GNATHOSTOMA SPINIGERUM : ANALYSIS OF PROTEIN PATTERNS BY TWO DIMENSIONAL GEL ELECTROPHORESIS

Chaisiri Wongkham[1], Wanchai Maleewong[2], Kosol Ieamviteevanich[3], Pewpan M Intapan[2] and Nimit Morakote[4]

[1]Department of Biochemistry and [2]Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002; [3]Loei Hospital, Ministry of Public Health, Loei 42000, Thailand; [4]Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50000, Thailand

Abstract. The protein extracts from male (MS) and female (FS) adults and advanced third-stage larvae (LS) of *Gnathostoma spinigerum* were separated by high resolution two-dimensional gel electrophoresis (2-DE). The polypeptide spots, as detected by silver staining, were subsequently identified. The spot patterns of LS, MS and FS were highly complex and consisted of more than 75, 44, 52 prominent spots, respectively. In addition, the stage-specific protein patterns were identified. This 2-DE database should provide an important reference for future biological and biochemical studies of *G. spinigerum.*

INTRODUCTION

Gnathostoma spinigerum, a spirurid nematode, is widely distributed in Asian countries, including Japan, China, Burma, Vietnam, Philippines, Malaysia and Thailand (Miyazaki, 1960; Daengsvang, 1981). Man acquires gnathostomiasis mainly by consuming raw meat containing the infective larvae of this parasite (Daengsvang, 1981). Cases occur annually, despite public educational programs. The worm usually migrates in subcutaneous tissue causing an intermittent migratory swelling; sometimes it reaches the central nervous system, producing various signs and symptoms, and the disease then becomes become life threatening (Boongird *et al*, 1977; Jaroonvesama, 1988; Schmutzhard *et al*, 1988). The protein analysis of this parasite had been revealed previously on the basis of one-dimensional polyacrylamide gel electrophoresis under reducing condition, which aim to identify the specific polypeptide for use as the antigen in immunodiagnosis of this disease (Nopparatana *et al*, 1988, 1991; Tapchaisri *et al*, 1991). However, there is no report of the protein composition of *G. spinigerul*&studied by two-dimensional gel electrophoresis (2-DE), which separates the protein according to their molecular weights and isoelectric points. The purpose of this study was to compare larval and adult worm protein extracts for the establishment of the data in 2-DE database. In addition, the stage-specific proteins were also identified.

Correspondence: Wanchai Maleewong.

MATERIALS AND METHODS

The adult *G. spinigerum* were isolated from naturally infected dog stomachs and separated by sex. The advanced third-stage larvae were obtained from mice inoculated orally with early third-stage larvae from infected copepods (Maleewong *et al*, 1988). The somatic adult extracts from male (MS) and female (FS) worms and advanced third-stage larvae (LS) were separately prepared by homogenization and extraction as described previously (Maleewong *et al*, 1997). Briefly, the worms were homogenized with a tissue grinder in a small volume of 0.1 M phosphate buffered saline pH 7.4, containing 0.1 mM of phenylmethylsulfonylfluoride, 0.1 mM of tosylamide-2-phenylethylchloromethylketone and 1 μm of N-(N- [-3-trans carboxyoxiran-2-carbonyl]-L-leucyl)-agmatine. The preparations were then sonicated with an ultrasonic disintegrator and centrifuged at 10,000*g* for 30 minutes at 4ºC. The supernatants were dialyzed against distilled water containing the same proteinase inhibitors, and the protein concentrations were determined by the method of Lowry *et al* (1951). These extracts were subsequently stored at -20ºC for further analysis.

