Characterization of a peroxidase in excretory-secretory product of adult *Paragonimus westermani*

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Abstract: When activity of peroxidase in ault *Paragonimus westermani* was monitored using o-dianisidine and H_2O_2 as substrates, its specific activity was 1.5 times higher in excretory-secretory product (ESP) than in crude extract. The enzyme was purified by two purification steps of Sephacryl S-300 Superfine gel permeation and DEAE-Trisacryl M anion exchange chromatographies. Its activity increased 16.9 fold with 32.3% recovery. The enzyme was inhibited totally by 1 millimoles of dithiothreitol (DTT), 2-mercaptoethanol and azide. Molecular mass was 16 kDa in reducing SDS-polyacrylamide gel electrophoresis (PAGE) or 19 kDa in TSK-Blue gel filtration high performance liquid chromatography (HPLC), respectively. Special staining for peroxidase by diaminobenzidine on SDS-PAGE confirmed the activity. The peroxidase was less reactive to a paragonimiasis serum when observed by SDS-PAGE/immunoblot. In addition, specific activities of superoxide dismutase (SOD) and catalase were also identified in the ESP. High activities of these antioxidant enzymes in ESP indicate that they are parts of defense mechanisms against reactive oxygen intermediates from host.

Key words: *Paragonimus westermani*, excretory-secretory product, reactive oxygen species, peroxidase, catalase, superoxide dismutase

INTRODUCTION

Paragonimus westermani is a trematode parasite which causes a chronic inflammatory lung disease in carnivorous mammals and man (Shim *et al.*, 1991). Complex pathophysiological interactions, occurred at the sites of infection, result in a granuloma. Recent evidences demonstrate that antioxidant enzymes and proteases of *P. westermani* are present in the worm (Song and Dresden, 1990; Chung *et al.*, 1991 & 1992) and presumed to play roles in pathogenesis of the paragonimiasis as well as in the parasite survival. Antioxidant enzymes such as superoxide dismutase (SOD), catalase, peroxidase and glutathione S-transferase catalyse the removal of reactive oxygen intermediates. Free oxygen radicals of superoxide radical (O_2^-), singlet oxygen ($^1O_2$), hydroxyl radical ($\cdot OH$) and hydrogen peroxide (H_2O_2), generated by a partial reduction of oxygen during the biological processes, pose a serious hazard to immediate vicinity (Harrison, 1992).

Many functioning proteins are preserved well in ESP of parasitic helminthes (Lightowlers and Richard, 1988; Marrero *et al.*, 1988; Marikovsky *et al.*, 1988; Conners and Yoshino, 1990; Orido, 1990). They are not only involved

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in a variety of pathological processes either by accelerating the catalysis of reactive oxygen intermediates (Callahan *et al.*, 1988) or by facilitating the parasites to salvage metabolites but in provoking of immune responses to the host. For example, ESP of *Fasciola hepatica* was shown to have immunoreactive molecules to the host (Marrero *et al.*, 1988) and that of *Schistosoma mansoni* could inhibit the phagocytosis during macrophage mediated killing processes (Marikovsky *et al.*, 1988; Conners and Yoshino, 1990). SOD of *Trichinella spiralis, Taenia taeniaeformis* and *Schistosoma mansoni* were secreted or released into culture media and possibly into the *in vivo* environment (Leid and Suquet, 1986; Callahan *et al.*, 1988).

Paragonimus was also known to excrete or secrete the several proteins, calcareous materials and lipid droplets (Orido, 1990). It therefore should contain various kinds of biologically active molecules which are required to cope with the potential lethal attacks from the host and to fulfill the physiological necessities of oneself. ESP of *P. westermani* seems to be more important because it might function as a first line of defense in the worm from oxygen mediated killing. In the previous study, we had purified SOD from adult extract of *P. westermani* (Chung *et al.*, 1991). The activities of oxygen scavenging enzymes such as SOD, catalase, peroxidase and glutathione peroxidase in early maturation stages of *P. westermani* have also been documented (Chung *et al.*, 1992). In this study, peroxidase was purified and characterized partially in ESP of adult *P. westermani*. Activities of SOD and catalase were also identified in ESP.

