Characterization of the 17 kDa oxygen transport protein of *Paragonimus westermani*

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폐흡충 (*Paragonimus westermani*)에서 17 kDa 산소운반단백질의 특성

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ABSTRACT

The hemoproteins from trematodes were valuable tools for research of protein function, structure and oxygen affinity. In the present study, the 17 kDa oxygen transport protein of *Paragonimus westermani* was purified by Resource Q ion exchange chromatography and subsequently by a Superose 6 HR gel filtration, and the purified protein was estimated to be 17 kDa on 7.5~15 % gradient sodium dodecyl sulfate gel electrophoresis. The absorption spectra of the oxygen transport protein were observed over the visible light range, 360~660 nm. In result, a high absorption peak was observed at pH 7.0, and those peaks for the ferrous oxy, deoxy, and carbonmonoxy forms were observed wavelength at 410, 425, and 420 nm, respectively. The studies of absorption spectra showed that the purified 17 kDa protein belong to oxygen-transport protein such as myoglobin. Immunoblotting studies showed that purified 17 kDa protein was not reactive against human paragonimiasis sera. In addition, the polyclonal antibody raised against the protein reacted to 17 kDa protein of egg, metacercariae, adult and adult excretory-secretory products. It has been revealed that the 17 kDa protein was essential component protein throughout the maturation stages. Immunohistochemical staining using the polyclonal antibody showed that the 17 kDa protein was distributed at paranchymal tissue, vitellaria as well as egg and testis. From these results, the protein may be involved in survival and reproduction of the parasite.

Key word: *Paragonimus westermani*, oxygen transport protein, hemoprotein, absorption spectra, myoglobin

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Ⅰ. Introduction

Paragonimus westermani is a trematode parasite of human lung and adult parasites are often found paired in worm capsules. Infection is occurred by eating raw or under-cooked crab or crayfish containing metacercaria (Kawashima, 1987). The clinical manifestations of the disease are cough, fever, dyspnea, chest pain, pleuritis, headache and epilepsy where the parasite invaded (Shim, 1991).

P. westermani has long been considered most important causative of paragonimiasis in Asia, including Korea. In Korea, the past metacercarial infections of fresh-water crayfish have been reported frequently to assess local folk remedy of paragonimiasis even though now crayfish juice is no longer used as a medicine for measles (Cho *et al.*, 1991). The life cycles of the parasite contained two intermediate hosts: first intermediate host is a snail (*Semisulcospira* spp.) and second intermediate hosts are crustacean species such as crab (*Eriocheir* spp.) and crayfish (*Cambaroides similis*). Final hosts are dog, cat, tiger and human (Choi, 1990).

When infected metacercariae are eaten by final host, the metacercariae excyst in the duodenum, penetrate the peritoneal cavity, and migrate to the lung. They become adults and are surrounded by a thick granulomatous wall. Adult worms can survive for approximately 5 years (Chung *et al.*, 1991).

P. westermani have been reported to accomplish an anaerobic metabolism even though it habitats in lung cyst. In this regards, it has been well documented that oxygen transport proteins such as hemoglobin (Hb) and myoglobin (Mb) are involved in oxygen transport as well as adaptation of low oxygen environment from hosts in several parasites (Rashid *et al.*, 1997; Chung *et al.*, 2003).

It has become evident that oxygen transport hemoproteins were widespread even in the lower phyla. Hemoproteins and Hb/Mb-like proteins have been characterized in an ever extending list of invertebrates, higher plants, fungi, protozoa, and bacteria. Comparisons with this protein family have revealed unexpected diversity in function and structure (Hardison, 1996; Bolognesi, 1997). The aggregation state is affected by heme ligand binding, pH conditions, anions, cations and nonheme proteins, possibly reflecting the very different habitats of these organisms (Parente *et al*., 1993). At variance with vertebrate oxygen carrying hemoproteins, hemoglobin and myoglobin often display a non-histidyl distal residue (Kapp *et al*., 1995). Trematodes possess a monomeric oxygen binding hemoproteins of 17 kDa, myoglobin or hemoglobin, which is distributed entire the body (Kiger *et al*., 1998). The trematodes lead a parasitic mode of life and feed upon the tissues and blood of their vertebrate hosts, therefore trematode hemoglobin was originally thought to derive from the host (Lee and Smith, 1965). Their life cycle exposed to oxygen before confronting snails as their first host; those arriving at their final destination in the vertebrates may still be exposed occasionally to higher oxygen levels (McGonigle and Dalton, 1995; Kiger *et al*., 1998).

The absorption spectra shows that the presence of heme structure (Fe-protoporphyrin IX) as a prosthetic group in the hemoproteins and the trematode hemoproteins are similar to other hemoglobin and myoglobin (Haider and siddiqi, 1976; Rashid *et al*., 1997). In addition, high oxygen affinity molecule of hemoproteins either by decreasing the oxygen dissociation rates through additional stabilization of the heme-O_2 complex in the nematode *Ascaris suum* (Yang *et al*., 1995; Shuocai *et al*., 1996) or by increasing the oxygen association rates, correlates with a large heme pocket in the leg-hemoglobin founding the nodules of leguminous plants (Gibson *et al*., 1989). It suggest that the trematode hemoglobin or

myoglobin play a role as reservoir, such as the myoglobin in seals or whales. One important difference between *A. suum* hemoglobin and vertebrate oxygen carriers is that the distal histidine E7 of vertebrate globins is replaced with a glutamine in *A. suum* hemoglobin domains. The distal histidine is a well studied residue known to stabilize the heme-bound oxygen through a hydrogen bond. Glutamine, the most common naturally occurring replacement for histidine, can also serve as a hydrogen bond donor to the bound oxygen in *A. suum* hemoglobin (Kloek *et al*., 1994).

