VALUE OF SNAIL ANTIGENIC STRUCTURE IN DETERMINATION OF ITS TREMATODE PARASITE

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SUMMARY

The present study dealt with molluscan host-parasite relationship from the immunological point of view where, the antigenic relationship between miracidium of Fasciola gigantica and Paramphistomum microbothrium and two molluscan hosts of different families; Lymnaea cailliaudi and Biomphalaria alexandrina was studied using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blot technique. A structural homology in snail and its compatible miracidium was observed by the use of SDS-PAGE. This homology resided in components of similar molecular weights between them. Cross-reaction between snail and miracidium was proved by the use of immunoblot in which rabbit anti-miracidium hyperimmune sera (HIS) were utilized. This cross-reaction was intensive with the compatible miracidium so that all the snail specific polypeptides were recognized. Meanwhile, less cross-reactivity was observed with incompatible miracidium. Although the two polypeptides of molecular weight 54 and 45 KDa were expressed by all the selected snail antigens, they reacted specifically with HIS raised against compatible miracidium only. Therefore, the technique of SDS-PAGE must be followed by western blot for assessment of antigenic community between snail and its trematode parasite. This fact play a major role in predicting the snail IH suitable for a trematode of unknown life cycle.

INTRODUCTION

The compatibility between trematodes and their snail intermediate hosts is of great parasitological and epidemiological importance. Penetration of a miracidium into its domesticated snail host does not appear to do a great deal of damage to the host, without any noticeable host response (Wright, 1971). On the other hand, when the miracidium penetrates an abnormal host snail, there is a rapid tissue response and infiltration of amoebocytes around the living parasite and within forty-eight hours it is

encapsulated by fibroblasts and destroyed. This was first described for *Schistosoma mansoni* in certain strains of *Biomphalaria* by Newton (1952) and subsequently for various other schistosomes in a variety of snails (Sudds, 1960) as well as for *Fasciola gigantica* by El-Bahy (1993) who concluded that these general responses occur to dead larvae as well as to incompatible strains of larvae.

From this view, the compatible miracidium is that able to inhibit the normal cellular defense action of the host or which fails to stimulate the snail immune mechanism. This is due to failure of the snail to recognize its domestic miracidia as foreign body. In this respect, there is evidence that snail tissues are capable of distinguishing between 'self' and 'not self' inside its body as mentioned by Tripp (1963). Yoshino and Bayne (1983) studied the mimicry of snail host antigens by miracidia and primary sporocysts of *S. mansoni* and reported that *S. mansoni* miracidia possess surface determinants that are antigenically similar to *B. glabrata* hemolymph macromolecules. These antigens persist during *in vitro* transformation to the primary sporocyst stage in the absence of snail components. Thereafter, it was confirmed that antibodies to sporocysts could bind to snail hemocytes (Bayne and Stephens, 1983). Dissous and Capron (1989) mentioned that two *S. mansoni* miracidial proteins of 43 and 39 KDa were shown to react with rabbit antibodies produced against *B. glabrata* proteins.

In this respect, domestic miracidium to special type of snail is that able to develop and complete its life cycle in this snail species without stimulating of the snail immune system. Theoretically, this originated from presence of antigenic similarity between the miracidium and its snail intermediate host. This play an important role in identification of new intermediate snail hosts to parasite of unknown life cycle. From this scope, the present study clarified the antigenic similarity between miracidium and its domestic and foreign snail hosts from the aspect of molecular weight using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and from the aspect of immune reaction using western blot technique after preparation of the required hyper-immune sera in rabbits.

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Table (1): Results of post mortem examination of skin reactor cattle using mammalian PPD tuberculin and filter sterilized lysozyme extract

Types of antigens used in skin test	Number	Reactor		PM Finding			
				VL *		NVL **	
		No.	%	No.	%	No.	%
1. Lysozyme extract	650	32	4.9%	25	78.1%	7.0	21.9%
2. Mammalian PPD tuberculin	650	31	4.76%	24	77.4%	7.0	22.6%

* VL: Visible Lesion.
** NVL: Non-Visible Lesion.

