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□ **Brief Communication** □

Component Proteins and Protease Activities in Excretory-Secretory Product of Sparganum

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Abstract: *Spirometra mansoni* plerocercoid (sparganum) was incubated in saline at 4°C or 37°C up to 100 hours. Protein contents in the excretory-secretory product (ESP) were rather constant (mean 7.7 mg of protein/gram of sparganum) in the preparations. Reducing SDS-PAGE of ESP showed similar protein subunit compositions with those in crude extract. Antigenic 36 and 31 kDa proteins were major bands in ESP. ESP exhibited specific activities of protease(2.9~5.3 units/mg) at pH 6.0 and pH 7.5. Presence of protease activity in ESP may be a supporting evidence that hitherto known cysteine protease of sparganum is possibly secreted.

Key words: *Spirometra mansoni* plerocercoid, sparganum, crude extract, excretory-secretory product, SDS-PAGE, protease

Serologic diagnosis of human sparganosis is now available because specific antibody test by ELISA is sufficiently sensitive and specific (Kim *et al.*, 1984). Crude extract of sparganum has been used as a diagnostic antigen. Of many antigenic protein components in the crude extract, 36 and 31 kDa proteins (in previous reports, they were described as 36 and 29 kDa; see Fig. 1 for change of molecular mass estimation) were most antigenic when observed by SDS-PAGE/immunoblot using patient sera (Choi *et al.*, 1988). These two antigenic proteins were purified by affinity chromatography, though their nature is yet to be studied. The 36 and 31 kDa proteins were localized in syncytial tegument and tegumental cells of the parasite by immunohistochemical staining using the monoclonal antibody (Kim *et al.*, 1992). Presence of the 36 and 31 kDa proteins at syncytial tegument suggests that they are secreted into microniche.

On the other hand, protease activities of the sparganum have been studied to explain its migration mechanisms through host tissues by tunnel formation (Fukase *et al.*, 1985 & 1986; Song and Choi, 1992). Neutral thiol or acidic cysteine proteases have been proved to exist in both crude extract and ESP of sparganum. Especially, Song and Choi(1992) described the presence of cysteine protease activity in ESP of sparganum. Their finding seems important because cysteine protease has been known as an intracellular lysosomal enzyme (Barrett, 1977; Bond and Butler, 1987). The above two findings prompted us to observe the composing proteins in ESP of sparganum to confirm that the antigenic proteins of 36 and 31 kDa are secreted and that protease activities exist in ESP.

From 50 terrestrial snakes, *Elaphe rufodorsata*, which were purchased in Chinju City, Korea, a total of 5.2 g of naturally infected spargana was collected. Of them, complete spargana with their scolices, neither damaged nor fragmented during removal, were selected and washed 3 times to clear adhered host tissue particles. Complete sparganum of wet weight 0.36 g was incubated in 4.5 ml sterile physiologic

