Original article **P58**

Production of monoclonal antibodies against cathepsin-L and cystatin of *Faasciola gigantica*

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Abstract

Fasciolosis caused by *Fasciola gigantica* is an important disease in cattle and buffaloes in Thailand. At present, an efficient molecular diagnostic test for fasciolosis is not available. This study aims to produce monoclonal antibodies specific to two important parasite antigens, cathepsin L and type 1 cystatin (FgStefin-1) and to evaluate their efficacy as diagnostic tools. Purified native cathepsin L and recombinant cystatin produced in *E. coli* will be used for immunization of BALB/c mice for three times in 3-week intervals using Freund's adjuvant. The spleen cells of the mice will be collected and fused with myeloma cells to obtain clones that produce antigen-specific monoclonal antibodies. These monoclonal antibodies will be analyzed for their sensitivity and specificity.

Keywords**:** *Fasciola gigantica*, cathepsin L, cystatin, monoclonal antibody, diagnosis

Introduction

Fasciola gigantica is the most common cause of liver fluke disease in cattle and buffalo in Thailand. The detection of parasite eggs in faecal samples is the most commonly used diagnostic method but it has relatively low sensitivity. Therefore, new diagnostic tools with higher diagnostic sensitivity are needed to detect the infection. The present study aims to produce monoclonal antibodies specific to two important and abundant parasite antigens, cathepsin L and type 1 cystatin (FgStefin-1) and to evaluate their efficacy as diagnostic tools.

Materials and methods

Expression of recombinant FgStefin-1 in *E.coli*

The protein was expressed in *E.coli* M15 transformed with pQE30-FgStefin-1 after induction with IPTG (1 mM final concentration). The soluble protein was purified by Ni-NTA affinity chromatography under native conditions.

Collection of excretion/secretion product and purification of cathepsin L

Adult *F. gigantica* were incubated at 37°C in RPMI-1640 medium, pH 7.3 containing 2% (W/V) glucose, 30 mM HEPES and 25 mg/l gentamycin. After 4 h incubation, the culture medium was centrifuge at 10,000 × g for 1 h at 4°C and the supernatant was used to precipitate cysteine proteinases. Chilled ethanol was added drop by drop to a final concentration of 60% (v/v) and the mixture incubated at -20°C for 24 h. After centrifugation at 6,000 × g (40 min, 4°C), the supernatant was mixed with 75% ethanol and incubated at -20°C overnight. After centrifugation at 6,000 × g (40 min, 4°C) the supernatant was discarded and the pellet air dried and resuspended in PBS, pH 7.2.

Zymography analysis

The proteins were incubate with reaction buffer (0.1 M sodium acetate, 1 mM EDTA adjusted to pH 5.5) before performing gel electrophoresis under non-reducing conditions on 10% polyacrylamide gels containing 0.1% gelatin from porcine skin. After electrophoresis,

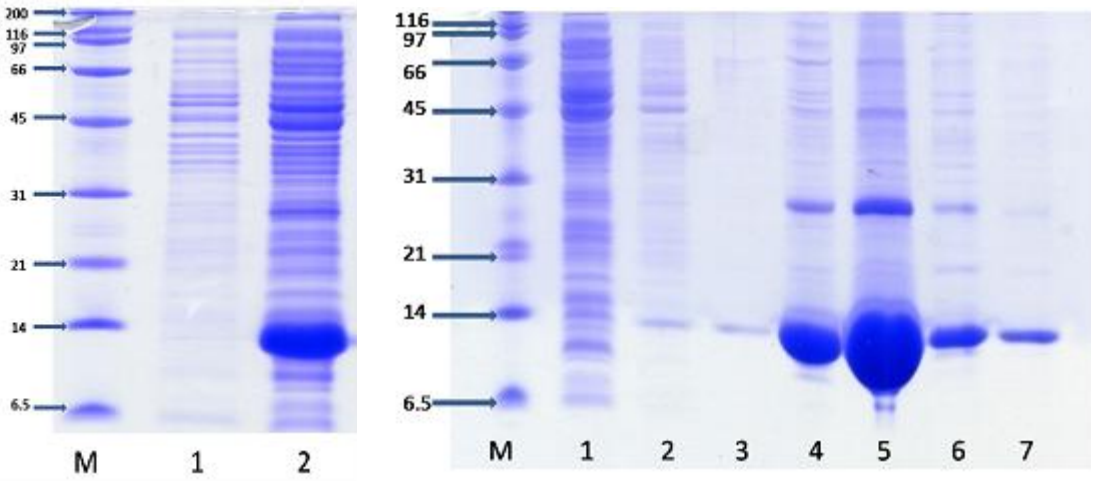
the gel was washed in 2.5% Triton-X 100 with agitation and then washed with developing buffer (0.1 M Sodium acetate, 1 mM EDTA, 2 mM DTT, adjusted to pH 5.5). The gel was incubated with developing buffer overnight at 37°C. The zymographic gel was then stained in 0.5% Coomassie Brilliant Blue R-250 and de-stained.