Table (2): ELISA test on sera from 32 skin test reactor cattle with or without gross lesions using mammalian, bovine PPD and lysozyme extract antigens

Samples	Lysozyme extract antigen	Bovine PPD	Mammalian PPD
With lesion (25):			
* True Positive	23	22	21
* False Negative	2	3	4
Without lesions (7):			
* False Positive	1	1	2
* True Negative	6	6	5
Total	32	32	32

Table (3): Statistical evaluation of ELISA results on cattle sera using 3 different antigens

	Types of coating antigen used:		
	Lysozyme extract	Bovine PPD	Mammalian PPD
Sensitivity	92 %	88 %	84 %
Specificity	85.8 %	85.7 %	71.4 %
Accuracy of positive predication	95.8 %	95.6 %	91.3 %
Accuracy of negative predication	75 %	66.7 %	55.6 %
Efficiency of predication	90.6 %	87.5 %	81.3 %
Error of predication	9.4 %	12.5 %	18.7 %

MATERIALS AND METHODS

Antigens preparations

Miracidial antigens

Eggs of *F. gigantica* and *P. microbothrium* were collected from gall bladder and rumen, respectively, of naturally infected buffaloes at Cairo abattoir. After several washing with tap water, the eggs were sieved using Fluke finder technique (Welch *et al.*, 1987). Eggs embryonation was carried out according to Boray (1963). Recovery of miracidia was possible after elapse of 14 days at 27°C in dark by exposure to direct sun-light (El-Bahy, 1988). The collected miracidia were sonicated in 0.01 M phosphate buffered saline, PH 7.4 (PBS) for 5 minutes under 150 watt interrupted pulse output at 50% power cycle using a sonifier cell disrupter. Thereafter, the sonicated miracidia were subjected to a high-speed centrifugation (10.000 rpm) for one hour at 4°C. The supernatant was concentrated in 6 - 8 KDa dialysis tubes by absorption against polyvinyl pylorridone. The protein content was measured by the method of Lowry *et al.* (1951) and stored at 70°C until used.

Snail antigens

Snails of two different families were selected; *Lymnaea cailliaudi* as susceptible intermediate host (IH) of *F. gigantica* and *Biomphalaria alexandrina* as susceptible IH of *P. microbothrium.* The field-collected snails were identified according to Brown (1994) and reared in the laboratory for production of laboratory-bred snails according to El-Bahy (1984). They were used for antigen preparation according to Khalil *et al.* (1985) with some modifications. Where, hepatopancreases and feet of laboratory bred mature non-infected snails were dissected. The collected tissues were homogenized in an equal amount of 0.01M PBS, PH 7.4 and sonicated for 5 minutes. Then, they were centrifuged at 5000 rpm for one hour at 4°C. The supernatant was dialyzed in 6 - 8 KDa dialysis tubes overnight at 4°C against 4 M urea buffer (Shalaby, 2002). Thereafter, they were concentrated and protein evaluated as before.

Preparation of rabbit hyper-immune serum (HIS)

Rabbit hyper-immune sera were raised against five antigens (*L. cailliaudi* feet, *L. cailliaudi* hepatopancreases, *B. alexandrina*

feet, *F. gigantica* miracidia and *P. microbothrium* miracidia) as described by Langley and Hillyer (1989) via initial subcutaneous injection in an equal volume of Freund's complete adjuvant and three consecutives intramuscularly injections in an equal volumes of Freund's incomplete adjuvant during 60 days. Level of specific antibodies in sera of immunized rabbits was evaluated before slaughter.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blot techniques

For determination of the antigenic relationship, on the base of molecular weight, between the tested miracidia and their snail hosts, the previous antigens were fractionated (40 and 100 ug/lane, respectively) using 12% SDS-PAGE according to Laemmli (1970) with the aid of high and low molecular weight standards (Pharmacia Biotech) and transferred onto nitrocellulose sheet for western blot technique according to Towbin *et al.* (1979). The nitrocellulose strips blotted with snail antigens were tested in group of four as in Fig.2:

The first strip was allowed to react with rabbit HIS raised against the same snail antigen.

