

Rapid purification of glutathione S-transferase from *Neodiplostomum seoulense*

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Abstract

The authors partially purified glutathione S-transferase (GST) of *Neodiplostomum seoulense* monitoring its activity using 1-chloro-2,4-dinitrobenzene (CDNB). The enzyme was partially purified using glutathione-agarose affinity column chromatography. The purification fold and recovery rate were 9.7 and 50.4%, respectively. The molecular weight of the purified GST was estimated to be 28 kDa on 7.5–15% gradient SDS-PAGE. From this study, the authors firstly reported the presence of GST from *N. seoulense* and further studies such as inhibition test, subclass of GST as well as immunological studies would be required. (*J Med Life Sci* 2014;11(2):197–199)

Key Words : glutathione S-transferase, *Neodiplostomum seoulense*, intestinal trematode

Introduction

Neodiplostomum seoulense is an intestinal trematode, and humans are infected by eating undercooked or raw snake or frogs¹. The normal habitat of the parasite is the duodenum¹ and the most pathological features are villous atrophy, mucosal inflammation and bleeding². Despite of causing gastrointestinal symptoms, many studies are not performed regarding biochemical, pathological characteristics of the functional proteins of the parasite until now. Few studies have been reported regarding functional proteins of the worm. Among them, a cysteine protease with molecular mass of 54 kDa from the parasite was purified and it was involved in nutrient uptake, rather than host tissue lysis³. Two cystatin-binding cysteine proteases were purified and useful for serodiagnosis of human *N. seoulense* infection⁴.

Glutathione S-transferases (GSTs) have been known from protozoa to vertebrates and catalyze conjugation of reduced glutathione to electrophilic substrates⁵ and also recognized in involving survival of parasites. However, GST of *N. seoulense* has not been illustrated until now. Here, the authors reported the some properties and rapid purification of GST from *N. seoulense* crude extracts.

Materials and Methods

1. Preparation of crude extracts

Adult worms of *N. seoulense* were prepared by the methods of Chai et al⁶. Briefly, metacercariae of *N. seoulense* were collected from intestine of snake, *Rhabdophis tigrina*, and then, BALB/c mice were infected with 50 metacercariae of *N. seoulense*. Adult worms were harvested in intestine of BALB/c mice and washed several times with cold phosphate buffered saline (PBS). The worms were then, homogenized with 20mM Tris-HCl buffer (pH 7.4), and centrifuged at 12,000 rpm for 30 min at 4°C. The resulting supernatant was used as crude extracts.

2. Assay of GST.

The reaction mixtures were consisted with 950 μ l of 0.1M Tris-HCl buffer (pH 7.4), 10 μ l of 1mM CDNB (Sigma, St Louis, USA), and 5 mM reduced glutathione (GSH, Sigma). The reaction mixtures were then incubated at 37°C for 30 min. After adding of 20–30 μ l of crude extracts, the enzyme activity was monitored spectrophotometrically at 340 nm for 5 min⁷. One unit of enzyme activity was defined as the amount of enzyme that catalyzed 1 μ mole of CDNB per minute under the above condition.

3. Purification of GST

To purify GST of the parasite, the crude extract was applied to glutathione-agarose (Sigma) column (1.6 x 2 cm long) previously equilibrated with 20 mM Tris-HCl buffer

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(pH 7.4). The column was eluted with flow rate 0.5 ml/min and each fractions were collected with 1.5 ml. The column was washed with the same buffer and absorbed proteins were gradually eluted with increasing of reduced glutathione up to 20 mM (5 to 20 mM). Fractions with high enzyme activity were pooled and analyzed the purity by 7.5–15% gradient SDS-PAGE.

Results

The total protein of crude extract in this experiment was 3.4 mg and total activity of GST was 12.9 (Table 1). The specific activity of GST in crude extract was 3.8.