MATERIALS AND METHODS

Preparation of ESP and crude extracts of adult *Paragonimus westermani*: A total of 50 adult worms of *P. westermani*, which had obtained from lungs of a dog at 13-week after an experimental infection, was incubated in sterile phosphate buffered saline (PBS, 0.01 M, pH 7.4) for 12 hours at 37°C. The incubation medium was centrifuged at 20,000 g for 1 hour. Resulting supernatant was used as crude enzyme of ESP of adult *P. westermani*. Protein content was measured by Lowry *et al.* (1951). All procedures for enzyme preparation were done at 4°C, unless otherwise specified except the incubation of the worms which had been carried out at 37°C.

Crude extracts of adult worms were prepared as described previously (Chung *et al.*, 1991). In brief, 3 g of 13-week old adult worms were homogenized with Potter-Elvejem homogenizer in 20 ml PBS (0.01 M, pH 7.4) containing 0.1 mM ethylenediamine tetraacetic acid (EDTA) (E. Merk, Rahway, NJ). The homogenate was centrifuged twice at 700 g for 5 minutes. Supernatant was obtained by centrifugation again at 20,000 g for 1 hour. Resulting supernatant was regarded as the crude enzyme. Protein content was measured (Lowry *et al.*, 1951).

Measurement of enzyme activities: Peroxidase activity was assayed as described by Poetter and Becker (1984). The reactant, which comprised 3.0 ml of potassium phosphate buffer (0.1 M, pH 7.0) containing 0.1 mM EDTA, 0.05 ml of 20.1 mM *o*-dianisidine (Sigma Chemical Co., St. Louis, MO), and 0.03 ml of 12.3 mM H_2O_2 (E. Merk, Rahway, NJ), was assayed at 405 nm using a spectrophotometer (Beckman M-35 with recorder, Palo Alto, CA). One unit of the enzyme activity was equivalent with the amount of 1 micromole of *o*-dianisidine oxidation. SOD activity was measured using a xanthine-xanthine oxidase system as described by McCord and Fridovich (1968). One unit of SOD was defined as the amount of enzyme that causes 50% inhibition of cytochrome *c* reduction. Catalase activity was measured as described by Aebi (1974). One unit of enzyme activity was defined as the amount of enzyme that degrade 1 micromole of H_2O_2 for 1 minute under the given condition. Specific activity of each enzyme was defined as the units of activity per milligram of protein.

Purification of peroxidase: Sephacryl S-300 Superfine gel filtration: Crude enzyme of ESP (11.5 mg of protein) was applied to 1.6 × 70 cm sized Sephacryl S-300 Superfine gel filtration (Pharmacia-LKB, Sverige, Sweden), which equilibrated with PBS (0.01 M, pH 7.4) containing 0.1 mM EDTA. The flow rate was 20

ml/hour · cm². 1.8 ml fractions were collected. Fractions showing peroxidase activity were pooled, dialysed against 0.05 M Tris-HCl (pH 7. 4) and lyophilized. It was rehydrated with 1.35 ml PBS (0.01 M, pH 7.4) containing 0.1 mM EDTA. Standard proteins (Pharmacia-LKB, Sverige, Sweden) used were thyroglobulin (669 kDa), β-amylase (220 kDa), bovine serum albumin (66 kDa), and cytochrome c (12.4 kDa).

DEAE-Trisacryl M anion exchange chromatography: 1.3 ml of partially clarified enzyme solution was brought to DEAE-Trisacryl M column (1.6 × 5 cm) and eluted with Tris-HCl (0.02 M, pH 7.2) containing 0, 0.02, 0.05, 0.1 or 0.2 M stepwise salt gradient fashion. Eluent were allocated in 2.0 ml fractions. The active fractions were pooled, dialysed against the same buffer, lyophilized and reconstitued with 0.2 ml Tris-HCl (0.1 M, pH 7.2).

High performance liquid chromatography (HPLC): DEAE-anion exchange column purified enzyme was then subjected to TSK-Blue gel filtration HPLC column (Spectra Physics, SP 8700, Japan). Ten microgram of the enzyme was eluted through the column at flow rate of 1 ml/minute. The elution was monitored by computing integrator (SP 4100) at 280 nm. Standard proteins of alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa), carbonic anhydrase (29 kDa) and cytochrome c (12.4 kDa) were also eluted.

