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A Novel Protein Found in Selenium-rich Silkworm Pupas

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Abstract: A novel protein was isolated and characterized in selenium-rich silkworm pupas. The peptide mass fingerprint of the protein was found to be new. Partial amino acid sequencing also confirmed to be a new protein. The novel protein had a molecular mass of about 80 kDa in the SDS-PAGE.

Keywords: Silkworm pupa, selenium, peptide mass fingerprint (PMF), amino acid sequence.

Selenium (Se) is well known for its cancer-preventive function[1]. In our previous work, silkworm pupas from Ziyang county of Shanxi Province, one of the two largest Se-rich areas in China, was found to have Se content 215 times higher than that from Luoyang which is a normal region in China[2]. Meanwhile, Se-rich amino acids extracted from Ziyang pupas could efficiently induce the apoptosis of human hepatoma cells SMMC-7721. In order to find out the effective component responsible for this carcinostatic activity, Se-rich proteins from Ziyang silkworm pupas were separated by tracing their Se contents and characterized through tryptic peptide mass fingerprint (PMF) analysis and partial amino acid sequencing. A novel protein was found in Ziyang silkworm pupas.

A portion of Se-rich silkworm pupas were homogenized with 30 mmol/L Tris-HCl buffer solution (pH 7.5) containing 2 mmol/L dithiothreitol (DTT), 1 mmol/L EDTA , 1 mmol/L $MgCl_2$, 0.1% Tween 20, and 1 mmol/L PMSF and centrifuged at 4℃ and 800 g for 15 min. The supernatant was lyophilized and the obtained solid was extracted with pre-cooled etheyl acetate at –20℃ for 10 h. The mixture was then centrifuged at 3000 g for 15 min and the pellet was vacuum-dried and extracted for another 12 h with 60 mmol/L Tris-HCl buffer (pH 7.5). After addition of pre-cooled acetone, the mixture was centrifuged and the clear supernatant was dialyzed against triple-distilled water before it was lyophilized.

For further purification[3,4], a portion of crude proteins was suspended in buffer A (30 mmol/L Tris-HCl, pH 7.5) and the solution was centrifuged at 4℃ under 15000 g for

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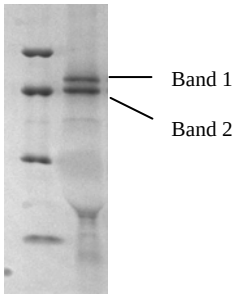
20 min. The supernatant was collected and applied onto a 2.6×20 cm DEAE Sepharose fast flow column and eluted with 0.005-0.6 mol/L sodium chloride linear gradient at a flow rate of 45 mL/h. Each protein peak monitored at a wavelength of 280 nm was collected for lyophilization. Se content in each peak was measured as reported previously[2]. Peak 4 was found to contain the highest Se and it was dissolved in buffer A and loaded onto a Sephedex G-75 column (1.6×80 cm). The column was eluted with buffer A at a flow rate of 30 mL/h. Protein peak 1 was found to have the highest containing of Se. The lyophilized peak 1 was stored at -80℃. Its purity was checked through SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (Hoefer Pharmacia Biotech, Sweden). In peak 1 two major proteins (band 1 and band 2 in **Figure 1)** were observed. The content of Se in protein increased after purification (**Table 1)**, indicating that these proteins are Se-containing proteins.

Figure 1 SDS-PAGE analysis on peak 1 proteins from Sephadex G-75 gel chromatography



1. Protein molecular markers (97, 66.2, 43, 31 kDa); 2. Proteins of peak 1 from Sephadex G-75 gel chromatography

Table 1 Selenium contents in Ziyang silkworm pupas and their protein fractions

Sample	Silkworm pupas	Crude proteins	Peak 4 from DEAE-Sepharose column	Peak 1 from Sephedex G-75 column
Se content （ng/mg）	6.67	15.1	27.0	37.9

