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Comparative Studies on Biotin-coupled Components in Adult Worms of *Paragonimus* Species

MAKOTO ITOH AND SHIGEFUSA SATO

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Abstract

Presence of avidin-binding components was electrophoretically shown in extracts of adult worms of *Paragonimus* species. The components were found to contain biotin which has specific binding capacity to avidin.

Electrophoretic patterns of the components were comparatively studied using *P. miyazakii,* diploid and triploid *P. westermani,* and *P. ohirai.* The patterns observed in extracts of the three species apparently differed each other. The components, 130kD and 82kD, were major and commonly observed in all the samples studied. However, two components, 65kD and 63kD, were detected only in extracts of *P. westermani,* and another component, approximately 64kD, was detected only in *P. ohirai* extracts. The patterns of diploid and triploid *P. westermani* were identical to each other. These observations suggested that the electrophoretic patterns of the biotin-coupled components are useful means for identification of adult worms of *P. miyazakii, P. westermani* and *P. ohirai.*

Key words: *Paragonimus miyazakii, P. westermani, P. ohirai,* avidin, biotin

Introduction

Biotin is known as the protective factor against egg-white injury, vitamin H and coenzyme R, and usually is coupled to proteins to play a role as a coenzyme in carboxylation, transcarboxylation and decarboxylation reactions (Moss and Lane, 1971). It has been widely found in many kinds of organisms, ranging from bacteria to plants and animals. However, there has been no report on such biotin-coupled components in helminthes.

In the course of a study which aimed to detect antibodies specific to antigens of lung flukes using avidin conjugate, we experienced non-specific binding of the conjugate to the antigens. Therefore, we suspected that there might be some components which bind avidin in extracts of the lung flukes.

In this study, we have shown the presence of the avidin-binding components in adult worms of three *Paragonimus* species, *Paragonimus miyazakii,* diploid and triploid *P. westermani,* and *P. ohirai* and have identified them as biotin-coupled components.

Department of Medical Zoology, Nagoya City University Medical School, Kawasumi, Mizuho, Nagoya 467, Japan.
伊藤 誠 佐藤重房 (名古屋市立大学医学部医動物学教室)

Materials and Methods

Parasites

Adult worms of *P. miyazakii,* diploid and triploid *P. westermani,* and *P. ohirai* were obtained from experimental definitive hosts, to which metacercariae of each *Paragonimus* species were administered. The host species, incubation periods, localities where their second intermediate hosts were captured, and numbers of worms examined are summarized in Table 1 with references.

Sample preparation

Adult worms of each *Paragonimus* species were homogenized in 1/15M phosphate buffered saline (pH 7.4) containing 0.5mM N-[N-(L-3-*trans*-carboxyoxiran-2-carbonyl)-L-leucyl]-agmatin (E-64), a cysteine proteinase inhibitor which prevents degradation of protein com-

Table 1 Summary of *Paragonimus* species used

Species	Second intermediate hosts	Localities	Experimental definitive hosts	Duration of infection	Number of adult worms examined	References
P. miyazakii	*Goethelphusa dehaani*	Dojima, Shizuoka	Rat	11 weeks	10	Ito and Mochizuki, 1975
P. miyazakii	*Geothelphusa dehaani*	Dojima, Shizuoka	Dog	5 months	10	Ito and Mochizuki, 1975
P. westermani (2n)	*Geothelphusa dehaani*	Hirukawa, Gifu	Dog	5 months	10	Shiwaku et al., 1986
P. westermani (2n)	*Geothelphusa dehaani*	Nenobi, Mie	Dog	5 months	10	Tomimura et al., 1989
P. westermani (2n)	*Geothelphusa dehaani*	Nakayama, Hyogo	Dog	5 months	1	Shibahara, 1982
P. westermani (3n)	*Eriocheir japonicus*	Tsushima, Nagasaki	Dog	5 months	10	Terasaki, 1980
P. ohirai	*Sesarma dehaani*	Kiso River, Mie	Rat	11 weeks	10	Matsuo and Makiya, 1985

ponents during heating process for sodium dodecyl sulfate polyacrylamide gel electrophoresis (Itoh *et al.*, 1990). Each homogenate obtained was centrifuged at 3,500×*g* for 5 min and the supernatant was used as samples. Protein concentrations of samples were adjusted to 10mg/ml and stored at −80°C until used.