SDS-PAGE and immunoblot assay: SDS-PAGE was done as described by Laemmli (1970) on 7.5~15% reducing separating gel. Constant current of 30 mA was supplied throughout the electrophoresis. The gel was stained with 0. 125% (W/V) Commassie blue R-250. For immunoblot assay, after SDS-PAGE on 7. 5~15% separating gel, the separated protein bands were electroblotted to nitrocellulose paper at 100 V for 2 hours at 4°C (Tsang *et al.*, 1983). Non-specific reaction was blocked by 1% bovine serum albumin for 2 hours. A paragonimiasis serum diluted at 1:150 in PBS/Tween 20 (0.05%, pH 7.4), whose diagnosis had been made by egg detection in sputum, was reacted for 2 hours. Peroxidase conjugated anti-human IgG (heavy- and light-chain specific, Cappel, PA) was reacted for

additional 2 hours at 1:1,000 dilution. The reaction was colored by 4-chloro-1-naphthol chromogen (Sigma Immunochemical Co., St. Louis, MO) dissolved in 0.1 M triethanolamine buffer.

Modulation of the enzyme activity by inhibitors: Cyanide effect (1 mM) on the enzyme activity and that of azide were observed according to Misra and Fridovich (1978). Final concentration of 1 mM DTT and 1 mM 2-mercaptoethanol, which are well known sulfhydryl agent, were added and the enzyme activity was measured. Effects of KCN (1 mM) and IAA (1 mM) were also assayed. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO)

Special staining for peroxidase: After SDS-PAGE on 10~15% separating gel, the gel was soaked in 0.01 M potassium phosphate buffer (pH 7.2) containing 2 mM o-dianisidine for 1 hour at room temperature. The gel was then placed to 0.01 M potassium phosphate buffer (pH 7.2) containing 2 mM diaminobenzidine and 0.1 mM H_2O_2 for 15 minutes (Misra and Fridovich, 1977).

RESULTS

Activity of peroxidase in ESP and in crude extract of adult *P. westermani*: When the total activity of peroxidase was assayed either in 11.5 mg protein in ESP of adult *P. westermani* or in 95.4 mg of the worm extracts, its activity was 557.3 units or 3090.1 units. The specific activity in the ESP was 48.5 and that of the worm extracts was 32.4 (Tables 1 and 2). Specific activity of the enzyme in ESP was therefore 1.5 times higher than that in the worm extracts.

Activities of SOD and catalase in ESP and in crude extract of adult *P. westermani*: As shown in Table 1, the activity of SOD in ESP was 127.2 units and that in the worm extract was 532.2 units. Protein content of the ESP and the worm extract were 11.5 mg and 95.4 mg, respectively. Specific activity of SOD in ESP was therefore 2 times higher than that in the worm extract (Table 1). Total activity of catalase in ESP and in worm extract were 38.5 and 505.6 units, respectively. Specific activity of catalase of ESP was 3.4 and that of the

Table 1. Comparison of activities of peroxidase, SOD and catalase in ESP and crude extract of adult *P. westermani*

Enzyme/ materials	Activity (units)	Protein (mg)	Specific activity (units/mg)
Peroxidase			
ESP	557.3	11.5	48.5
Adult*	3090.1	95.4	32.4
SOD			
ESP	127.2	11.5	11.1
Adult	534.2	95.4	5.6
Catalase			
ESP	38.5	11.5	3.4
Adult	505.6	95.4	5.3

Adult*: Crude worm extract of *P. westermani*.

crude extract was 5.3. The enzyme in ESP had a slightly lower specific activity than that in the worm extract (Table 1).

Purification of peroxidase: A total of 11.5 mg of protein in the ESP was subjected to Sephacryl S-300 Superfine gel filtration; 2.7 mg of the enzyme was clarified partially (Fig. 1 and Table 2). Specific activity of the enzyme increased 77.8 with recovery rate of 47.2% (recovered enzyme: 210.0 units). DEAE-Trisacryl M exchange chromatography was continued as shown in Fig. 2 and Table 2. A total of 180 units of peroxidase was eluted in 0.02 M salt concentration. Specific activity was 818.2 (protein content: 0.22 mg). The enzyme purified 16.9 fold with 32.3% recovery. When the purified enzyme was applied to TSK-Blue analytical gel filtration HPLC, it had a molecular mass of 19 kDa (Fig. 3). Yields and purification data were summarized in Table 2. Fig. 5 illustrated the SDS-PAGE finding of the purified peroxidase. The purified peroxidase

exhibited a single homogenous band at 16 kDa (Fig. 4).