Recently, the 17 kDa protein of *Clonorchis sinensis* has been characterized as myoglobin and it may be involved in survival of *C. sinenesis* (Chung *et al*., 2003). In *P. westermani*, 17 kDa molecules had been reported as cysteine protease and superoxide dismutase (Chung *et al*., 1991; Song and Kim, 1994; Chung *et al*., 1997). However, the characterization of myoglobin or oxygen transport protein from *P. westermani* has not been reported in elsewhere until now.

In the present study, 17 kDa protein of *P. westermani* was purified and characterized as myoglobin through absorption spectra analysis. Furthermore, it was also investigated that localization and presence of 17 kDa protein according to maturation stages have been evaluated using polyclonal antibody against the protein.

Ⅱ. Materials and Methods

1. Preparation of metacercariae

Metacercariae of *Paragonimus westermani* were isolated from naturally infected crayfish *Cambaroides similis*, caught in a Duryun Mountain, Cheollanam-do. The crayfish were crushed by motors, and emulsified in physiolosical saline, filtered through double-layered gauze and collected from sediments. Intact metacercariae were collected from the sediments under the dissecting microscope. The metacercariae were orally fed to dogs and adult flukes were recovered from their lung 12 weeks later. The worms were collected from the worm cyst of the lung. The collected worms were washed several times with sterile cold physiolosical saline solution (0.85 % NaCl). The adult worms were stored at liquid nitrogen until use.

2. Preparation of the crude extracts of *P. westermani*

The crude extracts of adult worms were prepared by homogenized in three volumes of 20 mM Na-acetate buffer (pH 6.5) containing 0.02 mM iodoacetamide (IAA), 1 mM diisopropyl fluorophosphate (DFP) to prevent proteolytic degradation. The homogenates were centrifuged at 15,000 rpm for 30 mins at 4 ℃. The resulting supernatant was used as crude extracts of *P. westermani* and frozen at -70 ℃ until used.

3. Purification of *P. westermani* oxygen transport protein

Purification of the 17 kDa protein was performed by a two-step column chromatography following by the methods of Chung *et al* (1997).

Step 1. Resource Q anion exchange column chromatography

The crude extract was loaded onto Resource Q anion exchanger column previously equilibrated with 20 mM Na-acetate buffer (pH 6.5). The anion exchanger column chromatography was performed using ÄKTA FPLC system (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The protein was eluted at a flow rate of 1 ㎖/min and the absorbed proteins were eluted with increasing sodium chloride molarity up to 1 M in the same buffer. Then, active fractions of oxygen transport protein were pooled and concentrated (Heto-Drywinner, Denmark). The concentrated fractions were dialyzed against 50 mM Tris-HCl buffer (pH 7.5) at 4 ℃ for 3 hrs.

Step 2. Superose 6 HR gel filtration column chromatography

The concentrated fraction was loaded onto Superpose 6 HR previously equilibrated with 50 mM Tris-HCl buffer (pH 7.5). The column was eluted at a flow rate of 0.2 ㎖/min. Purity of the obtained 17 kDa was analyzed by 7.5~15 % gradient sodium dodecyl sulfate polyacylyamide gel electrophoresis (SDS-PAGE). The gels were stained with Coomassie brilliant blue R-250.

4. Absorption Spectra

Absorption spectra of purified protein were measured using a spectrophotometer (Shimadzu UV-1601, Japan). The oxidized form was obtained by addition of a 10-fold excess of ferricyanide to a 10 ㎍/㎖ protein sample, followed by incubation at 37 ℃ for at least 2 hrs. The spectra of the oxidized form according to pH variation were performed with phosphate buffer (pH < 8) and Tris-HCl buffer (pH > 8). Oxygen off-rate was measured by injecting an oxy form sample into a cuvette containing about 2 mM sodium dithionite equilibrated under nitrogen. Spectra were recorded every 5 seconds.

5. Immunoblotting assay with patients sera

The crude extracts of *P. westermani* and purified 17 kDa protein were separated by 7.5~15 % gradient SDS-PAGE and transferred onto polyvinylidene difluoride membrane (PVDF, Amersham pharmacia Biotech, Uppsala, Sweden) using transfer blotter at 0.4 A for 40 mins (Hoffer, San Francisco, CA). The membranes were stripped and blocked with blocking buffer for 3 hrs. The membranes were incubated overnight with twenty patients of paragonimiasis sera diluted at 1 : 100 in blocking buffer. After incubation, the membranes were washed three times with washing solution (phosphate buffered saline containing 0.05 % Tween 20; PBS/T). The membranes were then incubated with peroxidase conjugated anti-human IgG diluted at 1 : 1000 in blocking buffer for 2 hrs. The membrane was developed by 4-chloro-1-naphthol (4C1N, sigma, St. Louis, MO) containing H_2O_2 in 10 mM phosphate buffer (pH 7.4). The reaction was stopped by washing with doubly distilled water.

6. Production of immune serum

The polyclonal antibody was produced using BALB/c mice with purified 17 kDa protein. Twelve BALB/c mice, 8 week old were immunized with 10 ㎍ purified protein emulsified with an equal volume of Freund's complete adjuvant, followed by boosting with incomplete adjuvant two weeks later. The mice received a boosting intravenous injection of 5 ㎍ protein and were bled 4 days after final boosting.

