fleischer, S. and Parker, L.). pp. 382-393. Academic Press, New York.

Dryer, S.E., Dryer, R.L. and Autor, A.P. (1980) Enhancement of mitochondrial, cyanide-resistant superoxide dismutase in the livers of rats treated with 2,4-dinitrophenol. *J. Biol. Chem.*, **255**:1054-1057.

Fairfield, A.S., Meshnick, S.R. and Eaton, J.W. (1983) Malaria parasites adopt host cell superoxide dismutase. *Science*, **221**:764-766.

Fridovich, I. (1975) Superoxide dismutase. *Ann. Rev. Biochem.*, **44**:147-159.

Geller, B.L. and Winge, D.R. (1982) Rat liver Cu, Zn-superoxide dismutase; subcellular localization in lysosome. *J. Biol. Chem.*, **257**:8945-8592.

Getzoff, E.D., Olson, A.J. and Tainer, J.A. (1986) Superoxide and superoxide dismutase in chemistry. In: *Biology and Medicine* (Ed: Rotilio, G.). pp. 135-140. Elsevier Science Publishers.

Halliwell, B. (1978) Biochemical mechanisms for the toxic action of oxygen on living organisms: The key role of superoxide dismutase. *Cell Biol. International Reports*, **2**:113-128.

Hjalmarsson, K., Marklund, S.L., Engstrom, A. and Edlund, T. (1987) Isolation and sequence of complementary DNA encoding human extracellular superoxide dismutase. *Proc. Natl. Acad. Sci. USA*, **84**:6340-6344.

Kazura, J.W. and Meshnick, S.R. (1984) Scavenger enzymes and resistance to oxygen mediated. *Mol. Biochem. Parasitol.*, **10**:1-10.

Kim, S.H., Kong, Y., Kim, S.I., Kang, S.Y. and Cho, S.Y. (1988) Immunoblot observation of antigenic protein fractions in *Paragonimus westermani* reacting with human patients sera. *Korean J. Parasit.*, **26**:239-243.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature (London)*, **227**:680-685.

Lee, Y.M., Ayala, F.J. and Misra, H.P. (1981) Purification and properties of superoxide dismutase from *Drosophila melanogaster*. *J. Biol. Chem.*, **256**:8506-8509.

Lowry, O.H., Rosenbrough, N., Lewis-Farr, A. and Randall, R.J. (1951) Protein measurement by Folin phenol reagent. *J. Biol. Chem.*, **193**:265-275.

McCord, J.M. and Fridovich, I. (1968) The reduction of cytochrome *c* by milk xanthine oxidase. *J. Biol. Chem.*, **243**:5753-5760.

McCord, J.M. and Fridovich, I. (1969) Superoxide dismutase: An enzymatic function for erythrocuprein(hematocuprein). *J. Biol. Chem.*, **244**:6049-6055.

Mckerrow, J.H. (1989) Parasite proteinase. *Exp. Parasit.*, **68**:111-115.

Misra, H.P. and Fridovich, I. (1978) Inhibition of superoxide dismutase by azide. *Arch. Biochem. Biophys.*, **189**:317-322.

Moon, J.S., Kwon, N.S., Lee, K.B. and Lee, H.S. (1982) Purification and properties of superoxide dismutase from human term placenta. *Chung-Ang J. Med.*, **7**:163-172. (in Korean with English abstract)

Rhoads, M.L. (1983) *Trichinella spiralis:* Identification and purification of superoxide dismutase. *Exp. Parasit.*, **56**:41-54.

Roder, J.C., Helfand, S.L., Werkmeister, J., McGarry, R., Beaumount, T.J. and Duwe, A. (1982) Oxygen intermediates are triggered early in the cytolytic pathway of human NK cells. *Nature(London)*, **298**:569-572.

Rotilio, J.C., Bray, R.C. and Fielden, E.M. (1972) A pulse radiolysis study of superoxide dismutase. *Acta Biochem. Biophys.*, **268**:605-609.

Salin, M.L. and McCord, J.M. (1974) Superoxide dismutase in polymorphonuclear leukocytes. *J. Clin. Invest.*, **54**:1005-1009.

Yokogawa, M. (1982) Paragonimiasis. In: *CRC handbook series in zoonoses: Parasitic zoonoses* (Vol. III). p.123. CRC Press, Boca Raton.

Yost, F.J. and Fridovich, I. (1973) An iron containing superoxide dismutase from *Escherichia coli*. *J. Biol. Chem.*, **248**:4905-4908.

=국문초록=

폐흡충 성충 Cu, Zn-Superoxide Dismutase의 정제 및 생화학적 특성

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정영배 · 송철용 · 이희성* · 공 윤** · 조승열**

폐흡충 성충의 인산완충액 추출액을 원심분리하여 만든 세포질 현탁액을 조효소(粗酵素)로 사용하여 xanthine-xanthine oxidase system으로 superoxide dismutase 활성을 측정한 결과 비활성도(比活性度; specific activity)는 4.3 units/mg이었다. 이 효소의 활성도를 30~85% ammonium sulfate 침전 및 DEAE-Trisacryl M anion exchange chromatography와 Sephadex G-100 column chromatography를 통과시키면서 측정하는 방법으로 superoxide dismutase를 정제하고, 정제한 효소의 생화학적 특성을 관찰하여 다음과 같은 결과를 얻었다.

1. 정제과정을 통해 세포질 내 superoxide dismutase를 150배 정제하였다. Sephadex G-100 gel filtration에 의해 계산한 이 효소의 분자량은 34 kDa이었고 환원성 SDS-PAGE상 subunit가 17 kDa이었다. 따라서 이 효소는 subunit 두개로 구성된 중합체로 판단하였다.

2. 이 효소는 1 mM 이상의 cyanide 농도에서는 효소 활성이 100% 억제되었고, 5 mM 농도의 azide에서는 11.1%, 10 mM azide에서는 22.2% 그 활성이 각각 억제되었다.

3. 이 효소는 완충액의 pH가 10.0일 때 효소 활성이 5.4배 증가되었다.

이상의 결과로 폐흡충의 세포질 내에 존재하는 superoxide dismutase는 구리와 아연을 함유한 superoxide dismutase라고 판단할 수 있었다.

〔기생충학잡지, 29(3):259-266, 1991년 9월〕