The second strip was allowed to react with rabbit HIS raised against the compatible miracidium.

The third one was allowed to react with rabbit HIS raised against the incompatible miracidium.

The forth one was allowed to react with rabbit pre-immune control sera.

The molecular weight of specific and non-specific polypeptides were determined using molecular weight standard curve as described by the producer (Pharmacia).

RESULTS

A) Antigenic relationship between snail and its domestic miracidium using SDS-PAGE.

Electrophoretic profile of snail and miracidial antigens was resolved by SDS-PAGE into multiple components at both high and low molecular weight ranges (Fig. 1). A structural homology was appeared between the snail and its compatible miracidium. As shown in Fig. 1, fractionated *L. cailliaudi* feet and hepatopancreases in comparison with its natural parasite *F.*

gigantica miracidial antigens revealed 10, 13 and 7 polypeptides in each antigen, respectively. These polypeptides molecular weight ranged from 22-160 KDa. The protein bands at molecular weight of about 85, 54, 45, 34, 28 and 22 KDa were common among the three tested antigens.

Concerning *B. alexandrina* feet and hepatopancreases in comparison with *P. microbothrium* miracidial antigens, there were 8,7 and 5 polypeptides in each antigen, respectively. Their molecular weights ranged from 21-97 KDa. Three polypeptides of molecular weight 78, 54 and 45 KDa were common among them. The components of 54 and 45 KDa were expressed by all selected snail and miracidial antigens.

B) Western blot technique for detection of miracidium-snail specific polypeptides.

To clarify the specificity of the recorded common miracidial and snail polypeptides, the adopted western blot technique (Fig.2) revealed that *L. cailliaudi* feet HIS reacted specifically with nine polypeptides present in its own antigen (*L. cailliaudi* feet) at molecular weight of 94, 85, 75, 70, 66, 57, 54, 51 and 45 KDa (Fig.2 Lane A). The most interested phenomenon that all these polypeptides were recognized by *F. gigantica* miracidium HIS (Fig.2 Lane B). Meanwhile, on reaction of the same polypeptides (*L. cailliaudi* feet) versus *P. microbothrium* miracidium HIS, only five were recognized at molecular weight of 75, 70, 66, 57 and 51 KDa (Fig.2 Lane C). On the other hand, no polypeptides were identified by pre-immune rabbit sera (Fig.2 Lane D).

From these reactions, the four polypeptides of 94, 85, 54 and 45 KDa were considered to be specific between *F. gigantica* miracidium and *L. cailliaudi* feet.

Concerning the immune reaction between *L. cailliaudi* hepatopancreases antigen and its homologous HIS, eight polypeptides of molecular weight 97-85, 70, 64, 54, 45, 42, 40 and 38 KDa (Fig.2 Lane E) were identified. All these polypeptides were recognized by *F. gigantica* miracidium HIS except that of 97-85 KDa (Fig.2 Lane F). Only four of them; 97-85, 64, 42 and 40 KDa were recognized by *P. microbothrium* miracidium HIS (Fig.2 Lane G). While, there was a non-specific reaction at 97-85 KDa on using pre-immune sera (Fig.2 Lane H).

From these reactions, the four polypeptides of 70, 54, 45, and 38 KDa were considered to be specific between *F. gigantica* miracidium and *L. cailliaudi* hepatopancreases.

On reaction of *B. alexandrina* feet antigen versus the different prepared HIS, the results in Fig.2 revealed that *B. alexandrina* HIS identified six polypeptides; 97, 85, 75, 70, 54 and 45 KDa (Fig.2 Lane I). All of these polypeptides were recognized by *P. microbothrium* HIS (Fig.2 Lane J).