7. Expression of oxygen transport protein according to maturation stages and cross-reaction with other parasites

The profiles of protein bands according to maturation stages including eggs, metacercariae, adult and adult excretory-secretory products (ESP) were observed on 7.5~15 % gradient SDS-PAGE by Coomassie brilliant blue R-250 staining. The proteins of maturation stages were separated by SDS-PAGE and transferred to PVDF membranes at 0.4 A for 40 mins. The membranes were blocked by blocking buffer for 3 hrs. The membranes were incubated overnight with anti-17 kDa protein antibody diluted at 1 : 100 in blocking buffer. After incubation, the membranes were washed three times with washing solution (PBS/T). The membranes were then incubated with peroxidase conjugated anti-mouse IgG diluted at 1 : 1000 in blocking buffer for 2 hrs. The reactive protein bands were visualized by 4C1N and the reaction was stopped by washing with doubly distilled water.

To observe cross-reactivity of the protein with various parasites, immunoblotting was performed using anti-17 kDa polyclonal antibody. The crude extracts of various parasites used in this study included *C. sinensis*,

Ophistochis viverrini, *Fasciola hepatica*, *Schistosoma japonicum*, *Metagonimus yokogawai*, Sparganum and cyst fluid of *Taenia solium* metacestode.

7. Immunohistochemical staining

Adults *P. westermani* were recovered from the lung cysts of dogs were fixed in 10 % neutral formalin and embedded in paraffin. The paraffin blocks were cut into ribbons with 5 ㎛ thickness. The ribbons were incubated with anti-17 kDa polyclonal antibody (1 : 50 diluted phosphate saline buffer) at humid chamber for 2 hrs. The negative control section was used with pre-immune BALB/c mice serum. The ribbons were washed PBS/T and incubated with Cy3-conjugated anti-mouse IgG (Zymed Lab Inc., South San Francisco, CA) diluted at 1 : 100 for 2 hrs. The ribbons were washed twice above buffer and observed with fluorescence microscope Olympus AX 70 (Olympus, Japan). The positively reacted ribbons were then stained with Hematoxylin-Eosin to visualize and compare the organs reacted with the anti-17 kDa antibody.

Ⅲ. Results

1. Purification of the 17 kDa protein

The crude extract was initially fractionated by Resource Q anion-exchange column and eluted with increasing NaCl molaity in 20 mM Na-acetate (pH 6.5). The eluted fractions were monitored on 7.5~15 % gradient gel. The constructed protein included 17 and 16 kDa. Active protein peaks could be obtained when proteins were eluted from the column in 0.2 M NaCl (Fig. 1A). The pooled proteins was further separated into several peak by gel filtration chromatography using on Superose 6 HR and the purified protein migrated as a single band on 7.5~15 % gradient gel. The apparent molecular weight of the purified protein was found to be 17 kDa determined by gel filtration and SDS-PAGE (Fig. 1B).

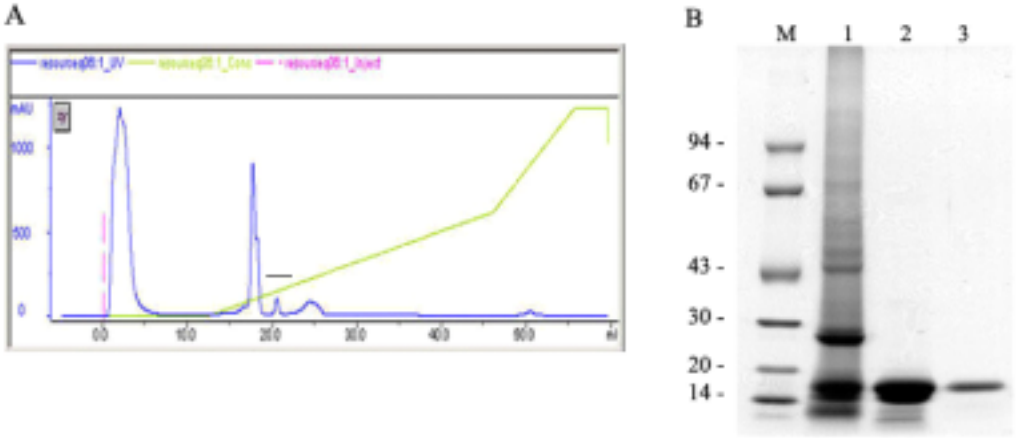

Figure 1. Purification of *P. westermani* 17 kDa protein. Panel A, Elution profile using Resource Q anion exchange column. Bar indicates partially purified the 17 kDa fraction. Panel B, 7.5~15 % gradient SDS-PAGE of purified 17 kDa protein. M, molecular weight marker, kDa ; lane 1, crude extracts; lane 2, Resoure Q anio-exchange column fraction; lane 3, purified 17 kDa protein using Superpose 6 HR gel filtration.

2. Absorption spectra

The purified 17 kDa protein of *P. westermani* was detected absorption spectrum at wavelength of visible ranges from 360 to 660 nm. In addition, 17 kDa protein known as myoglobin from *C. sinensis* was used as standard myoglobin in absorption spectra analysis. As shown in Fig. 2, both purified 17 kDa protein of *P. westermani* and *C. sinensis* myoglobin showed highly absorption peak at pH 7.0 in visible ranges. In oxy-form derivatives, purified 17 kDa protein of *P. westermani* showed highly peak at 410 nm (Fig. 3A). Upon reduction of the protein with dithionite, the purified protein showed a peak at 425 nm. In addition of CO to the reduced protein altered the spectrum to the characteristic of CO-form with a peak at 420 nm (Fig. 3A). These spectra analysis were very similar results obtained from *C. sinensis* myoglobin (Fig. 3B).