2-DE was performed by the combination of isoelectric focusing (first dimension) and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE; second dimension) as developed by O' Farrell (1975), with some modifications. Ultrathin gels (0.6 mm, thick), which contained 7.3% acrylamide, 0.225% N, N′-methylenebisacrylamide

(Pharmacia Biotech, Sweden), and 8% v/v carrier ampholytes (Biolyte pH 3-10, Bio-Rad Laboratories, USA) was prepared on the Gel Bond films (FMC, USA) and the isoelectric focusing was performed in the LKB 2117 Multiphor II Electrophoresis System (Pharmacia Biotech). Each of the protein samples, with the equivalent of 200 µg protein in 5 µl of 2% v/v carrier ampholyte, pH 3-10 in distilled water, was applied to the anode side of each gels and focused at 2,000 Vh with cooling. After isoelectric focusing, the gels were equilibrated for 15 minutes in a sample buffer (0.062 M Tris-HCl, pH 6.8, 10% glycerol, 5% 2 mercaptoethanol, 2% SDS and 0.001% bromphenol blue) before further applying the second step. In the second dimension, SDS-PAGE was performed with 10 % to 18% (w/v) linear gradient polyacrylamide gel, according to the method described by Laemmli (1970). After electrophoresis, the gels were stained with a silver staining kit (Silver Stain Plus Kit, Bio-Rad Laboratories). Protein spots were analyzed using the gel documentation system and 2D gel analysis software (GelWorks™, UVP, UK) along with the autodetection procedure.

RESULTS

Using the 2-DE, the spot patterns of LS, MS, and FS were highly complex and consisted of more than 75, 44, and 52 prominent protein spots, respectively (Figs 1-3). Most of the protein spots, from all three extracts were present in the acidic and neutral area. It was revealed that the number of polypeptide spots from the LS, with an apparent molecular mass of 30 to 43 kDa and pI of 4.65 to 7.0, were greater than the MS and FS. The polypeptide spots, with an apparent molecular mass of 20.1 to 30 kDa and pI of 6.0 to 7.0, were demonstrated in the FS, but were not in the MS (Figs 2, 3).

DISCUSSION

The results of 2-DE, in the present study revealed that the protein spot patterns were apparently different among larvae, female and male adult worms. However, little attention had been previously given to study the phenotypic difference between the stages of *G. spinigerum*, especially from larvae to adult at the molecular level. Therefore, this data might be useful as a genetic basis for understanding gnathostome worms. Further analyses of *G. spinigerum* antigenic polypeptides recognized by individual infected human gnathostomiasis, other parasitic infections and normal healthy sera using 2-DE and subsequent immunoblotting are now in progress. In conclusion, the information in the present study, provided a framework for further analyses of the biology, biochemistry, and immunology in gnathostome worms.

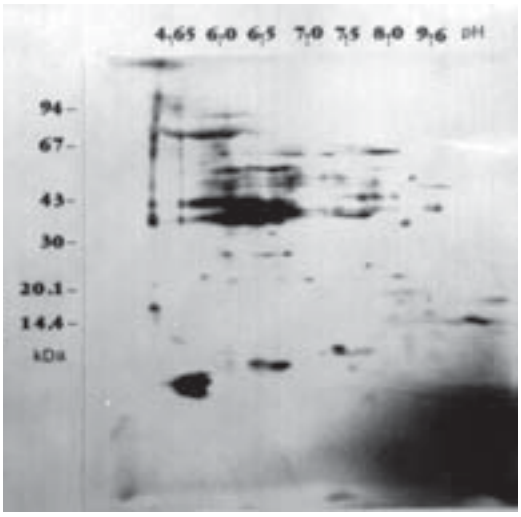

Fig 1–Silver staining of protein spot patterns of 2-DE of *Gnathostoma spinigerum* advanced third-stage larvae. The pH markers are indicated on the top. The molecular mass markers are indicated on the left in kilodaltons (kDa).

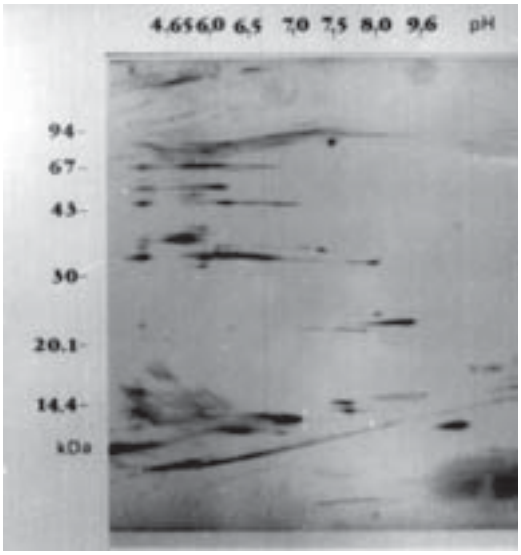

Fig 2–Silver staining of protein spot patterns of 2-DE of *Gnathostoma spinigerum* adults male. The pH markers are indicated on the top. The molecular mass markers are indicated on the left in kilodaltons (kDa).