Detection of avidin-binding components

Each sample was mixed with an equal volume of sample buffer (2.5% SDS, 25% glycerol, 2.5% 2-mercaptoethanol in 0.125M Tris-HCl buffer pH6.8). The mixture was boiled for 3 min and then, supplied to 10% polyacrylamide gel electrophoresis (80×80mm, ACI Japan Inc., Japan). The separated protein components in the gel were electrically transferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was incubated in casein buffer (1% casein in 50mM Tris-HCl buffer pH7.6) at room temperature for 30 min, and then in the buffer containing peroxidase labeled avidin-D (500 fold diluted, Vector Lab. Inc., U.S.A.) at 37°C for 30 min. For a blocking study, biotin was added to the peroxidase labeled avidin-D solution (10 μg/ml at a final concentration) prior to the incubation. After washing, remaining conjugates were detected by 4-chloro-1-naphthol.

Detection of biotin-coupled components

Protein components in the extracts were separated by SDS-PAGE and trans-blotted to a PVDF membrane as described above. The membrane was incubated in the casein buffer at room temperature for 30 min, then in the solution containing goat IgG against biotin (500 fold diluted, Vector Lab. Inc., U.S.A.) at 37°C for 1 hour. After washing, the membrane was incubated in peroxidase conjugated anti-goat IgG solution (1,000 fold diluted, Cappel Lab. Inc., U.S.A.) at 37°C for 1 hour. As a substrate, 4-chloro-1-naphthol was used.

Results

Electrophoretic patterns of avidin-binding components detected in pooled samples of adult worms of *P. miyazakii,* diploid and triploid *P. westermani,* and *P. ohirai* are shown in Fig. 1. Two major components, 130kD and 82kD, were commonly observed in all the samples examined. However, differences were observed in other components. Two components, 65kD and 63kD, were specific to *P. westermani* samples, and a 64kD component was observed only in samples of *P. ohirai.* The patterns of the components of diploid *P. westermani* were identical with those of the triploid.

Binding of avidin conjugate to the com-

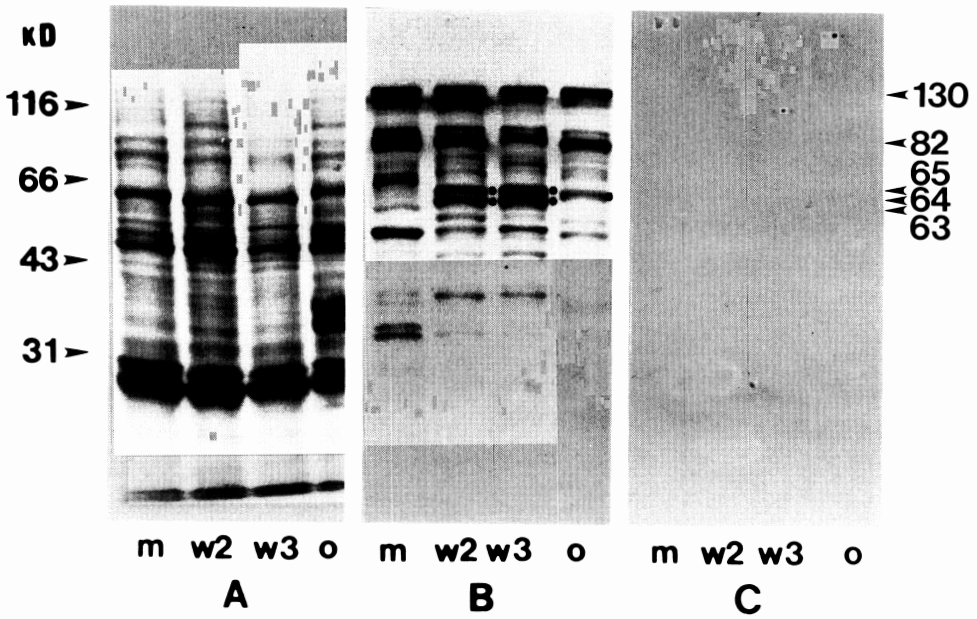

Fig. 1 Components in samples of adult worms of each *Paragonimus* species separated by SDS-PAGE and detected by peroxidase labeled avidin-D after blotting. A: whole protein staining with coomassie brilliant blue, B: band patterns of avidin-D bound components, C: inhibition of avidin-D binding to the components by biotin (a final concentration was 10 μg/ml), m: samples of *P. miyazakii,* w-2: samples of diploid *P. westermani,* w-3: samples of triploid *P. westermani,* o: samples of *P. ohirai.* Dots indicate specific components of *P. westermani* and *P. ohirai.*

ponents was inhibited by addition of free biotin (Fig. 1). A further experiment was conducted to ensure that the detected components actually contain biotin. As shown in Fig. 2, patterns of components detected by anti-biotin antibodies corresponded with those detected by the avidin conjugate.