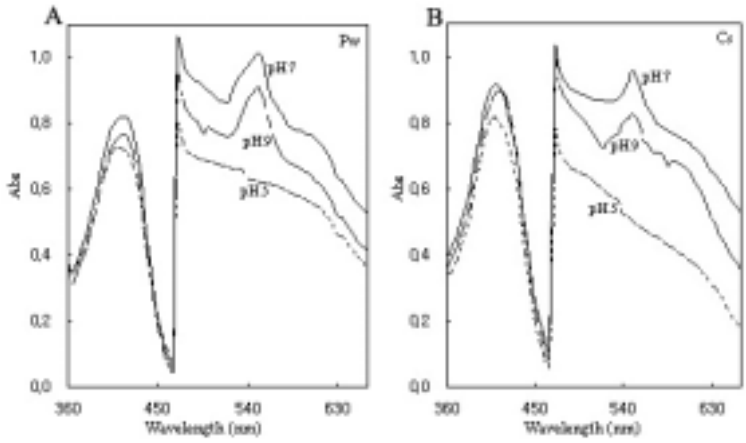

Figure 2. Absorption spectra of oxidized purified 17 kDa protein from *P. westermani* (A) and *C. sinensis* myoglobin (B) at different pH values. The absorption spectra were measured wavelength from 360 to 650 nm and assayed at different pH values from acidic to alkaline condition (pH 5, 7 and 9).

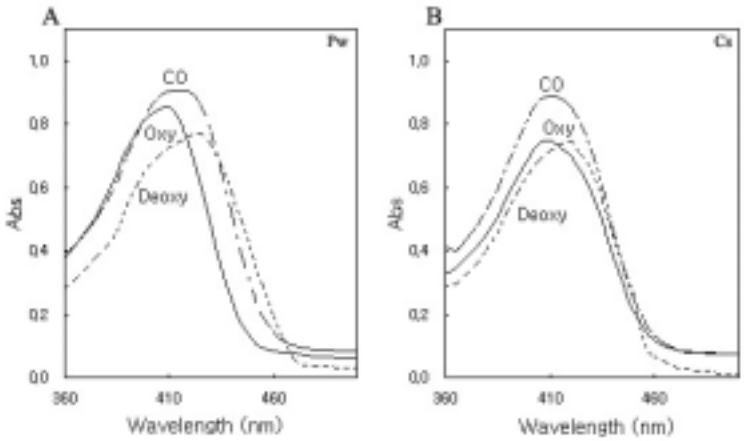

Figure 3. Absorption spectra of purified 17 kDa protein from *P. westermani* (A) and *C. sinensis* myoglobin (B). The absorption spectra were measured wavelength from 360 to 580 nm and assayed at Oxy, oxy-form; Deoxy, deoxy-form; CO, CO-form.

3. Antigenecity of the purified 17 kDa protein

To determine antigenecity of 17 kDa protein, immunoblotting was performed with several patients sera of paragonimiasis. As shown in Fig. 4 antigenic protein bands of crude extracts showed various range from high to low molecular weight proteins. The major antigenic protein bands included 90, 43, 34-32, 28 kDa, and 8 kDa (Fig. 4, panel A). However, it was found that purified 17 kDa protein did not react against patients sera of paragonimiasis (Fig. 4, panel B).

4. Production of polyclonal antibody

The polyclonal antibody of BALB/c mice were produced following immunization schedules. The purity of polyclonal antibody was assessed by immunoblotting with crude extracts of the adult worm. Several mice sera were collected and used as anti-17 kDa polyclonal antibody (Fig. 5).

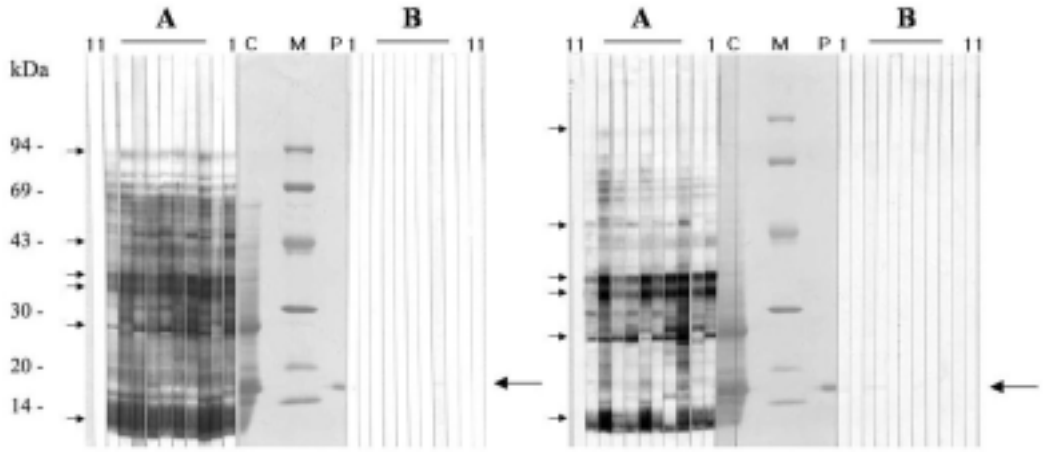

Figure 4. Immunoblotting of purified 17 kDa oxygen transport protein with patients sera of paragonimiasis. Panel A, crude extracts; Panel B, purified 17 kDa oxygen transport protein. Strips 1~10, positive human sera of paragonimiasis; strip 11, healthy human serum. M, molecular weight marker, kDa; C, crude extracts; P, purified 17 kDa oxygen transport protein. Arrows indicate purified 17 kDa oxygen transport protein bands and small arrows indicate major antigenic protein bands.

