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Research Note

# Distribution of the Cysteine Protease in *Spirometra erinacei* Plerocercoid (Cestoda; Diphyllobothriidae)

TOHRU FUKASE[1)], YOSHIFUMI MATSUDA[2)], SUMIYUKI AKIHAMA[2)] AND HIROSHI ITAGAKI[1)]

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**Key words:** *Spirometra erinacei,* plerocercoid, cysteine protease

We previously reported some hydrolyzing enzymes in plerocercoids of the pseudophyllidean tapeworm *Spirometra erinacei* (Fukase *et al.,* 1984). One of the enzymes was then purified and examined for its properties and it was considered to be a cysteine protease (Fukase *et al.,* 1985). This cysteine protease seems to be identical with that reported by Nakamura and Yanagisawa (1982a, b) and Nakamura *et al.* (1984, 1985), which hydrolyzes azocoll, actin, myosin, and myofibril. However, the physiological functions of the cysteine protease in host animals are not enough known. Kwa (1972), on the other hand, detected a casein hydrolyzing enzyme(s) in the protoscolex of *S. erinacei* plerocercoids, although the properties of the enzyme(s) were not cleared at all. In the present study, we examined whether our previous cysteine protease exists in the protoscolex as well as Kwa (1972)'s enzyme(s).

Plerocercoids were collected from two Japanese rat snakes, *Elaphe quadrivirgata,* captured in Okayama Prefecture, Japan, and two lots (Lots 1 and 2) of them were used in the present study, Lots 1 and 2 consisting of 331 and 339 plerocercoids respectively. Plerocercoids were washed in tap water and then cut into two parts: the protoscolex and the remainder of the body. Both the parts were separately freezed until use. To identify the species of the plerocercoids used, two domestic cats were each fed with three intact plerocercoids of each lot and autopsied 20 days after infection. The adult worms obtained at autopsy were identified as *S. erinacei.*

Chemicals used were all obtained commercially from the same manufacturers, and the assay of enzyme activity and estimation of protein concentration were done by the same methods as described in our previous paper (Fukase *et al.,* 1985).

The two parts of plerocercoids, the protoscolex and the remainder of the body, were separately crushed in a porcelain mortar, and Triton X-100 was added to the resulting preparation to get a final concentration of 1%. This preparation was then homogenized, and deionized water was added to the homogenate in a ratio of 3 ml per gram of plerocercoids. The diluted homogenate was shaken vigorously for 1 hr and then centrifuged at 11 000 × *g* for 20 min at 4°C. The supernatant was used as crude enzyme preparation. For further

[1)]*Department of Parasitology, School of Veterinary Medicine, Azabu University, Fuchinobe, Sagamihara 229, Japan.*

[2)]*First Department of Biochemistry, Meiji College of Pharmacy, Nozawa, Setagaya, Tokyo 154, Japan.*

purification, this crude enzyme preparation was adjusted to 1 mS/cm in conductivity and to pH 7.5 and then applied to a DEAE-cellulose column (1.2 x 10 cm) equilibrated with 0.02 M Tris-HCl buffer at pH 7.5. After rinsed with the same buffer, the enzyme(s) was eluted with 1 M NaCl solution and 3 ml fractions were collected. Each fraction was assayed for amidolytic activity toward *N*-α-benzoyl-DL-arginine-*p*-nitroanilide (Bz-Arg-*p*NA), and active fractions were collected and pooled. The solution obtained by DEAE-cellulose adsorption and elution was applied to a Sephadex G-200 column (2.2 x 90 cm) equilibrated with 0.01 M phosphate buffer at pH 7.0. The column was eluted with the same buffer and 5 ml fractions were collected. Each fraction was measured for Bz-Arg-*p*NA amidolytic activity.

In the protoscolex, Bz-Arg-*p*NA amidolytic activity was detected faintly in the crude enzyme extract, but not in the eluate obtained by DEAE-cellulose adsorption and elution. In the body without the protoscolex, on the other hand, Bz-Arg-*p*NA amidolytic activity was detected in the solution obtained by DEAE-cellulose adsorption and elution as well as in the crude extract. The activity was further detected in Sephadex G-200 gel filtration with an elution profile similar to that in our previous study in which whole plerocercoids were used (Fukase *et al.*, 1985). Namely, the fractions obtained were divided into two active groups of fractions: the first eluted fractions with lower Bz-Arg-*p*NA amidolytic activity and the secondly eluted fractions with higher activity (Fig. 1). The latter group of fractions is considered to contain the cysteine protease, because we previously purified the enzyme from this group of fractions (Fukase *et al.*, 1985).

In the present study, Bz-Arg-*p*NA amidolytic activity owing to the cysteine protease was definitely detected in plerocercoids without the protoscolex. In the protoscolex, on the other hand, Bz-Arg-*p*NA amidolytic activity was not detected by DEAE-cellulose adsorption and elution. Accordingly, it seems that the cysteine protease is present densely in the other part of plerocercoid than the protoscolex.