Intraspecies variations were hardly observed in the patterns of avidin-binding components (data are not shown).

Although some difference in darkness of bands were observed, there was no difference in the basic patterns between the *P. miyazakii* flukes obtained from different definitive hosts, rats and a dog, and among the *P. westermani* flukes from three different localities, Hirukawa, Nenobi and Nakayama (Fig. 3).

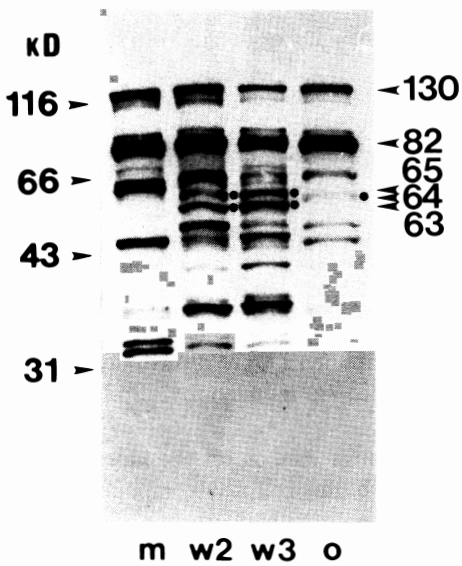

Fig. 2 Components in samples of adult worms of each *Paragonimus* species detected by goat anti-biotin antibodies and peroxidase labeled anti-goat IgG antibodies. Abbreviations used are indicated in the legend to Fig. 1. Dots indicate specific components of *P. westermani* and *P. ohirai.*

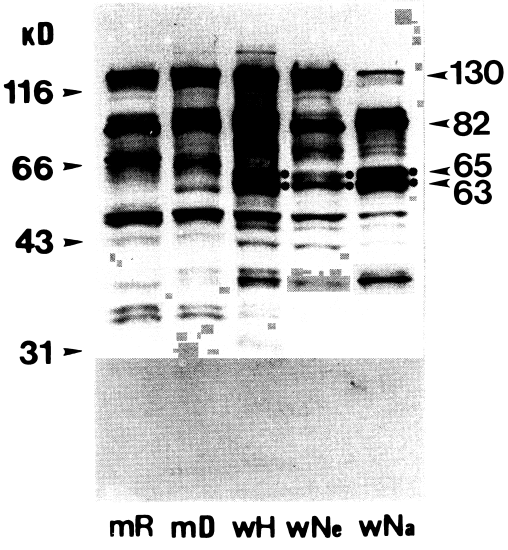

Fig. 3 Components detected by peroxidase conjugated avidin-D in samples of adult worms of *P. miyazakii* from different hosts and diploid *P. westermani* of which metacercariae were from different localities. *P. miyazakii* adult worms recovered from rats: mR or a dog: mD; *P. westermani* adult worms of which metacercariae were from crabs captured at Hirukawa: wH, Nenobi: wNe, or Nakayama: wNa. Dots indicate specific components of *P. westermani.*

Discussion

This is the first report which has shown avidin binding components in *Paragonimus* species. Possibilities of non-specific binding of the avidin conjugate to components contained in the samples were excluded, since addition of a glycoside or raised ionic strength (Raymond and Whitehead, 1990) did not interfere the binding (data are not shown). The specificity of the binding was further confirmed by an observation that avidin binding to the components was completely inhibited by free biotin. The avidin-binding components were directly identified as biotin-coupled components by anti-biotin antibodies.

Comparative studies on the electrophoretic patterns of biotin coupled components of the *Paragonimus* members showed differences among them. Although the 130kD and 82kD components were observed in all the samples studied, the 65kD and 63kD components were specific to *P. westermani,* and the 64kD components to *P. ohirai.* The 63–65kD components were not detected in samples of *P. miyazakii.* These differences were always apparently observed in every samples studied.

Little variation on the basic pattern was observed between *P. miyazakii* flukes obtained from different definitive hosts, among *P. westermani* flukes from different localities, and among individual samples within the same species.

Through the studies of C-banded karyotypes (Hirai *et al.*, 1985) and isozymes (Agatsuma and Habe, 1985), they suggested that triploid *P. westermani* is allotriploid that originated from interspecific hybridization between the diploid *westermani* and unknown species. Our present results suggest that the unknown species is so closely related to the diploid *westermani,* because no difference was seen in the patterns between them. Close relationship between them has been already suggested by Agatsuma and Habe (1985), and Hirai and Agatsuma (1991).

The present results strongly suggest that electrophoretic patterns of the biotin-coupled components are good markers for adult worm identification among *P. miyazakii, P. westermani* and *P. ohirai.*

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