Biochemical and immunological properties of the peroxidase: The effects of various inhibitors such as DTT, 2-mercaptoethanol, IAA, KCN and azide to peroxidase activity were observed. Because the peroxidase is a well known heme protein, we thought that cyanide, and sulfhydryl agents such as DTT and 2-mercaptoethanol, could inhibit the activity. As presented in Table 3, the enzyme activity disappeared completely when 1 mM DTT or 1 mM 2-mercaptoethanol was added. Similar result was observed by addition of 1 mM azide in the reactant. KCN (1 mM) and IAA (1 mM) showed also the inhibitory modulation to peroxidase activity up to 93.8% and 76.9%, respectively. Fig. 6 exhibited the result obtained from peroxidase staining of purified enzyme of ESP of adult *P. westermani*. As shown in lane P, the purified enzyme reacted strongly to o-dianisidine substrate. Lane C_r exhibited also the peroxidase activity in the worm extract at the same electrophoretic mobility as in the ESP.

Antigenicity of the purified peroxidase was assessed by western blot using a confirmed paragonimiasis serum. Typical finding was seen in Fig. 8 on which the crude ESP, worm extract and purified enzyme were used as antigens. A patient serum reacted to multiple bands of 64, 52, 42, 32, 30, 28, 26, 24, 22, 11 and 8 kDa to the crude ESP (lane C) while faint reaction was observed to the purified peroxidase (lane P). Adult worm extract revealed also the multiple reacting bands (lane A).

DISCUSSION

In this study, we purified a peroxidase from

**Table 2.** Purification of peroxidase in ESP of adult *P. westermani*

|	Activity (units)	Protein (mg)	Specific activity (units/mg)	Purification fold	Recovery (%)
ESP					
	557.3	11.5	48.5	1	100
Sephacryl S-300 column chromatography					
	210.0	2.7	77.8	1.6	47.2
DEAE-anion exchange chromatography					
	180.0	0.22	818.2	16.9	32.3
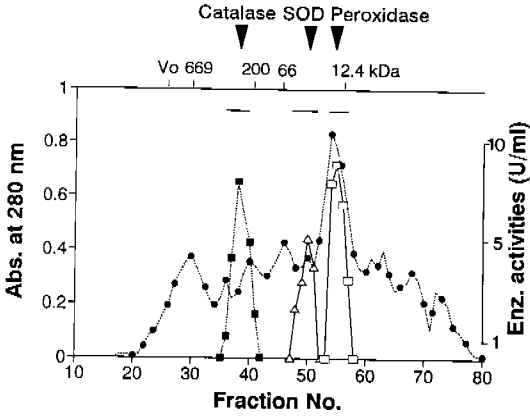
Fig. 1. Elution profile of ESP of adult *P. westermani* in a Sephacryl S-300 Superfine gel permeation chromatography (1.6 × 70 cm). The column was equilibrated with 0.01 M PBS (pH 7. 4) containing 0.1 mM EDTA and eluted with the same buffer at a flow rate of 20 ml/hour · cm2. Allocations of 1.8 ml were assayed for enzyme activities. ●: Protein content. Inverted arrowheads indicate the respective enzymes. Bar (-) indicates the pooled fraction of respective enzyme. ■: SOD activity, △: Catalase activity, □: Peroxidase activity.
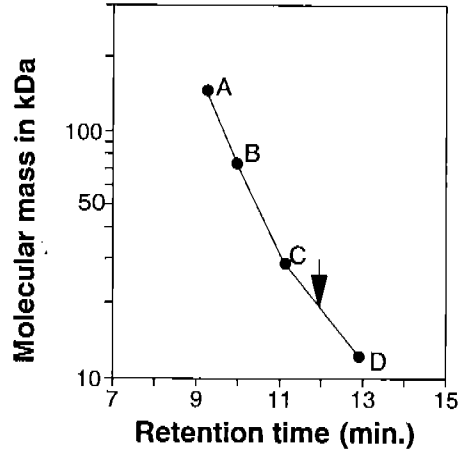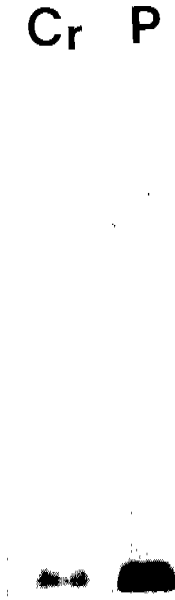