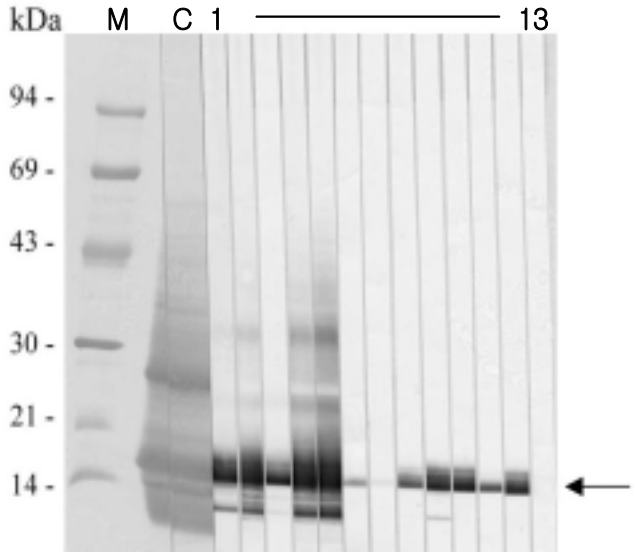

Figure 5. Production of 17 kDa polyclonal antibody using BALB/c mice. Strips 1~12, number of anti-17 kDa mice sera; strip 13, negative mice serum. Arrow indicates 17 kDa protein bands. M, molecular weight marker, kDa; C, crude extracts.

5. Expression of oxygen transport protein according to maturation stages and cross-reaction with other parasites

Immunoblotting analysis using anti-17 kDa polyclonal antibody was performed to detect presence of 17 kDa oxygen transport protein in maturation stages of the parasite and react with other helminthic parasites. At the first, the antibody reacted to the protein of egg extracts, metacercariae, adult crude extracts and adult ESP. The polyclonal antibody recognized 17 kDa protein bands according to maturation stages (Fig. 6). Furthermore, the antibody could only recognize 17 kDa protein of *P. westermani* while that of other parasites could not recognize (Fig. 7).

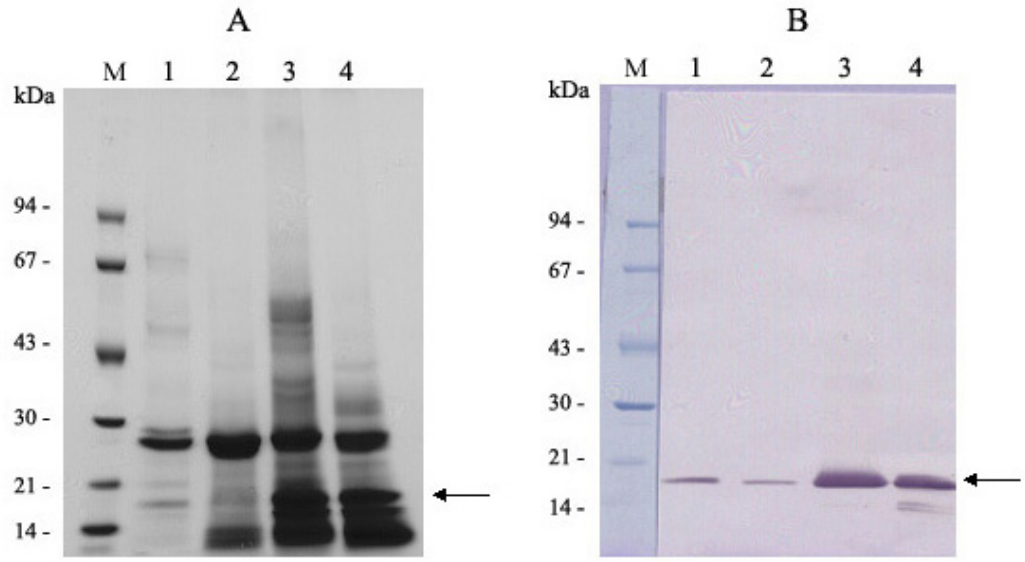

Figure 6. SDS-PAGE and immunoblotting of maturation stages of *P. westermani*. Panel A, 7.5~15 % SDS-PAGE profile; panel B, immunoblotting with anti-17 kDa antibody. M, molecular weight marker, kDa; Lane 1, egg extracts; lane 2, metacercarial extracts; lane 3, adult extracts; lane 4, adult ESP. Arrows indicate 17 kDa protein bands.