On testing the specificity of these *Biomphalaria* snail polypeptides versus the foreign miracidium HIS (*F. gigantica)*, only four polypeptides at molecular weight of 97, 85, 75 and 70 KDa were recognized (Fig.2 Lane K). No reaction could be recorded on using pre-immune rabbit sera (Fig.2 Lane L). On the other hand, the polypeptides of 54 and 45 KDa were considered as specific bands between *B. alexandrina* feet and its own miracidium.

DISCUSSION

The present study dealt with the molluscan host-parasite immunological relationship between miracidia of *F. gigantica* and *P. microbothrium* and their molluscan hosts; *L. cailliaudi* and *B. alexandrina* , respsctively.

Several investigators had demonstrated the antigenic community between snail and its trematode parasites (Iwanage *et al.* 1992, Iwanaga 1994, Weston *et al.* 1994). It had been suggested that at least some of these shared antigens reflect a genetic accommodation between the host and the parasite "molecular mimicry" as a result of the pressure of selection (Damian 1987). Chacon *et al.* (2002) confirmed the presence of common antigens between *S.mansoni* and its vector, *B. glabrata* and discussed the relevance of this vector for serodiagnosis and immunoprophylaxis. In the current research, as it was expected, a structural homology in snail and its compatible miracidium was observed by the use of SDS-PAGE. This homology resided in components of similar molecular weights between them as 85, 54, 45, 34, 28 and 22 KDa in *L. cailliaudi* feet and hepatopancreases and *F. gigantica* miracidial antigens as well as 78, 54 and 45 KDa in *B. alexandrina* feet and hepatopancreases and *P. microbothrium* miracidial antigens. In that sense, Lackie (1980) reported that the invertebrate immune system was based on the recognition of rather gross differences of physical and/or chemical parameters. There would then be a certain range of histocompatibility within which the surfaces are recognized as similar. Thus, the possibility would exist that the tissue surfaces of two organisms would have such a configuration as to appear immunologically compatible.

Cross-reaction between snail and miracidium was proved in the present study by the use of immunoblot in which rabbit anti-miracidium antisera were utilized. This cross-reaction was intensive with the compatible miracidium so that all the snail specific polypeptides were recognized by its domestic miracidium HIS. Meanwhile, less cross-reactivity was observed with incompatible miracidium. Indeed, *F. gigantica* miracidium HIS recognized all the nine specific polypeptides in *L. cailliaudi* feet antigen's immunoblot. While, *P. microbothrium* miracidium HIS reacted faintly with only five polypeptides in *L. cailliaudi* feet antigen's immunoblot. In the meantime, *P. microbothrium* miracidium HIS reacted intensively with all specific polypeptides of *B. alexandrina* feet antigen. This proved a very important point clearing that high similarity in antigenic composition present between the snail and the species of trematode miracidium able to develop inside it. This appeared to be in line with the findings of Henning *et al.* (1978) that immunoelectrophoresis showed cross-reactions between the different stages in the life cycle of *S. mansoni* and extracts of hepatopancreas from its intermediate host snails *B. glabrata* and *B. alexandrina*. This was attributed to the presence of cross-reacting determinants (Stein and Basch, 1979). Furthermore, Chacon *et al.* (2002) found that sera from outbred mice immunized with a soluble *B. glabrata* antigen (SBgA) of non-infected *B. glabrata* snails recognized molecules of SBgA itself and *S. mansoni* adult worm antigen (AWA) by western blot. Recognition of several molecules of the SBgA was inhibited by pre-incubation with AWA (155, 60, 36, 30 and 16 KDa).

