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Proteomic Study for Low Molecular Weight Peptides in the Mealworm *Tenebrio molitor*

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Received September 15, 2020 /Revised October 21, 2020 /Accepted October 21, 2020

In this study, we examined low molecular weight peptides using proteomics in order to identify their original proteins, derive their peptides, and determine the functions of the proteins in *Tenebrio molitor*, the mealworm (larvae, pupae, or adult) from which the peptides were extracted. Fifty-four proteins were finally identified through an analysis of proteome to derive the analyzed peptides. The proteins that induced low molecular weight peptides were identified to be the most abundant in adults only, and the next highest were derived from a group containing both adults and larva. However, other groups, including pupa, were detected to have a lower frequency of peptides. As a result of orthologous classification of the detected proteins, the general function prediction was only investigated at the highest frequency among the examined proteins. Proteins related to chromatin structure and dynamics were detected by their higher frequency among functional classes. The next highest frequency was shown by proteins related to amino acid transport and metabolism and carbohydrate transport and metabolism. Therefore, it is assumed that proteins correlated with chromatin, amino acid, and carbohydrate metabolisms are easily induced into low molecular weight peptides, and that their peptides could play a role as bioactive substances.

Key words : Bioactive peptide, ortholog, proteome, peptide, *Tenebrio molitor*

Introduction

Insects have been highlighted as an alternative resource that can solve a food problem caused by shortage of livestock products in the future. Especially, insect's larvae have been variously utilized for purposes such as improvement of liver function and health promotion as a classically Chinese herbal medicine resources [4]. Since protein content of insect (larva, pupa, and adult) is composed of approximately 50% in a dry weight, insects have been highly valuable for application as alternative protein source [2, 5]. Fat content of insects maintains approximately 30~40%, in which proportion of saturation/unsaturation fatty acids is approximately 0.5 [6]. Especially, since oleic acid and alpha-linolenic acid as major unsaturated fatty acids maintain

high contents with 50 and 28%, respectively, insects are optimal for health supplement [6]. *T. molitor* larvae have a slightly lower fat content compared to beef, but have a slightly higher protein and metabolic energy [7]. In terms of amino acid composition, *T. molitor* larvae maintain a high content of isoleucine, leucine, valine, tyrosine and alanine compared to beef, whereas they have a low content of glutamic acid, lysine and methionine. In fatty acids, *T. molitor* larvae maintain a low content of palmitoleic acid, palmitic acid, stearic acid, etc. compared to beef, whereas they have a high content of linoleic acid, an essential fatty acid. Minerals in *T. molitor* larvae remained similar to beef, while vitamins were generally high.

Since low molecular weight peptides in *T. molitor* influence on various physiological processes as autocrine/paracrine signaling molecules, molecular adaptors, or anti-bacterial peptides, we examined less than 10 kDa peptides among protein extracts of *T. molitor*. As we tracked the proteins to cause the low molecular weight peptides and tracked functions of the proteins in the body, we examined possible of applications of them as basic data for functional peptides.

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Materials and Methods

Pretreatment of *T. molitor*

An aliquot (5 g) of larvae (13~16th instar and 1 kg), pupa (7 days after pupation and 1 kg), or adults (1 month and 1 kg) of the freeze-dried mealworm were suspended with 45 ml of PBS buffer, pH 7.4, and then homogenized for 5 min at 12,000x g by a homogenizer (T25basic, IKA, Damstadt, Germany). The supernatant of the homogenized samples were collected by centrifugation for 20 min at 2,000 × g and then filtered under 10 kDa by centricon (Merckmillipore, Damstadt, Germany). To examine proteome, the filtered fluids were dried with SpeedVac Vacuum concentrator (ThermoFisher scientific, Massachusetts, USA), and then the treated *T. molitor* extract (30 mg) was resolubilized in a 10 mM Hepes buffer, pH 8.0, containing 6 M urea (GE), 2 M thiourea (Sigma-Aldrich Korea, Seoul, Korea), and reduced, alkylated, and digested essentially as described previously [3]. To reduce disulfide bonds, 100 mM dithiothreitol was added to a final concentration of 10 mM in the protein solutions and incubated for 1 hr at 56°C. Each sample was digested with trypsin (1.2 mg (>800 U)/50 mg sample protein) for 18 hr at 37°C.