Fig. 3. Determination of molecular mass of peroxidase by TSK-Blue analytical gel filtration HPLC. Ten microgram of the enzyme was eluted through the column at a flow rate of 1 ml/minute. Standard proteins used were alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa), carbonic anhydrase (29 kDa) and cytochrome *c* (12.4 kDa). Inverted arrow (↓) indicates the retention time of the peroxidase.
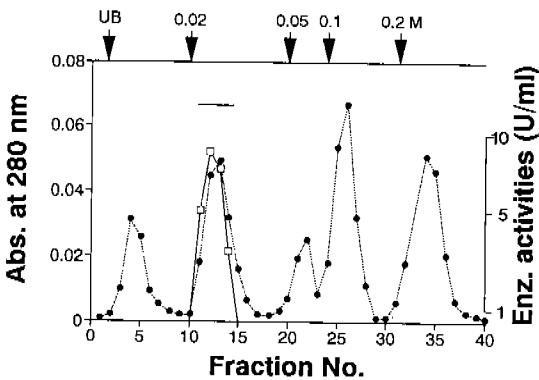
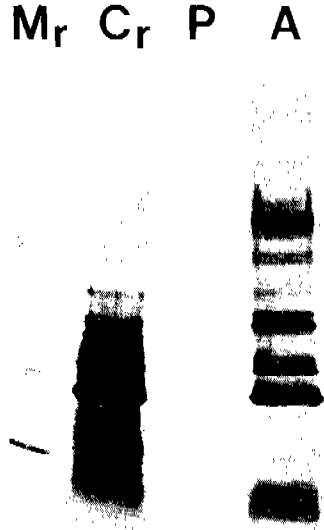**Fig. 2.** Elution profile of peroxidase in ESP of adult *P. westermani* on DEAE-Trisacryl M anion exchange chromatography. Partially clarified enzyme solution containing high activity of peroxidase was applied to a 1.6 × 5 cm sized DEAE-Trisacryl M column and eluted through 0. 02 M Tris-HCl buffer (pH 7.2) containing 0, 0.02, 0.05, 0.1 or 0.2 M stepwise salt concentration. 2.0 ml fractions were assayed for peroxidase activity. Inverted arrow indicate the salt gradient. Bar (-) indicates the pooled fraction containing high peroxidase activity.
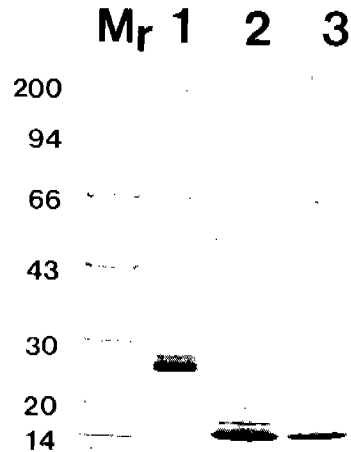
Fig. 4. SDS-PAGE findings of the purified peroxidase on 7.5~15% gel in reducing condition. Throughout the electrophoresis, constant current of 30 mA was supplied. The gel was stained with Coomassie blue R-250 and destained with methanol/acetic acid solution. M_r: Molecular mass in kDa, Lane 1 showing the crude ESP. Lane 2 exhibits Sephacryl S-300 filtered fraction and lane 3 demonstrates the purified peroxidase.

Cr   P

M$_r$ C$_r$ P   A

Fig. 6. SDS-PAGE/immunoblot using a confirmed patient serum to the ESP, adult worm extract and the purified peroxidase showing a typical finding. After transfer the resolved proteins to nitrocellulose paper by electrophoresis at 100 V for 2 hours at 4°C, a patient serum, diluted at 1:150 in PBS/T, was reacted overnight as a probe. M$_r$: Molecular mass in kDa, Cr: Crude ESP, P: Purified peroxidase, A: Whole worm extract of adult P. westermani.