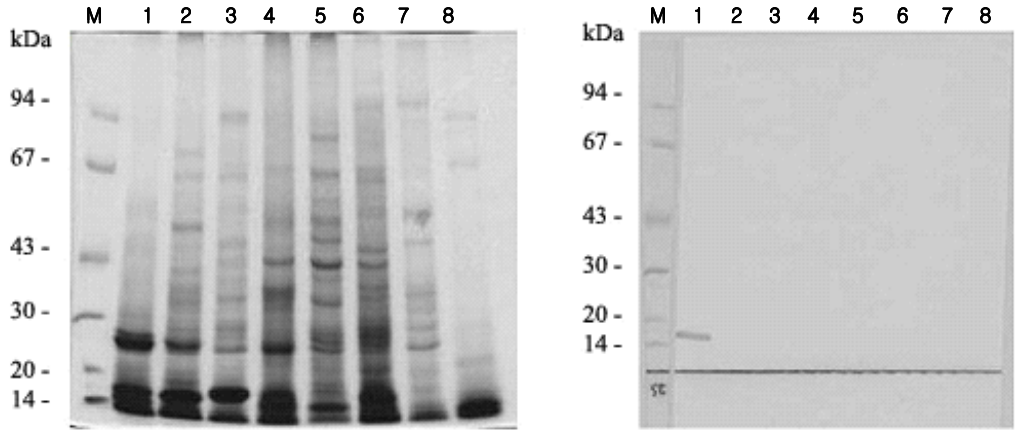

Figure 7. Species-specific reactivity of anti-17 kDa polyclonal antibody with proteins of human-infecting other parasites. Panel A, SDS-PAGE finding of crude extracts of other parasite; panel B, immunoblotting with anti-17 kDa polyclonal antibody. M, molecular weight marker, kDa; lane 1, *P. westermani*; lane 2, *C. sinensis*; lane 3, *Ophistochis viverrini*; lane 4, *Fasciola hepatica*; lane 5, *Schistosoma japonicum*; lane 6. *Metagonimus yokogawai*; lane 7, Sparganum; lane 8, cyst fluid of *Taenia solium* metacestode.

6. Immunolocalization of the 17 kDa protein

The anti-17 kDa protein polyclonal antibody was used as s primary antibody for immunolocalization of 17 kDa protein in *P. westermani*. Immunohistochemical studies showed that the 17 kDa protein was distributed at testis, vitellaria and parenchymal tissues of the worm (Fig. 8). The positive reaction was also somewhat observed at eggs. Pre-immune serum of BALB/c mice did not reacted any organ of the parasite.

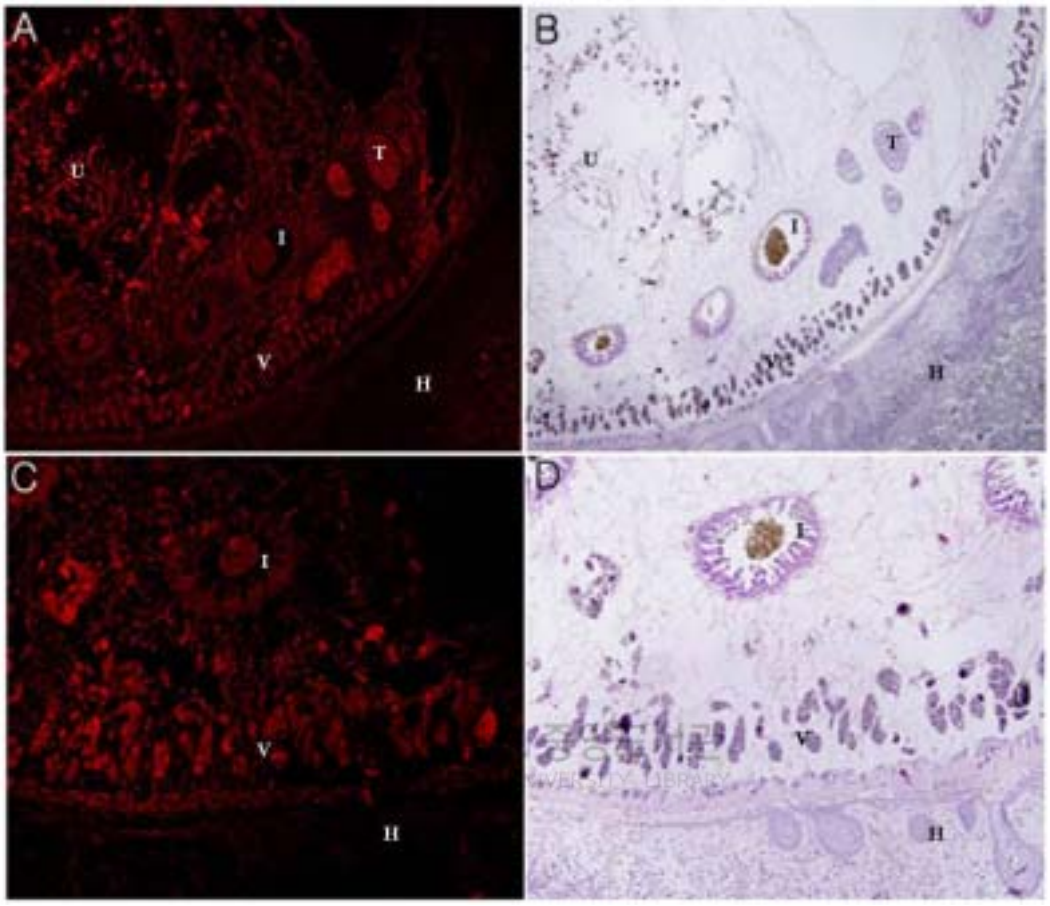

Figure 8. Immunolocalization of 17 kDa oxygen transport protein using anti-17 kDa antibody. Adult *P. westermani* were recovered from the lung cysts of dogs were fixed in 10 % neutral formalin and embedded in paraffin. Panel A, reacted with anti-17 kDa antibody panel B, the same slide stained with H-E staining (Panel A & B, 40x; panel C & D, 100x). H, host tissue; I, intestine; U, uterus; T, testis; V, vitellaria.