It was worthy to mention that although the two polypeptides of molecular weight 54 and 45 KDa were expressed by all selected snail antigens, they reacted only with HIS raised against compatible miracidium and in the meantime, these two polypeptides were detected also in the domestic miracidia to these snails with high degree of specificity between the snail and its own miracidia. However, such parasite/snail species or strain compatibility is genetically dependent (Bayne *et al.*2002). It is possible that the recovered peptides from snail antigen against the heterologous (foreign) miracidia HIS represent enzymes such as myeloperoxidase or fibrinogen-related proteins particularly from *B.* alexandrina. For such defensive components, large number of genes is maintained in the genome of planorbid snails such as *Helisoma trivolvis* and *B. glabrata* (Adema, 2002). Based on such explanation, the lymnaeid snail, *L. cailliaudi* is refractory to its

foreign miracidium; *P. microbothrium*. The observations together with the data presented in this study lead to believe in the importance of the common molecules in identification of new intermediate snail hosts to parasite of unknown life cycle. It was interesting to confirm that these common molecules present between the given miracidia and their suspected snail IH.

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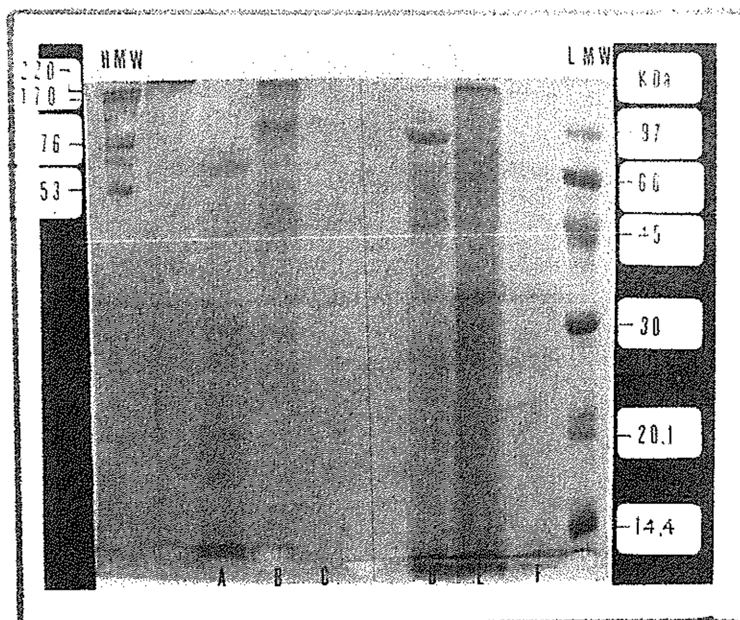

Fig. 1: SDS-PAGE of different snail and miracidial antigens.

Lane A. *B. alexandrina* hepatopancreases antigen.
Lane B. *B. alexandrina* feet antigen.
Lane C. *P. microbothrium* miracidial antigen.
Lane D. *L. cailliaudi* hepatopancreases antigen.
Lane F. *L. cailliaudi* feet antigen.
Lane F. *F. gigantica* miracidial antigen.
HMW. High Molecular Weight marker.
LMW. Low Molecular Weight marker.