Fig. 5. Finding of the purified enzyme in special staining for peroxidase. Separating gel of 10~15% was used for protein separation in reducing condition. After SDS-PAGE, the gel was soaked in 0.01 M potassium phosphate buffer (pH 7.2) containing 2 mM o-dianisidine 2 mM 3,3-diaminobenzidine and stained with 0.1 mM H$_2$O$_2$ and C$_r$: Crude extract of adult P. westermani, P: Purified peroxidase from the ESP.

Table 3. Effect of inhibitors on peroxidase activity in ESP of adult P. westermani

Inhibitors[a]	Activity (units/ml)	Relative activity (%)	Inhibition (%)
None	400	100	–
DTT	0	0	100
2-mercaptoethanol	0	0	100
IAA	92.3	23.1	76.9
KCN	24.6	6.2	93.8
Azide	0	0	100

a) Concentration of inhibitors was 1 mM

ESP of adult P. westermani by 2 step column chromatographies of Sephacryl S-300 Superfine gel filtration and DEAE-Trisaryl M anion exchange chromatography utilizing o-dianisidine and H$_2$O$_2$ substrates. As revealed by inhibitor profiles presented in Table 3, the enzyme, purified in this study, was most similar to cytochrome c peroxidase. The enzyme activity was inhibited totally by azide, DTT and 2-mercaptoethanol which are well known reducing agents as well as peroxidase inhibitors. Special staining for peroxidase confirmed the observation (Fig. 5). Molecular mass of the enzyme was 16 kDa in SDS-PAGE or in 19 kDa in gel filtration HPLC. This finding suggested strongly that the purified enzyme was a cytochrome c peroxidase because of relatively smaller molecular mass; many plants peroxidase including horseradish have a molecular mass of ca. 35 kDa and that of glutathione peroxidase is about 80 kDa. In addition, so far peroxidases found in cestode parasites have been regarded as cystochrome c-linked peroxidase (Paul and Barrett, 1980).

Excreted/secreted antioxidant enzymes in biological systems seemed to play a key role in defense against oxidants. For examples, Kazura and Meshnik (1984) stated that co-culturing adult T. spiralis with the new born

larvae protected the larvae from acetaldehyde-xanthine oxidase induced killing. This finding suggested that SOD, excreted/secreted from adults, might protect the new born larvae from the attack. The differences in the activity and in the amount of SOD between the new born and the adult *T. spiralis* are considered to be a mechanism of different resistance to killing by the oxidants (Callahan *et al.*, 1988; Chung *et al.*,'1991).

Microorganisms differ greatly in their susceptibility to oxygen-mediated damages because the levels of endogenous scavenging enzymes are different each other (Murray, 1981). We showed recently that the worms of an early maturation stages of *P. westermani* were equipped with higher activities of antioxidant enzymes (Chung *et al.*, 1991 & 1992). To understand properly the roles of oxygen scavenging enzymes in host-parasite interactions in paragonimiasis, we measured the activities of 3 oxygen radical scavenging enzymes in ESP of *P. westermani.*

Table 1 demonstrated clearly that ESP of adult *P. westermani* contained a variety of antioxidant enzymes such as SOD, catalase and peroxidase. Specific activity of SOD, catalase and peroxidase of the ESP were 11.1, 3.4 and 48.5 while those of the adult worm extract were 5.6, 5.3 and 32.4, respectively. Activities of SOD and peroxidase in ESP were higher than those of adult worm extract. This finding may be an evidence that ESP of *P. westermani* play a major defense mechanism of the worm against oxygen mediated killing. SOD was found to have a molecular mass around 32 kDa which were comparable not only to that of the worm extract but that of other parasites such as *T. spiralis, A. suum, O. volvulus* and *T. taeniaeformis*. Molecular masses of SODs from these parasites were reported to be around 33 kDa (Callahan *et al.*, 1988). It is well known that the enzyme distributed widely in oxygen metabolizing organisms regardless their phylogenetic origin and their being related biological reactivities, and physicochemical structures (Callahan *et al.*, 1988; Simurda *et al.*, 1988; Henkle *et al.*, 1991). The nucleotide sequences of *S. mansoni* and *O. volvulus* cDNA of SOD revealed also that the encoded proteins shared predicted hydrophobic signal sequences (Simurda *et al.*, 1988; Henkle *et al.*, 1991).