Fluorometric assay

The substrate Z-Arg-Arg-AMC (Calbiochem) was added to a final concentration of 400 µM and cathepsin L and cysteine protease activity in ES product were measured by a modified method from Barrett and Kirschke. Cathepsin L (1 ng/reaction) and ES product (10 ng/reaction) were incubated in 340 mM sodium acetate, 60 mM acetic acid, 4 mM EDTA, 8 mM DTT, pH 5.5 for activation of the enzyme. E-64 a specific cysteine proteases inhibitor was used as positive control. All samples had a 100 µl final reaction volume and were incubated for 30 min at 37°C in black 96-well microtiter plates before measurement of activity at wavelengths 355 nm and 460 nm.

Results

Expression of recombinant FgStefin-1 in *E.coli* M 15



(A) **(B)**

Figure1. SDS-PAGE analysis of the expression and purification of FgStefin-1. A, non-induced (1) and IPTG induced (2) bacterial protein. B, purification by Ni-NTA affinity chromatography flow through (1) wash (2, 3) and elution (4, 5, 6, 7) fractions.

Purification of cathepsin L

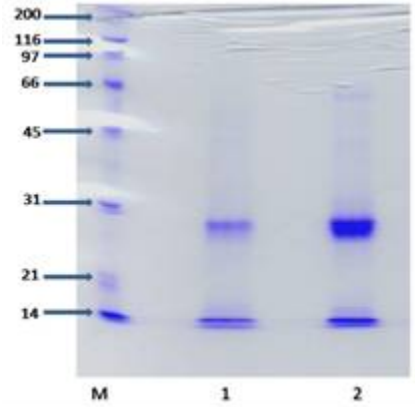


Figure 2. Purification of cathepsin L: ES (1), cathepsin L (2)

Figure 3. Zymography analysis: (1) ES and cysteine protease inhibitor E64, (2) ES, (3) cathepsin L and cysteine protease inhibitor E64, (4) cathepsin L

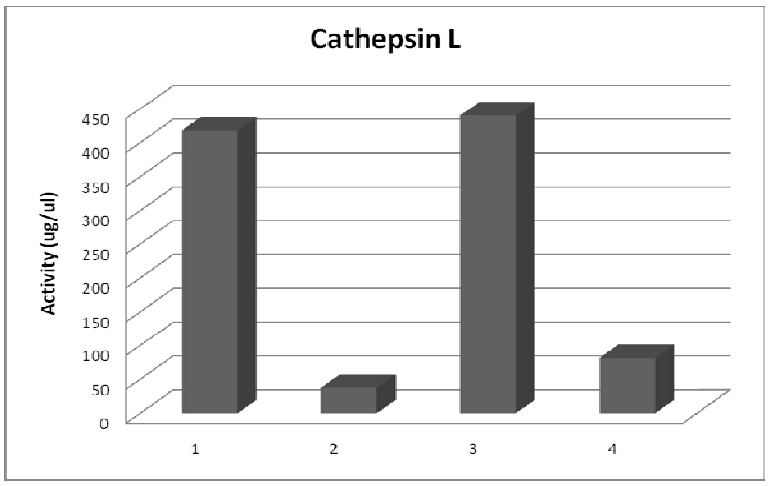


Figure 4. Fluorometric assay: (1) ES activity was detected using the substrate Z-Arg-Arg-AMC. (2) ES and cysteine protease inhibitor E64. (3) Cathepsin L activity was detected using the substrate Z-Arg-Arg-AMC. (4) Cathepsin L and cysteine protease inhibitor E64.

Discussion

Type 1 cystatin (FgStefin-1) and cathepsin L are important proteins in *F. gigantica*. They are secreted in large amounts by the parasite and induce a strong immune response in the infected host. Both proteins are also stable. These properties make them interesting candidates for diagnostic application. Monoclonal antibodies against these proteins are preferable to polyclonal antisera as they may show higher specificity.

Conclusion

Type 1 cystatin (FgStefin-1) and cathepsin L were purified as recombinant or native protein, respectively. Expression of recombinant FgStefin-1 in *E. coli* was induced by IPTG and the protein was purified by Ni-NTA affinity chromatography. Native cathepsin L was precipitated from excretion/secretion product by ethanol and tested by zymography analysis and fluorometric assay for its activity. Both proteins will be used for immunization of BALB/c mice to produce monoclonal antibodies that can be applied for diagnosis of fasciolosis.

Acknowledgements

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