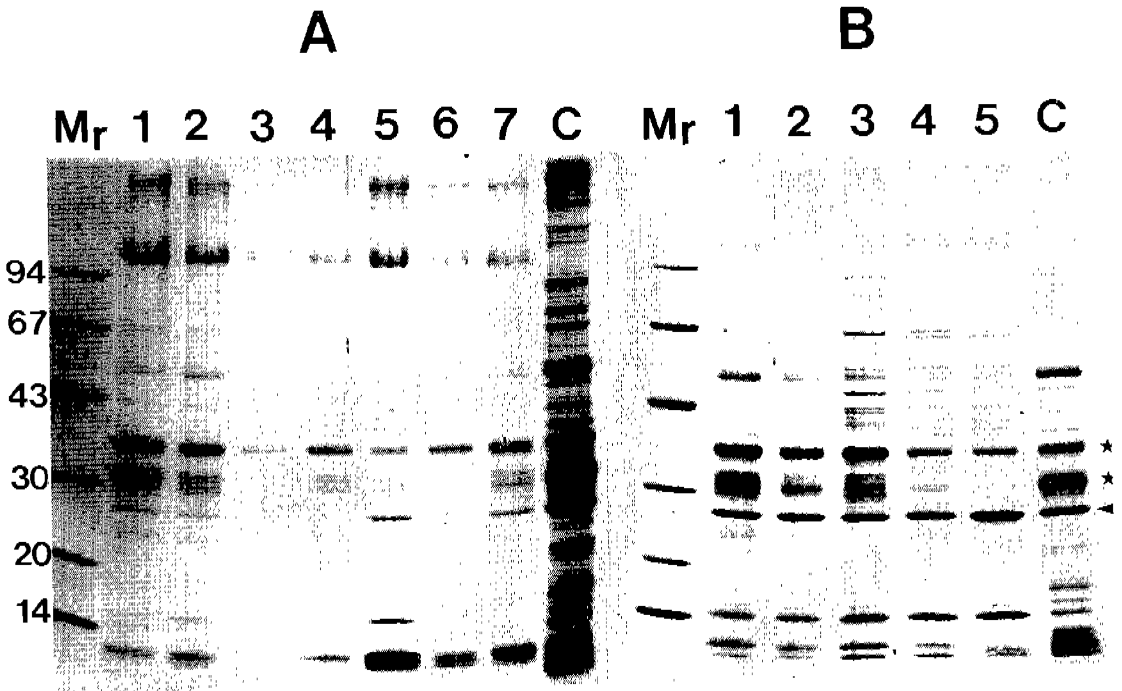

Fig. 1. Reducing SDS-PAGE findings of ESP and crude saline extract of sparganum (80 μg protein in each lane) in linear gradient separating gel of 7.5~15%. Coomassie brilliant blue R-250 stained. (A): ESP collected at 4°C and crude saline extract of sparganum. Mr: Molecular mass marker, 1: ESP of an hour incubation, 2: ESP of 2-hour incubation, 3: ESP of 5-hour incubation, 4: ESP of 8-hour incubation, 5: ESP of 12-hour incubation, 6: ESP of 24-hour incubation, 7: ESP of 48-hour incubation, C: crude saline extract. (B): ESP collected at 37°C and crude extract of sparganum. 1: ESP of 30-minute incubation, 2: ESP of an hour incubation, 3: ESP of 2-hour incubation, 4: ESP of 3-hour incubation, 5: ESP of 24-hour incubation, C: crude extract of sparganum. ★: Antigenic proteins of 36 and 31 kDa of Choi *et al.*(1988) and Kim *et al.*(1992), which are recognized not only in crude extract but also in ESP. ◀: Possible cysteine protease of Song and Choi (1992). Estimation of molecular mass of 29 kDa protein in previous reports was changed to 31 kDa when the molecular mass markers of Pharmacia(Sweden) is used instead of Sigma product.

saline at 37°C. Incubation medium was harvested according to the schedules shown in Table 1. Incubation was carried out separately at 4°C in 20 ml of saline using complete worms of wet weight 0.3 g. In 4°C incubation, the medium of 20 ml was replaced as shown in Table 1. Incubation medium was regarded as ESP. Immediately after harvest, ESP was dialysed against 50 mM Tris buffered saline (pH 7.4) and centrifuged at 4°C(10,000 *g*) to remove particles, and stored at −70°C until used.

Protein contents in ESP was measured by the method of Lowry *et al.*(1951). As shown in Table 1, the total protein amounts secreted in each ESP were similar each other regardless of incubation temperature or time. The ranges of protein secretion were calculated as 5.6~10.0 mg/g (mean: 7.7 mg of ESP protein/g of wet sparganum tissue).

Composition of protein subunits in ESP, together with crude saline extract of sparganum, was observed by reducing SDS-PAGE of Laemmli (1970) (Fig. 1). The proteins of 36 and 31 kDa were present in all ESP prepared at 4°C or 37°C and at different incubation time. SDS-PAGE in different preparations of ESP exhibited almost identical findings with minimal differences in the banding patterns. Differences in banding patterns between ESP and the crude extract were recognized in high molecular masses above

Table 1. Protein contents in ESP of sparganum collected in different temperature and time interval

Incubation time*	Incubation at 4°C*		Incubation at 37°C**	
	Prot. conc. (mg/ml)	Total protein (mg)	Prot. conc. (mg/ml)	Total protein (mg)
30 min	—	—	0.53	2.1
1 hour	0.085	1.7	0.80	3.2
2 hours	0.12	2.4	0.56	2.2
3 hours	—	—	0.50	2.0
5 hours	0.11	2.2	—	—
8 hours	0.14	2.8	—	—
12 hours	0.15	3.0	—	—
24 hours	0.14	2.8	0.54	2.2
48 hours	0.15	3.0	—	—

* ESP was collected in the given time intervals after change of the previous medium.
* 0.3 g of wet sparganum was incubated in 20 ml of sterile saline. Actual incubation time was 100 hours from the beginning.
** 0.36 g of wet sparganum was incubated in 4 ml of saline. Total incubation time was 30.5 hours.