It is interesting that catalase activity either in ESP or in the crude extract of *P. westermani* were comparable to other eukaryotes. Catalase that has a single substrate of H_2O_2, distributed ubiquitously in tissue of all species. In the previous observations concerning to parasites catalase, however, Barrett (1980) described that any evidence of catalase was not recognized in *Fasciola hepatica.* Mkoji and co-workers (1988) stated also that *Schistosoma mansoni* had no catalase activity. They explained that the toxicity of hydrogen peroxide in these parasites might be removed through the actions of cytochrome *c*-linked peroxidase instead of catalase. To elucidate the different mechanisms of hydrogen peroxide removal among 3 phylogenetically related trematodes species of *P. westermani, F. hepatica* and *S. mansoni*, further studies are needed.

Despite its diverse physiological and biological functions, the peroxidase of *P. westermani* induced a weak antibody response in an infected patient as far as our SDS-PAGE/immunoblot finding was concerned. As exhibited in Fig. 7, crude ESP and adult extract of *P. westermani* showed many antigenic bands to the infected patient serum such as 64, 42, 32, 30, 28, 26, 24, 11 and 8 kDa. Of them, the purified enzyme disclosed faint antibody reaction to the infected serum.

REFERENCES

Aebi H (1974) Catalase. In: *Methods of enzymatic analysis.* (Bergmeyer, H.U., ed.), **2**: 674-678. Academic Press, New York.

Barrett J (1980) Peroxide metabolism in the liver fluke, *Fasciola hepatica. J Parasitol* **66**: 697.

Callahan HL, Crouch RK, James ER (1988) Helminth anti-oxidant enzymes: A protective mechanism against host oxidants? *Parasitology Today* **4**: 218-225.

Chung YB, Song CY, Lee HS, Cho SY (1992) Activities of scavenging enzymes in early maturation stages of *Paragonimus westermani. Korean J Parasit* **30**: 355-368.

Chung YB, Song CY, Lee HS, Kong Y, Cho SY (1991) Purification and characterization of a Cu/Zn superoxide dismutase from adult

Paragonimus westermani. Korean J Parasit **29**: 259-266.

Conners VA, Yoshino TP (1990) *In vitro* effect of larval *Schistosoma mansoni* excretory-secretory products on phagocytosis-stimulated superoxide production in hemocytes from *Biomphalaria glabrata. J Parasitol* **76**: 895-902.

Harrison ED (1992) Regulation of antioxidant enzymes. *FASEB J* **6**: 2675-2683.

Henkle KJ, Liebau E, Meuller S, Bergmann B, Walter RD (1991) Characterization and molecular cloning of a Cu/Zn superoxide dismutase from the human parasite *Onchocerca volvulus. Infect Immun* **59**: 2063-2069

Kazura JW, Meshnick SR (1984) Scavenger enzymes and resistant to oxygen mediated. *Mol Biochem Parasitol* **10**: 1-10.

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* **227**: 680-685.

Leid RW, Suquet CM (1986) A superoxide dismutase of metacestodes of *Taenia taeniaeformis. Mol Biochem Parasitol* **18**: 301-311.

Lightowlers MW, Richard MD (1988) Excretory-secretory products of helminth parasites: Effects on host immune responses. *Parasitology* **96**: 123-166

Lowry OH, Rosenbrough N, Farr LA, Randall RJ (1951) Protein measurement by Folin phenol reagent. *J Biol Chem* **193**: 265-275.

Marikovsky M, Arnon R, Fishelson Z (1988) Proteases secreted by transforming schistosomula of *Schistosoma mansoni* promote resistance to killing by complement. *J Immunol* **141**: 273-278.

Marrero CAR, Santiago N, Hillyer GV (1988) Evaluation of immunodiagnostic antigens in the excretory-secretory products of *Fasciola hepatica. J Parasitol* **74**: 646-652.

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

Misra HP, Fridovich I (1977) Superoxide dismutase and peroxidase: A positive activity stain applicable to polyacrylamide gel electrophoregrams. *Arch Biochem Biophys* **183**: 511-515.