Proteomic analysis

The digested peptides were separated and identified by liquid chromatography integrated with electrospray ionization mass spectrometry (LC/ESI ion Trap MS). Tryptic peptides (30 µg) were loaded on a trapping column with 75 µm inner diameter, packed with 5 µm C18 particles (Acclaim PepMap100, ThermoFisher scientific, Massachusetts, USA) and analyzed using a 15 cm analytical column packed with 2 µm C18 particles (Acclaim PepMap RSLC, ThermoFisher scientific). Reversed phase chromatography was performed using an Ultimate 3000 RSLC nano system (ThermoFisher scientific) with a binary solvent consisting of 0.1% formic acid (buffer A) and 80% ACN in 0.1% formic acid (buffer B). The peptides were separated by a linear gradient of buffer B from 5% up to 95% for 180 min with a flow rate of 0.3 ml/min. Liquid chromatography was coupled to a Q Exactive Plus hybrid quadrupole-Orbitrap mass spectrometer (Q Exactive Plus, Thermo Scientific). Q Exactive Plus was operated in data-dependent mode with MS scans acquired at a resolution of 70,000 an ion target value of 1e6 and maximum ion injection time for the MS scan was set to 250 ms.

Bioinformatic analysis

For protein identification, MS/MS spectra were searched by MASCOT 2.4 (Matrix science, www.matrixscience.com) [1]. Genome sequences of *T. castaneum* and Uniprot insect DB were obtained from NCBI (<https://www.ncbi.nlm.nih.gov/>) and Uniprot database (<https://www.uniprot.org/>), respectively, for protein identification. Mass tolerance of parent ion or fragment ion was 0.8 Da. Cabamidomethylation of cysteine and oxidation of methionine were considered in MS/MS analysis as variable modifications of tryptic peptides [1].

Results and Discussion

The number of queries analyzed from proteome were 38,578~42,678, and total residues were 9,906,812, and sequences after taxonomy filter were 16,696. Signal threshold for analysis of proteome was 0.01, and false discovery ratio was 5.37±1.33, 10.08±2.49, and 8.69±0.51 for larva, pupa, and adult samples, respectively. The number of proteins confirmed by peptide levels were 94~107, 71~77, and 119~125 from larva, pupa, and adult samples, respectively. These results presented less than half value when compared to result of 382 proteins obtained by analysis of proteome from *Scleroderma guani* parasitization [8].

The original proteins to derive the peptides were finally matched with total 54 through MASCOT filtration via genome sequence of *T. castaneum* DB (<https://www.ncbi.nlm.nih.gov/>). Among them, the most number was identified in adult only, and the next higher number was maintained by the larva and adult group (Fig. 1). Proteins to be identified in groups including pupa showed totally low detection ratio. From the results, it is assumed that adult has the most low molecular weight peptides and the next larva has higher, but pupa maintains the lowest value. In fact, as a result of analysis in this laboratory, it was found that peptides less than 10 kDa possess various antibacterial activities (unpublished data). Therefore, it is possible that the peptides shown in the current analysis may be involved in various physiological activities including innate immunity.

As a result of orthologous cluster, general function prediction only showed the most count of 23 (Fig. 2). In addition, orthologous clusters predicted by high ratio were detected by proteins associated with chromatin structure and dynamics, amino acid transport and metabolism, and carbohydrate transport and metabolism. Proteome of mealworms

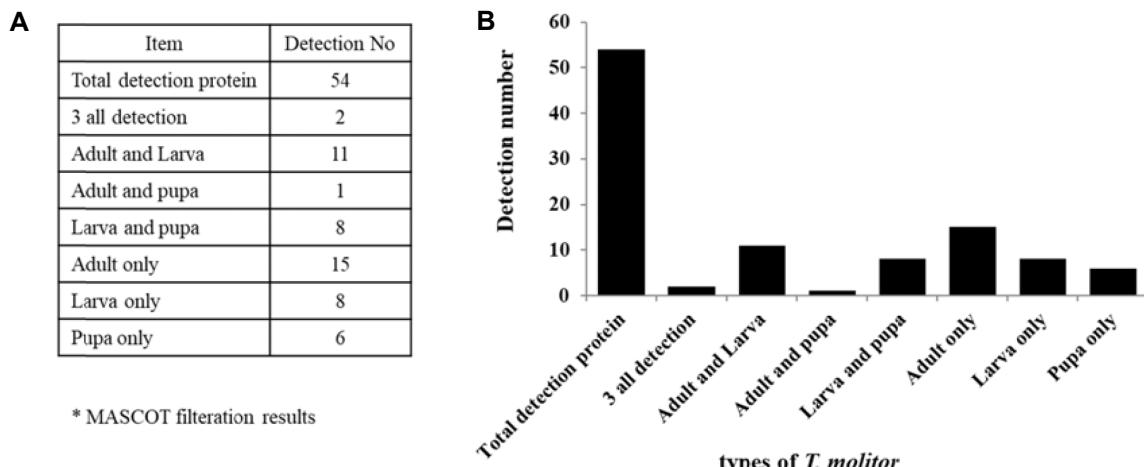


Fig. 1. Result of proteome analysis from *T. molitor*. (A) Result of proteome and (B) bargraph of proteome result. The result was finally obtained by MASCOT filtration. X- and Y-axes in B indicate types originated from *T. molitor* and detected number of protein, respectively.