66 kDa and low molecular masses around 29 kDa. Evidence of protein denaturation was not found up to 24 hours incubation at 37°C as far as the findings of SDS-PAGE are concerned.

Protease activities were measured in ESP preparations and crude saline extract, at pH 6.0 and pH 7.5, by the method of Sauer and Senft (1972) using bovine hemoglobin (Sigma, U.S.A.) as a substrate. One unit of the protease activity was defined as the amount of absorption rate through 1 cm path in which 5 mg hemoglobin was hydrolysed for an hour. Specific activity was defined as the unit of activity per milligram of protein.

Specific activities of protease in differently prepared ESP were presented in Table 2. Preparations of ESP collected either at 37°C or at 4°C exhibited comparable activities each other (specific activities in range of 2.9~5.3 units/mg protein) when measured at acidic pH of 6.0 or neutral pH 7.5 (Table 2). Specific activity of protease in the crude saline extract was 4.7 units/mg protein. This study does not confirm the secretion of hitherto known cysteine protease of sparganum into ESP specifically. However,

Table 2. Specific activities of protease in different ESP preparations of sparganum, measured at pH 6.0 and pH 7.5

Incubation time of sparganum	Specific activities* (unit/mg) in ESP collected at			
	4°C		37°C	
	pH 6.0	pH 7.5	pH 6.0	pH 7.5
30 min.	—	—	4.1	4.7
1 hour	4.3	4.0	4.0	5.3
2 hours	4.6	4.0	3.3	3.7
3 hours	—	—	3.5	3.3
5 hours	2.9	3.5	—	—
8 hours	3.3	4.2	—	—
12 hours	4.4	3.9	—	—
24 hours	3.9	4.5	4.2	4.8
48 hours	3.9	4.4	—	—

* Specific activities of protease of crude saline extract of sparganum was 4.7 units/mg protein at both pH 6.0 and pH 7.5, respectively.

this proteolytic enzyme of sparganum, which is active at acidic pH, is possibly included in ESP because 28 kDa protein of cysteine protease (Song and Choi, 1992) was consistently observed in SDS-PAGE of the ESP preparations.

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=국문초록=

스파르가눔 분비배설항원의 단백질 조성 및 단백질분해효소 활성

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스파르가눔증의 항체검사에서 항원으로 사용하는 스파르가눔 충체추출액의 구성 단백질 중, 환자혈청과 반응시켜 관찰한 바, 분자량 36 및 31 kDa인 단백질이 항원성이 높았다. 또한 이 두가지 단백질은 스파르가눔 충체의 표피와 표피세포에 분포하여 충체 외부로 분비되는 항원일 것으로 추정하였다. 이 연구단보에서는 이들 두가지 단백질이 분비배설항원에 나타남을 증명하고자 스파르가눔을 4°C 및 37°C 생리식염수에 30분~100시간 동안 배양하여 분비배설항원을 만들고, 분비배설항원내 단백질 분비량, 단백질의 조성 및 단백질분해효소의 활성을 각각 관찰하였다. 충체 g당 분비배설항원의 단백질량은 관찰 범위안에서 배양온도에 관계없이 평균 7.7 mg이었다. SDS-폴리아크릴아미드 겔 전기영동상 분비배설항원의 단백질 구성은 분자량 66 kDa 이상인 단백질에서 차이가 있을 뿐 충체추출액의 것과 거의 유사하였다. 물론 분자량 36 및 31 kDa인 단백질도 분비배설항원의 주구성성분이었다. 분비배설항원의 단백질분해효소 비활성도(比活性度)는 2.9~5.3 units/mg으로 충체추출액의 것과 차이가 없었다. 이미 보고된 스파르가눔의 cysteine protease가 체외로 분비되는 효소라는 것이 사실이라고 추정하게 하는 결과로 생각하였다.

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