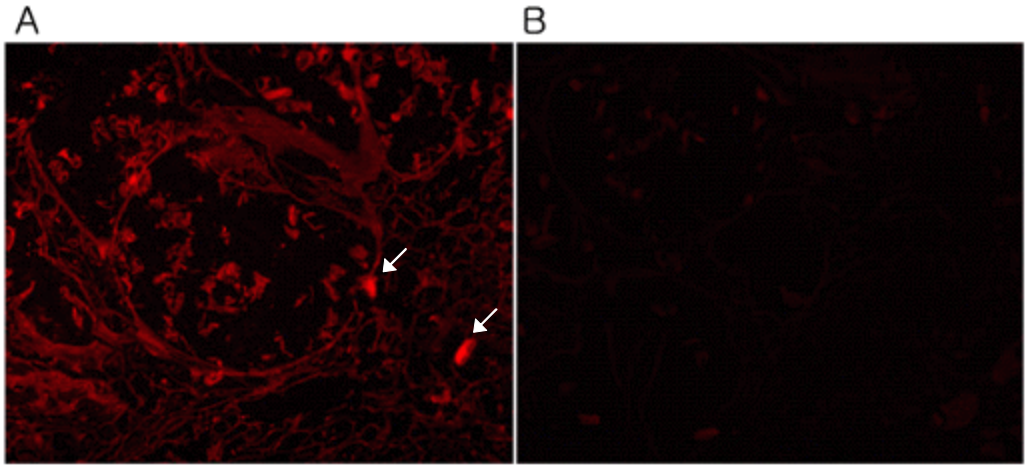

Figure 9. Localization of 17 kDa oxygen transport protein using anti-17 kDa antibody. Panel A, reacted with anti-17 kDa antibody; panel B, reacted with pre-immune serum (100x). Arrows indicate eggs of the parasite.

Ⅳ. Discussion

In the present study, a 17 kDa protein from the crude extracts of adult *P. westermani* was purified by two steps purification, ion-exchange and gel filtration chromatography. The molecular weight of the purified protein was estimated to be 17 kDa by gel filtration and SDS-PAGE and the estimated molecular weight of the protein was very similar to the trematode myoglobin such as *Paramphistomim epicilitum*, *Isoparorchis hypselobari* and *C. sinensis* (Rashid *et al*., 1997; Chung *et al*., 2003).

The absorption spectra obtained for the deoxy, oxy and carbon monoxide forms of the purified protein are also similar to the properties of human hemoglobin and myoglobin, trematodes *P. epicilitum, I. hypselobari* and especially myoglobin of *C. sinenensis*. (Dewilde *et al*., 1998; Rashid *et al*., 1997; Chung *et al*., 2003). In contrast, the spectrum of the oxidized form is sensitive to pH value. As a function of pH, they will show an acid-alkaline transition when a water molecule is replaced by an OH- as the heme iron ligand. The pH of this transition is also influenced by the residue at the distal position (E7). For human hemoglobin and sperm whale myoglobin, both displaying an E7 His, this pH is about 8 - 9, whereas for Aplysia myoglobin, having an E7 Val, it is pH 7.6 (Brantley *et al*., 1993; Dewilde *et al*., 1998). The apparent pH for the acid-alkaline transition of *P. westermani* oxygen transport protein is near pH 7.0 (Fig. 2A). The high stability of the bound ligand in the *A. suum* hemoglobin is obtained by the formation of three interactions between the oxygen molecule and the protein: the usual coordination bond with the iron atom, a hydrogen bond with the distal (E7) residue, and one with the B10 Tyr (Yang *et al*., 1995). A measurement of the oxygen dissociation process by a time constant of 30 seconds was determined for the oxy to deoxy

transition, however, the oxy-form samples were incubated at 37 °C to study the transition from oxy to the oxidized form and the rate for this process was 2 hrs. This value is therefore not consistent with the correlation of a lower oxidation rate for a higher oxygen affinity (Brantley *et al*., 1993). The same is true for hemoglobin *A. suum*, indicating that another mechanism may cause the oxidation in the case of these very high affinity proteins. From the absorption spectra analysis, it is concluded that the *P. westermani* 17 kDa oxygen transport protein is fully functional with a very high oxygen affinity. Therefore, the extremely high oxygen-binding affinity of the trematode myoglobin may enable the parasite to survive in the oxygen-depleted environment of the host (McGonigle and Dalton, 1995). The high oxygen affinity indicates that trematode hemoproteins in the transport of oxygen would not provide an efficient oxygen delivery system, whereas vertebrate hemoglobins participate in oxygen transport as an essential aspect of respiration.

Studies of immunoblotting analysis, it was evident that the 17 kDa oxygen-transport protein of the crude extracts did not react with the human sera of paragonimiasis. In reaction with the human sera of paragonimiasis, the crude extracts showed major antigenic protein bands including 94, 53, 34-32, 28 kDa and 8 kDa protein (Fig. 4, Panel A). However, purified 17 kDa oxygen transport protein showed either a very weakly reactive band or no reactive (Fig. 4, Panel B). From this result, 17 kDa oxygen transport protein could not be used as a diagnostic antigen although 17 kDa cysteine proteinase had been reported as valuable diagnostic antigen (Song and Kim, 1994).

Immunoblotting analysis revealed that anti-17 kDa antibody react specifically with 17 kDa oxygen transport protein of *P. westermani* while that of other parasites did not reacted (Fig. 7). Moreover, it was confirmed that the protein was present in maturation stages including egg,

metacercaria, adult and adult ESP even though relative quantities of the protein is slightly differentially expressed (Fig. 6). These finding indicated that the 17 kDa oxygen transport protein is produced in maturation stages and might be play an important physiological role of oxygen transport. Further studies including molecular cloning will be required for elucidation of oxygen transport protein in maturation stages of the parasite.