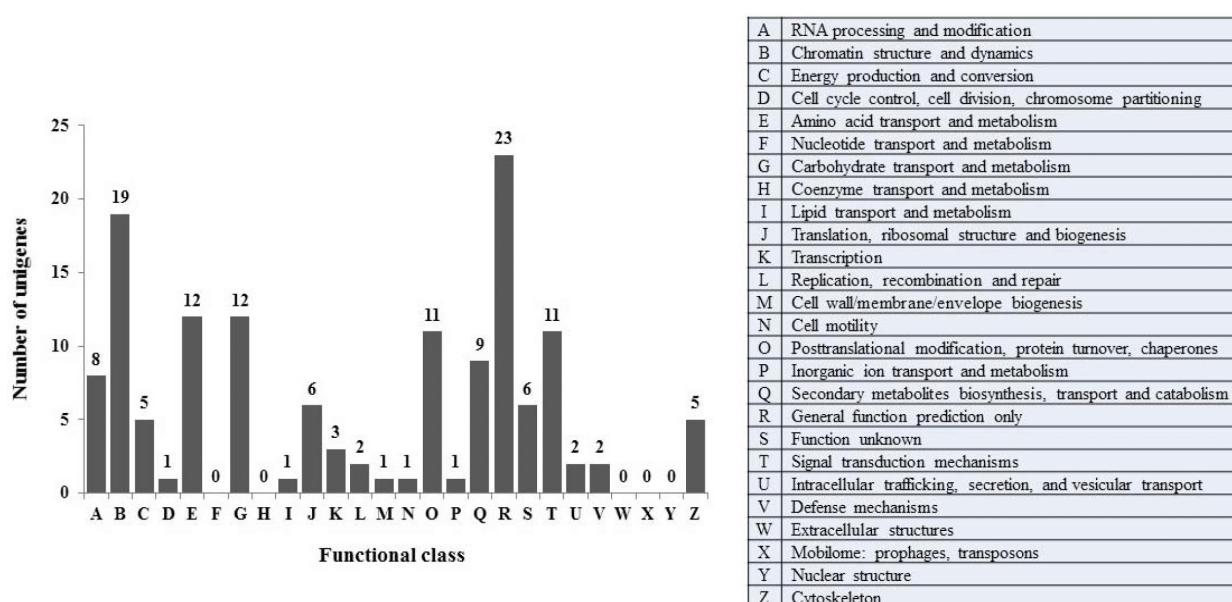


Fig. 2. Cluster of orthologous group. The orthologous clustering was done by the clusters of orthologous groups (COG) database (<http://www.ncbi.nlm.nih.gov/COG/>) and kyoto encyclopedia of genes and genomes (KEGG) database (<http://www.genome.jp/kegg>).

has been only studied for differential protein expression in *T. molitor* pupa parasitized by *Scleroderma guani* [8]. Therefore, we suggest that the data obtained in this study are a value as a reference for study of mealworm proteome in future.

Acknowledgement

This work was supported by the research invigoration program of 2020 Gyeongnam National University of Science

and Technology.

The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

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초록 : 갈색거저리 유래 저분자단백질체의 분석

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본 연구에서는 저분자펩타이드로부터 유래되는 단백질을 확인하기 위해 갈색거저리의 유충, 번데기, 성충의 저분자 단백질체 분석을 수행하였다. 저분자 펩타이드 분석으로부터 유래된 54 단백질이 최종적으로 확인되었다. 확인된 단백질 중 성체에만 존재하는 단백질이 가장 높은 빈도로 존재하였고, 그 다음은 성체와 유충에 동시 존재하는 단백질이 높은 빈도로 탐색되었다. 그러나 번데기를 포함하는 그룹들은 모두 낮은 빈도로 감지되었다. 분석된 단백질에 orthologous classification의 결과에서 일반적 기능 예견만(general function prediction only) 보이는 단백질이 가장 높은 빈도로 조사되었다. 크로마틴 구조와 동적상태(chromatin structure and dynamics)에 연관된 단백질은 비교적 높은 빈도로 탐색되었다. 또한, 아미노산 수송과 물질대사(amino acid transport and metabolism) 및 탄수화물 수송 및 물질대사(carbohydrate transport and metabolism)와 연관된 단백질도 높은 빈도로 분석되었다. 그러나 뉴클레오타이드 수송 물질대사, 코엔자임 수송 및 물질대사, 세포외 구조, 모빌로좀(mobilome), 및 핵 구조와 연관된 단백질은 전혀 탐지되지 않았다. 따라서 크로마틴, 아미노산, 탄수화물 물질대사와 연관된 단백질들이 보다 쉽게 저분자 펩타이드로 전환되어 체액 중에 잔존될 수 있는 것으로 보이며, 이들 펩타이드들이 생리활성물질로써 기능을 수행할 수 있을 가능성성이 높은 것으로 추정된다.