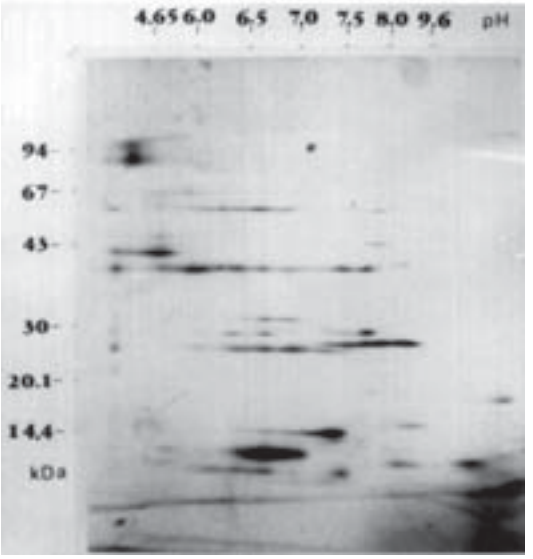

Fig 3–Silver staining of protein spot patterns of 2-DE of *Gnathostoma spinigerum* adults female. The pH markers are indicated on the top. The molecular mass markers are indicated on the left in kilodaltons (kDa).

ACKNOWLEDGEMENTS

We are grateful to the Faculty of Medicine, Khon Kaen University for supporting this research.

REFERENCES

Boongird P, Phuapradit P, Siridej N, Chirachariyavej T, Chuahirun S, Vejjajiva A. Neurological manifestations of gnathostomiasis. *J Neurol Sci* 1977; 31: 279-91.

Daengsvang S. Gnathostomiasis in Southeast Asia. *Southeast Asian J Trop Med Public Health* 1981; 12: 319-32.

Jaroonvesama N. Differential diagnosis of eosinophilic menigitis. *Parasitol Today* 1988; 4: 262-6.

Laemmli UK. Cleavage of structural protein during the assembly of the head of bacteriophage T4. *Nature* 1970; 227: 680-5.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 193: 265-75.

Maleewong W, Intapan PM, Tomanakarn K, Wongkham C. Antigenic components of somatic extract from adult *Fasciola gigantica* recognized by infected human sera. *Asian Pac J Allerg Immunol* 1997; 15: 213-8.

Maleewong W, Sithithaworn P, Tesana S, Morakote N. Scanning electron microscopy of the early third-stage larvae of *Gnathostoma spinigerum*. *Southeast Asian J Trop Med Public Health* 1988; 19: 643-7.

Miyazaki I. On the genus *Gnathostoma* and human gnathostomiasis with special references to Japan. *Exp Parasitol* 1960; 9: 338-70.

Nopparatana C, Setasuban P, Chaicumpa W, Tapchaisri P. Purification of *Gnathostoma spinigerum* specific antigen and immunodiagnosis of human gnathostomiasis. *Int J Parasitol* 1991; 21: 677-87.

Nopparatana C, Tapchaisri P, Setasuban P, Chaicumpa W, Dekumyoy P. Antibody responses in human gnathostomiasis. *Southeast Asian J Trop Med Public Health* 1988; 19: 219-24.

O'Farrel PH. High resolution two-dimensional electrophoresis of proteins. *J Biol Chem* 1975; 250: 4007-21.

Schmutzhard E, Boongird P, Vejjajiva A. Eosinophilic meningitis and radiculomyelitis in Thailand, caused by CNS invasion of *Gnathostoma spinigerum* and *Angiostrongylus cantonensis*. *J Neurol Neurosurg Psychiatry* 1988; 51: 80-7.

Tapchaisri P, Nopparatana C, Chaicumpa W, Setasuban P. Specific antigen of *Gnathostoma spinigerum* for immunodiagnosis of human gnathostomiasis. *Int J Parasitol* 1991; 21: 315-9.