Immunohistochemical staining studies showed that the protein was distributed at eggs, vitelleria, testis and parenchymal tissues of the parasite (Fig. 8 and 9). It is likely that the oxygen transport protein serve as transfer of oxygen to the parenchymal tissue of the parasite in adult stage. Taken together with immunoblotting study, it seems likely that the oxygen transport protein might be also important role in reproduction.

In conclusion, it was confirmed that 17 kDa oxygen transport protein of *P. westermani* was found to be distributed at parenchymal tissue and reproductive organ. It is believed that the abundance of myoglobin plays an important role as an oxygen reservoir under anaerobic conditions. On going studies of molecular cloning and crystal structure of the *P. westermani* 17 kDa oxygen transport protein might be required for understanding biological roles of oxygen transport protein.

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Ⅵ. 초 록

흡충류에서 hemoproteins은 단백질의 기능과 구조 및 산소 친화력에 관하여 다양한 연구가 이루어져 있다. 폐흡충에서는 기능적인 단백질에 많은 연구가 이루어져 있으나, 숙주 조직 내에서 생존을 영위하기 위해 호흡대사에 결정적으로 필요한 헤모글로빈과 같은 산소 운반 단백질 (oxygen transport protein)의 연구는 거의 보고되지 않았다. 폐흡충의 산소운반 단백질의 특성을 규명하기 위하여 성충의 조효소 분획을 Resource Q ion exchange와 superose 6 HR gel filtration 을 이용하여 17 kDa 단백질을 정제하였고, 7.5~15 % gradient gel에서 17 kDa 밴드를 확인하였다. 정제한 폐흡충의 17 kDa 단백질과 간흡충 (*Clonorchis sinensis*) myoglobin을 파장 360~660 nm 범위에서 흡광도 비교 분석하였다. 그 결과, pH 의존도에서는 pH 7.0 에서 높은 흡광도 값을 나타내었고, oxy-, deoxy- 그리고 carbonmoxy- 형태의 폐흡충 단백질은 410, 425, 그리고 420 nm에서 간흡충의 myoglobin과 비슷한 흡광도을 보여주어 폐흡충의 17 kDa 단백질이 산소운반 단백질임을 확인하였다. 정제된 산소운반 단백질은 폐흡충증의 환자 혈청과의 반응에서는 미약하거나 반응을 하지 않았으므로, 항원 단백질로서의 가능성이 없음을 알 수 있었다. 또한 BALB/c 마우스에 면역시켜 특이 면역 혈청을 제작하여 실험한 결과, 정제된 산소운반 단백질은 폐흡충의 충란, 피낭유충, 성충에 존재하였고 다른 흡충류 및 조충류와 교차반응이 없는 종 특이성을 가지는 단백질임을 확인하였다. 특이 항체를 이용하여 폐흡충 성충 내 산소운반 단백질의 분포도를 조사한 결과, egg, testis, vitelleria 그리고 실질조직 (paranchymal tissue)에 분포하는 것으로 관찰되었으며 폐흡충의 생식 및 발육과 충체 내에서의 산소 운반 및 전달에 중요한 역할을 수행할 것으로 판단된다.

감사의 글

어느덧 대학원의 2년이라는 시간이 훌쩍 지나가고 졸업을 하게 되었습니다. 대학원 2년은 저에게 있어 보람과 힘든 시간의 연속으로 저를 조금 더 성숙하기 위한 과정이었으며, 여전히 배워야할 것들이 많다는 걸 새삼 느낍니다. 이 시간 동안 많은 깨달음과 가르침을 주신 모든 분들께 지면으로나마 감사의 말씀 드립니다.

이 논문이 완성되기까지 부족한 저에게 많은 관심 가져주시고 격려해주신 김세재 교수님께 진심으로 감사드립니다. 그리고 미흡한 논문을 세심하게 다듬어 주신 정영배 교수님과 이선령 교수님께 깊은 감사 말씀 드립니다. 그리고 학부 4년, 대학원 2년 동안 많은 가르침을 주신 오문유 교수님, 이용필 교수님, 오덕철 교수님, 김문홍 교수님, 김원택 교수님, 이화자 교수님, 고석찬 교수님께 감사의 마음을 전합니다.

석사 과정동안 관심과 조언으로 많은 도움을 주신 분자 생물학 실험실에 감사의 말을 전합니다. 부족한 저에게 많은 가르침을 주셨던 박지원 선생님, 강신해 선생님, 박수영 선생님, 정형복 선생님, 최수연 선생님 그리고 형이라고 하는 영준오빠... 그리고 선배이지만 친구 같은 성일, 준호, 대학입학 동기인 경만, 미경, 주엽, 은주, 현재, 혜선, 혜진에게도 수고 했다는 말을 함께 전하고 싶습니다. 또한 의학과의 교수님들과 여러 연구원 선생님께도 감사의 마음을 전합니다.

석사과정 2년동안 서로 의지하며 도와준 대주오빠, 민철오빠, 홍림오빠, 원종오빠, 재희에게 지금까지 모두 고생했다고 언젠가 성공해서 웃는 얼굴로 보는 날이 오길 기원합니다.

마지막으로 항상 사랑과 정성으로 보살펴 주시며 나의 버팀목인 부모님과 동생들에게 말로 다 표현할 수 없는 고마움과 사랑의 마음을 전하며, 이 논문을 바칩니다.

감사합니다.