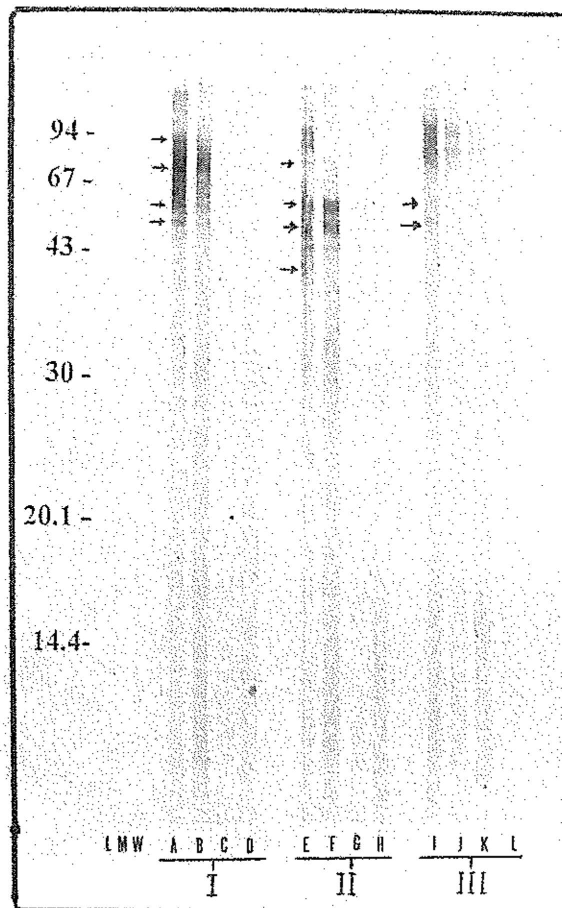

Fig. 2: Pattern of recognition of different snail antigens by homologus and miracidial HIS using western blot.

I. *L. cailliaudi* feet antigen.
II. *L. cailliaudi* hepatopancreases antigen.
III. *B. alexandrina* feet antigen.
Lane A. With *L. cailliaudi* feet HIS.
Lane B, F & K. With *F. gigantica* miracidium HIS.
Lane C, G & J. With *P. microbothrium* miracidium HIS.
Lane D, H & L. With Pre-immune control sera.
Lane E. With *L. cailliaudi* hepatopancreases HIS.
Lane I. With *B. alexandrina* feet HIS.
LMW. Low Molecular Weight marker.

الملخص العربي

أهمية التركيب الأنتيجينى للقواقع في تحديد الديدان المفلطحة التى يمكنها النمو بها

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تناولت هذه الدراسة العلاقة بين الديدان المفلطحة و عوائلها الوسيطة من القواقع من وجهة النظر المناعية، حيث تم دراسة العلاقة الأنتيجينية بين الطور الهدبى الخاص بطفيل الديدان الكبدية العملاقة و عائله الوسيط (قوقع الليمنيا) و ديدان الكرش (بارمفستومم ميكروبوثريم) و عائله الوسيط (قوقع البيومفلاريا) باستخدام التحليل الكهربي في البولي أكريلاميد جيل وطريقة الويستيرن بلوت.

أظهر التحليل الكهربي بأستخدام الجل وجود تماثل تركيبي من حيث الوزن الجزيئى للبروتينات المكونة له وذلك بين القوقع و الطور الهدبى الأليف له. وبأجراء الأختبار المناعى لهذة البروتينات (الويستيرن بلوت) ظهرت تفاعلات متبادلة بينهما باستخدام أمصال متخصصة محضرة معمليا ضد الطور الهدبى في الأرانب. و كانت هذه التفاعلات ذات درجة عالية من التخصص مقارنة مع الطور الهدبى الأليف للقوقع. في الوقت الذي كان فيه تفاعلات أقل مع الطور الهدبى الغير أليف.

كذلك اوضحت الدراسة انه بالرغم من وجود البروتينات دات الوزن الجزيئين 45 و 54 كيلو دالتون مشتركة بين القواقع المختبرة, إلا إنها أظهرت تفاعلات متخصصة مع المصل المحضر ضد الطور الهدبى الأليف لها فقط. لذلك وجد أن التحليل الكهربي في البولي أكريلاميد جل يجب أن يتبع بطريقة الويستيرن بلوت للتحقق من التواصل الأنتيجينى بين القوقع و الطفيل الغازي .

و يعتبر هذا البحث أتجاها جديدا للاستخدام المناعي لتحديد ملائمة الطور الهدبى للديدان المفلطحة للنمو في نوع محتمل من العوائل الوسيطة (القواقع) مما يفتح الطريق لتحديد العوائل الوسيطة للكثير من الديدان المفلطحة غير معلومة دورة الحياة والتأكد من ذلك بالطرق البيولوجية بطريقة اكثر تحديدا.

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