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

Mkoji GM, Smith JM, Prichard RK (1988) Antioxidant systems in *Schistosoma mansoni*: Evidence for their role in protective of the adult worms against oxidant killing. *Int J Parasitol* **18**: 667-673.

Murray HW (1981) Interaction of *Leishmania* with a macrophage cell line: Correlation between intracellular killing and generation of oxygen intermediates. *J Exp Med* **153**: 1690-1695.

Orido Y (1990) Development of the excretory bladder of the lung fluke *Paragonimus ohirai* (Trematoda: Treglotrematidae). *J Parasitol* **76**: 201-211.

Paul JM, Barrett J (1980) Peroxide metabolism in the cestodes *Hymenolepis diminuta* and *Moniezia expansa. Int J Parasitol* **10**: 121-124.

Poetter J, Becker R (1984) Peroxidase. In: *Methods of enzymatic analysis.* (Bergmeyer, H. U., ed.), **3**: 286-293. Academic Press, New York.

Shim YS, Cho SY, Han YC (1991) Pulmonary paragonimiasis: A Korean perspective. *Seminars Rep Med* **12**: 35-45.

Simurda MCH, van Keulen DM, LoVerde PT (1988) *Schistosoma mansoni*: Identification and analysis of an mRNA and a gene encoding superoxide dismutase (Cu/Zn). *Exp Parasit* **67**: 73-84.

Song CY, Dresden MH (1990) Partial purification and characterization of cysteine proteinase from various developmental stages of *Paragonimus westermani. Comp Biochem Physiol* **95B**: 473-476.

Tsang VCW, Peralta JM, Simons AR (1983) Enzyme-linked immunoelectrotransfer blot techniques (EITB) for studing specificities of antigen and antibodies separated by gel electrophoresis. *Method in Enzymol* **92**: 377-391.

=국문요약=

폐흡충 성충이 분비배설하는 anti-oxidant enzymes의 특성 관찰 및 peroxidase의 정제

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　폐흡충의 분비배설물은 항원성이 높아 폐흡충증의 진단용 항원으로 가치가 있다고 보고되었다. 이 까닭은, 분비배설물에는 숙주내에서 여러 생물학적 반응을 일으키는 물질뿐 아니라 충체의 일부 구성 성분 및 여러 효소 등이 포함되어 있기 때문이라 가정할 수 있다. 이 연구는 폐흡충 성충의 분비배설물에서 숙주의 산소라디칼을 분해시키는 효소인 catalase, superoxide dismutase (SOD), peroxidase 등이 존재하는지를 확인하고 그 활성도를 측정하였으며 그 중 peroxidase를 정제하여 생화학적 특성의 일부 및 항원성을 관찰하였다. 폐흡충 성충 50마리를 37℃ 부란기에 12시간 배양한 뒤 배양액을 원심분리하고 이를 분비배설 조효소(粗酵素)로 사용하였다. 분비배설물에서 catalase, SOD와 peroxidase의 활성도를 측정할 수 있었고 그 비활성도(比活性度, specific activity)는 각각 11.1, 3.4 및 48.5 이었다. 분비배설물의 peroxidase 비활성도는 충체추출액의 비활성도보다 1.5배 높았다. 이 효소를 Sephacryl S-300 Superfine gel filtration, DEAE-Trisacryl M anion exchange chromatography로 정제하였다. 정제한 peroxidase의 분자량은 HPLC에서는 19 kDa이었고 SDS-전기영동에서는 16 kDa이었다. 정제한 효소를 전기영동한 후 diaminobenzidine으로 specific staining한 결과 이 효소는 충체추출액의 효소와 같은 영동이동거리를 나타내었다. 즉 이 효소는 충체로부터 분비되는 것임을 알 수 있었다. 한편, 폐흡충 감염 환자의 혈청과 반응시킨 immunoblot에서 분비배설물의 구성 단백질중 84, 64, 42, 32, 30, 28, 26, 24, 22, 11 및 8 kDa가 항원성을 보인 반면 정제한 peroxidase는 미약한 반응을 보였다. 이상의 결과로 폐흡충 peroxidase는 분비배설되어 SOD, catalase와 함께 산소 독성을 제거하는데 작용하고 있으나 폐흡충 감염 환자에서 특이 항체 반응을 일으키는데에는 미약한 작용을 한다는 것을 알 수 있었다.