In order to characterize the two major proteins shown on SDS-PAGE, they were cut off the gel and digested separately with trypsin for 20 h. The peptides were extracted with the solution of 0.1% TFA and 50% ACN, and then mixted with the matrix-solution containing 2, 5-dihydroxy benzoic acid (DHB) in 0.1% TFA and 50% ACN solution for peptide mass fingerprint (PMF) analysis on Reflex III MALDI-TOF-MS[5] (Bruker, Germany). The obtained data were analyzed and searched through Mascot on the net (http://www.matrixscience.com). The protein of band 2 on SDS-PAGE was identified to be a sex-specific storage-protein 2 precursor (match score: 217) in silkworm pupas with mass weight of 83412. The match score for the other major protein in silkworm pupas was very low, indicating that this protein is a new one, which does not exist in the

database up to now (**Figure 2**). The protein of band 2 showed a molecular mass (about 80 kDa) in the SDS-PAGE (**Figure 1**). To be sure that this is a new protein, we repeated the PMF experiment in another lab, and the result further confirmed that it is a new protein.

To further characterize the new protein, its partial sequences were determined through CapLC-ESI-MS/MS (Micromass, UK)[6]. The obtained data were also analyzed and searched through Mascot on the net. Six partial sequences of the new protein were listed in **Table 2**, which also suggested that they are new peptides that could not be found in the database at present. Is this new protein responsible for or related to the carcinostatic activity of Se-rich amino acids from Ziyang silkworm pupas? What is its biological function? It remains to be clarified.

Figure 2 The peptide mass fingerprint of the novel protein found in Ziyang Se-rich silkworm pupas

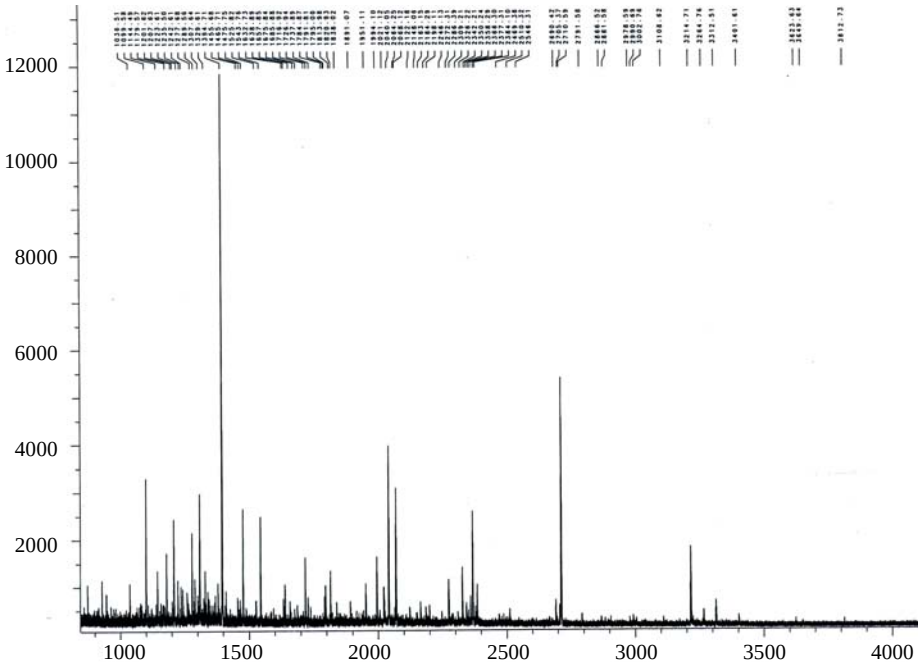


Table 2 Partial amino acid sequences of the novel protein found in Ziyang silkworm pupas

Peptide	Amino acid sequence
Peptide 1	PVDAAFVEK
Peptide 2	LPEFSWYSPLR
Peptide 3	PGQSQLTR
Peptide 4	TFFKYLQQLVEK
Peptide 5	PNAFYQLYK
Peptide 6	MFFLVSESVNHR

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