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Proteome identification of the silkworm middle silk gland

Jian-ying Li [a,b], Lu-peng Ye [b], Jia-qian Che [b], Jia Song [b], Zheng-ying You [b], Shao-hua Wang [b], Bo-xiong Zhong [b,*]

[a] Institute of Developmental and Regenerative Biology, Hangzhou Normal University, Hangzhou 310036, PR China
[b] College of Animal Sciences, Zhejiang University, Hangzhou 310029, PR China

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ABSTRACT

To investigate the functional differentiation among the anterior (A), middle (M), and posterior (P) regions of silkworm middle silk gland (MSG), their proteomes were characterized by shotgun LC–MS/MS analysis with a LTQ-Orbitrap mass spectrometer. To get better proteome identification and quantification, triplicate replicates of mass spectrometry analysis were performed for each sample. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (Vizcaíno et al., 2014) [1] via the PRIDE partner repository (Vizcaino, 2013) [2] with the dataset identifier PXD003371. The peptide identifications that were further processed by PeptideProphet program in Trans-Proteomic Pipeline (TPP) after database search with Mascot software were also available in .XML format files. Data presented here are related to a research article published in Journal of Proteomics by Li et al. (2015) [3].

Specifications Table

Subject area	*Biology*
More specific subject area	*Insect proteomics*

* Corresponding author. Tel./fax: +86 571 86971302.
E-mail address: bxzhong@zju.edu.cn (B.-x. Zhong).

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Type of data	*Excel data sheets with identified proteins and corresponding peptides from each analyzed sample.*
How data was acquired	Ettan MDLC nanoflow/capillary LC system (GE Healthcare, Pittsburgh, PA) coupled with a LTQ mass spectrometer (Thermo Fisher Scientific) with a nano-electrospray ionization (ESI) source.
Data format	Analyzed
Experimental factors	No sample pretreatment applied.
Experimental features	The sample proteomes were fractionated using 1D SDS-PAGE followed by tryptic digest. Digested peptides were fractionated using MDLC system prior to LC–MS/MS. Data mining of the acquired MS output was performed by bioinformatics analysis.
Data source location	*Hangzhou, China*
Data accessibility	Data are available with this article and related to [3].

Value of the data

- High-confidence proteome identifications of the silkworm middle silk gland.
- The identified tissue-specific proteins are valuable for understanding of the functional differentiation among different regions of middle silk gland.
- Label-free quantitation of the three regions of silkworm middle silk gland to determine their relative abundances.
- In-depth proteome comparison with posterior silk gland will contribute to better understanding of the mechanism of silk protein synthesis.

1. Data, experimental design, materials and methods

In order to disclose the mechanism of high efficient synthesis of silk proteins, in-depth proteomic analysis of the silkworm middle silk gland (MSG) was performed with shotgun LC–MS/MS. The silkworm MSGs at the 3rd day of the fifth instar were dissected and cut into anterior (MSG-A), middle (MSG-M), and posterior (MSG-P) sections. The proteins were separated by 1D SDS-PAGE followed by in-gel trypsin digestion. The digested peptides were analyzed using a Nano-LC–MS/MS system with a LTQ-Orbitrap mass spectrometer. The generated raw MS data were deposited to the ProteomeXchange Consortium [1] via the PRIDE partner repository [2] with the dataset identifier PXD003371. We finally identified 643, 594, and 823 proteins from the MSG-A, -M, and -P, respectively, with a FDR of lower than 0.5% [3]. The differential expression of proteins was analyzed with a label-free quantification method. The differentially expressed proteins were further subjected to functional enrichment analysis (Fig. 1).

1.1. Sample preparation

The silkworm MSGs at the 3rd day of the fifth instar were dissected in pre-cooled physiological saline under a dissecting microscope. Each MSG was cut into three sections at the two turnings. To remove the secreted sericin proteins in the gland lumen, the MSG sections were immersed in pre-cooled 60% ethanol for 1–2 min to stiffen the sericins and draw them out with nippers. The protein extraction protocol was according to the description in our previous articles [4,5]. Briefly, the tissues were mechanically homogenized on ice in lysis buffer that containing 2.5% SDS, 10% glycerin, 5% β-mercaptoethanol, and 62.5 mM Tris–HCl pH 6.8. The homogenate was further subjected to sonication treatment in an ice-bath. The protein concentration was determined by using the 2-D Quant Kit (Amersham Biosciences, Piscataway, NJ, USA) according to the manufacturer's instructions.