To purify GST using glutathione-agarose column, the column was washed firstly with 20 mM Tris-HCl buffer (pH 7.4) containing 0.15 M NaCl to remove nonspecifically bound protein, and bound GST was eluted by 5 mM reduced glutathione and finally eluted 20 mM reduced glutathione to obtain the GST as much as possible. The purified GST was then excessively dialyzed against 20 mM Tris-HCl buffer (pH 7.4) to remove excess NaCl and reduced glutathione, and then, it was lyophilized and dissolved small amount of same buffer. Thus we purified GST with single-step purification using the column. The purified GST showed that recovery rate and purification fold was 57.4%, 9.7, respectively (Table 1).

Table 1. Purification of *N. seoulense* GST

	Total activity (units)	Total protein (mg)	Specific activity (units/mg protein)	Purification (fold)	Recovery (%)
Crude extract	12.9	3.4	3.8	1	100
glutathione-agarose	7.4	0.2	37	9.7	57.4

To confirm the purity of the GST, SDS-PAGE was performed using 7.5–15% gradient gel to visualize from low to high molecular mass proteins (Fig. 1). In this experiment, many proteins of crude extracts were distributed at from high molecular mass to low molecular weight protein such as 14 kDa (Fig. 1). The purified GST of the fluke migrated at 28 kDa protein band (Fig. 1, lane 2).

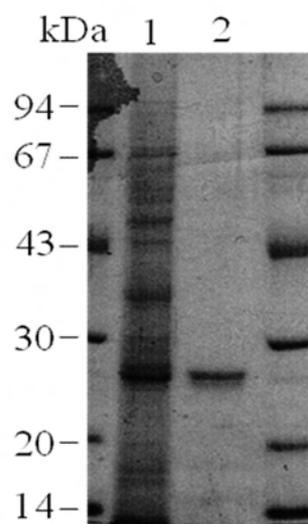


Figure 1. Purification of *N. seoulense* GST. Lane 1; crude extract, lane 2; purified GST using glutathione-agarose. SDS-PAGE was performed using 7.5–15% gradient gel under reducing condition.

Discussion

In the present study, GST was purified from crude extract of *N. seoulense* adult using glutathione-agarose affinity chromatography. The molecular weight of GST of *N. seoulense* adult was estimated to be 28 kDa by SDS-PAGE and the molecular mass is very similar size in other trematodes such as *Paragonimus westermani*²⁰, *Clonorchis sinensis*⁹.

The cytosolic GST of *P. westermani* adult have been purified using CDNB as substrate. The molecular weight of the GST is 28 kDa and their activity was inhibited by bromosulfophthalein, cibacron blue and albendazole but not praziquantel. Therefore, it belongs to sigma-class GST⁶. In addition, the polyclonal antibody against 28 kDa GST of *P. westermani* only reacted with 28 kDa GST. The antibody did not react with that of *Schistosoma japonicum*, *Fasciola hepatica* and *C. sinensis* indicating that it showed species-specific immunoreaction in Western blotting.

However, our results showed only rapid purification of GST and tried to firstly detect the activity and presence of the enzyme from *N. seoulense*. Large quantities of purified GST will be required for more biochemical and immunological studies such as inhibitor test, classification of GST and species-specific antigenic reactions.

Molecular cloning of 28 kDa GST from *C. sinensis* had

been reported that it consisted of 212 amino acid residues, and recombinant 28GST showed GST activity with CDNB as substrate and was sensitive against GST inhibitors⁹⁾. Moreover, recombinant Cs28GST was same property with native 28 kDa GST recognized by clonorchiasis patients sera and it was located in the mesenchymal tissue and tegumental syncytium. And also, 26 kDa GST was also co-purified during purification of 28 kDa GST and 26 kDa GST was different enzyme in terms of its molecular, antigenic properties⁹⁾.

In this regard, it is also possible that 26 kDa GST of *N. seoulense* adult is also present and can be co-purified in our experiment. The purified 28 kDa GST was appeared tailing band as if it contained small amount of 26 kDa GST (Fig. 1, lane 2). It is also required for further studies the presence of 26 kDa GST of the fluke. In this study, we purified and confirmed for presence of 28 kDa GST from *N. seoulense* and further studies would be required for more biochemical, immunological studies to understand precise role of GST in the parasite.

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