Kwa (1972) detected a casein hydrolyzing enzyme(s) in the protoscolex of *S. erinacei* plerocercoids. His enzyme(s) probably differs from the present cysteine protease because of the difference in location in the worm body. Some kinds of amidases were previously reported in whole plerocercoids of *S. erinacei*

Table 1 Distribution of the cysteine protease activity in *Spirometra erinacei* plerocercoids

| Procedure | Lot 1 (331 plerocercoids) | | | | | | Lot 2 (339 plerocercoids) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Protoscolices (1.2 g) | | | Plerocercoids without protoscolex (11.5 g) | | | Protoscolices (1.4 g) | | | Plerocercoids without protoscolex (11.8 g) | | |
| | Total $A_{280}$ | Activity Total* | Activity Specific* | Total $A_{280}$ | Activity Total* | Activity Specific* | Total $A_{280}$ | Activity Total* | Activity Specific* | Total $A_{280}$ | Activity Total* | Activity Specific* |
| Extraction | 208 | 156 | 0.8 | 896 | 4656 | 5.2 | 172 | 180 | 1.0 | 732 | 3586 | 4.9 |
| DEAE-cellulose adsorption and elution | 44.1 | n.d.** | – | 204 | 2832 | 13.9 | 32.6 | n.d.** | – | 202 | 2660 | 13.2 |
| Sephadex G-200 gel filtration | – | – | – | 121 | 2237 | 18.5 | – | – | – | 118 | 1995 | 16.9 |

*Total and specific activities are represented as nmol of substrate hydrolyzed per minute and nmol of substrate hydrolyzed per minute per $A_{280}$, respectively.
**n.d.: not detected

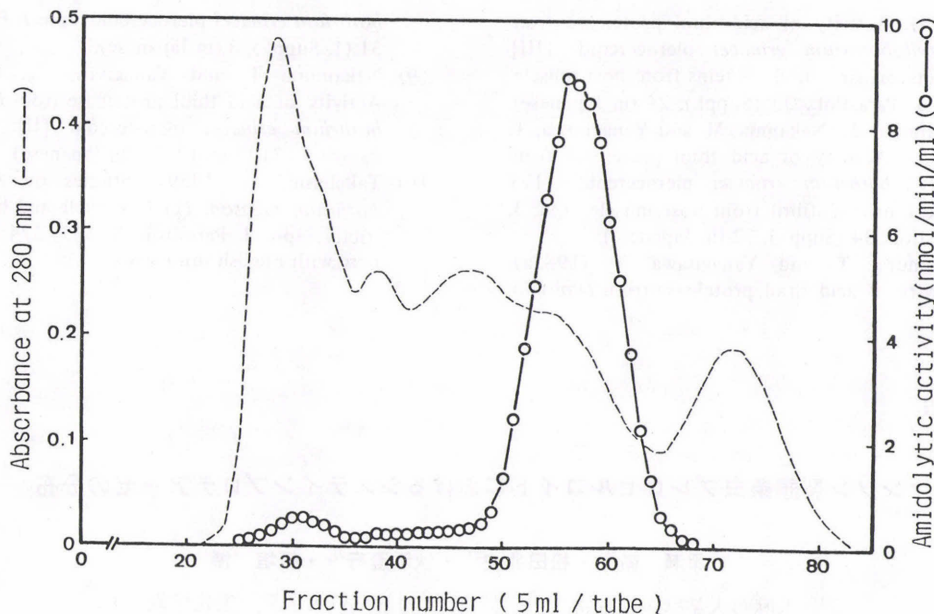


Fig. 1 Elution profile in Sephadex G-200 gel filtration of the enzyme preparation from plerocercoids without the protoscolex in Lot 1
------: absorbance at 280 nm, o——o: Bz-Arg-$p$NA amidolytic activity.

(Fukase *et al.,* 1984), and Kwa (1972)'s enzyme(s) may be included in those enzymes.

*S. erinacei* plerocercoids without the protoscolex do not develop into adults in final hosts (Iwata, 1972), and the body except the protoscolex is immediately lost after plerocercoids are ingested by final hosts (Takahashi, 1959). So that, the protoscolex is thought to have some particular functions for plerocercoids to develop into adults, whereas the physiological significance of the remaining part of the plerocercoid body is not well-known. The present study revealed that the cysteine protease is present densely in the other portion of plerocercoids than the protoscolex. In adult *S. erinacei,* Maki *et al.* (1982) detected a hemoglobin hydrolyzing enzyme, possibly a carboxyl protease, but no cysteine protease has been reported. Accordingly, the present cysteine protease seems to be peculiar to plerocercoids and to have a function lysing the tissues of intermediate and paratenic hosts for penetration and/or nutrition.

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短　報

# マンソン裂頭条虫プレロセルコイドにおけるシステインプロテアーゼの分布

深瀬　徹[1]・松田兆史[2]・秋浜澄行[2]・板垣　博[1]

([1] 麻布大学獣医学部寄生虫学教室, [2] 明治薬科大学第一生化学教室)

マンソン裂頭条虫 *Spirometra erinacei* のプレロセルコイドの原頭節部分と原頭節以外の部分について，DEAE-セルロース吸着・溶出およびセファデックスG-200ゲル濾過を用いて，システインプロテアーゼの存在を検討した．原頭節部分においては，*N*-α-benzoyl-DL-arginine-*p*-nitroanilide(Bz-Arg-*p*NA) 水解活性は，粗抽出液ではわずかに認められたものの，DEAE-セルロース吸着後の溶出液では検出されなかった．一方，原頭節以外の部分においては，Bz-Arg-*p*NA水解活性は，DEAE-セルロース吸着・溶出によっても，また，セファデックスG-200ゲル濾過によっても認められた．この原頭節以外の部分に認められたアルギニンアミダーゼ活性の大部分は，DEAE-セルロースへの吸着性ならびにセファデックスG-200ゲル濾過における溶出位置から，先に報告したシステインプロテアーゼのものであると考えられる．したがって，本酵素は，プレロセルコイドの原頭節部分に集中して存在しているのではなく，原頭節以外の部分に多量に存在しているものと考えられる．