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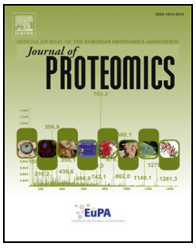

Comparative proteomic analysis reveals the suppressive effects of dietary high glucose on the midgut growth of silkworm

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ABSTRACT

The silkworm, *Bombyx mori*, is an important model of *lepidoptera* insect, and it has been used for several models of human diseases. In human being, long-term high-sugar diet can induce the occurrence of diabetes and other related diseases. Interestingly, our experiments revealed the high glucose diet also has a suppressive effect on the development of silkworms. To investigate the molecular mechanism by which high-glucose diet inhibited the midgut growth in silkworms, we employed comparative proteomic analysis to globally identify proteins differentially expressed in normal and high-glucose diet group silkworms. In all, 28 differently proteins were suppressed and 5 proteins induced in high-glucose diet group. Gene ontology analysis showed that most of these differently proteins are mainly involved in metabolic process, catalytic and cellular process. A development related protein, imaginal disk growth factor (IDGF), was further confirmed by western blot exclusively expressing in the normal diet group silkworms. Taken together, our data suggests that IDGF plays a critical role in impairing the development of silkworms by a high-glucose diet.

Biological significance

Glucose has been thought to play essential roles in growth and development of silkworm. In this paper, we certified firstly that high-glucose diet can suppress the growth of silkworm, and comparative proteomic was employed to reveal the inhibition mechanism. Moreover, an important regulation related protein (IDGF) was found to involve in this inhibition process. These results will help us get a deeper understanding of the relationship between diet and healthy. Furthermore, IDGF may be the critical protein for reducing the blood sugar in silkworm, and it may be used for screening human hypoglycemic drug. The work has not been submitted elsewhere for publication, in whole or in part, and all the authors have approved the manuscript.

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1. Introduction

Glucose, a simple dietary monosaccharide, is the primary source of energy for the body's cells. It can be absorbed directly into the bloodstream during digestion and the mean normal blood glucose level in humans is about 5.5 mM [1]. Long-term high-sugar diet can increase the blood glucose levels and induce type 2 diabetes in animal models [2]. Diabetes type 2 is a metabolic disorder that is characterized by high blood glucose levels in the context of insulin resistance and relative insulin deficiency [3]. In recent years, the relationship between gut microbiota and diet in the pathogenesis of diabetes has emerged into an important research area [4,5], and several researches have demonstrated some gut bacteria can relieve the symptoms of diabetes [6], or cause obesity and other chronic complication [7,8].

Like humans, silkworm has its own regulatory mechanisms of hemolymph sugar [9]. Trehalose, the main hemolymph sugar, is synthesized from glucose in the fat body and released into the hemolymph by the trehalose transporter [10], but it is surprising that the glucose is generally not detected in hemolymph [11]. The level of hemolymph sugar can be regulated by bombyxin, a peptide hormone with structural similarity to human insulin, but its physiological roles remain unclear [12]. With the deepening of this research, many scholars have found that injection of glucose can promote release of bombyxin into the hemolymph [13]. However, the growth of silkworm can be impaired with increase of glucose levels when they were fed a diet containing high glucose [11], and the reason is still unknown.

Diet has a major impact on the nutrition and metabolism of the intestinal tract especially for the intestinal flora, and many diseases are related with the changes of diet. Similarly, high glucose diet can also affect the normal development of silkworms [11]. This may imply that glucose in food is not only a nutrient, but also a signaling molecule to regulate the development of silkworm. The silkworm midgut is the primary site of food digestion and absorption, and it is the frontline where silkworm initiates its defense against external signal molecules, so many researchers used this tissue in their studies. In the present study, to globally identify proteins involved in impairing growth of silkworm, a group of hyperglycemic silkworms was constructed by feeding a high-glucose diet. Subsequently, proteomic method was employed to analyze midgut proteins from the silkworms fed with normal diet and high-glucose diet, respectively. Thirty-three proteins were found to be differentially expressed, among which imaginal disk growth factor (IDGF) was expressed exclusively in normal silkworms, but not in the hyperglycemic silkworms. Our data suggests that IDGF deficiency may be involved in impairing the development of hyperglycemic silkworms by a high-glucose diet.

2. Materials and methods

2.1. High-glucose diet preparation and silkworm rearing conditions

Silkworms, HNW strain, were kept in disposable plastic container at 25 ± 1 °C and with 75 ± 2% relative humidity. On the first day of fifth instar, 30 silkworms were reared on a normal diet from a domestic provider in China, which contains antibiotics. Another 30 larvae were reared on a 30% (*w*/*w*) glucose diet prepared by mixing the normal diet and D-glucose, as described by Matsumoto Y. et al. [11]. 72 h later, these larvae were dissected to obtain midgut and stored at −80 °C for later use.

2.2. Protein sample preparation

The silkworm midgut and other tissues (hemolymph, fat body, and silk gland) from the normal diet group (NDP) and high-glucose diet group (HDP) were grounded in liquid nitrogen with the homogenization buffer (20 mM Tris–HCl, pH 7.5, 250 mM sucrose, 10 mM EDTA, 1 mM PMSF, 1 mM β-mercaptoethanol and 1% (*v*/*v*) Triton X-100), as described by Cilia et al. [14]. Then the mixture was vortexed for 30 min and centrifuged 15,000 rpm for 20 min. The supernatant was added equal volume Tris-saturated phenol to precipitate proteins. The phenol layer containing proteins was collected, incubated with methanol solution (containing 100 mM ammonium acetate) and centrifuged 15,000 rpm for 20 min to pellet proteins. The pellet was washed with cold acetone (containing 1 mM DTT), lyophilized, dissolved in protein lysate containing 7 M urea, 2 M thiourea, 4% (*w*/*v*) chaps and 1% (*w*/*v*) DTT and centrifuged 15,000 rpm for 20 min. The supernatant, as the sample of total midgut protein, was pooled and stored at −80 °C for later use. The protein concentration was determined using RC DC™ (Bio-Rad, USA) kit.

2.3. Two-dimensional electrophoresis

Two-dimensional electrophoresis (2-DE) was performed with 17 cm (linear, pH 5–8) IPG gel strip (Bio-Rad, USA), as described by Liang et al. [15]. Total midgut protein (2500 μg) was loaded onto IPG strip using active rehydration (50 V for 13 h), and the isoelectric focusing (IEF) was performed at 17 °C with a voltage gradient of 250 V for 0.5 h, 1000 V for 1 h, 10,000 V for 5 h, and then continued for a total of 60 kVh. The IPG gel strip was equilibrated for 15 min with equilibration buffer (6 M urea, 0.375 M Tris–HCl, 20% (*v*/*v*) glycerol, 2% (*w*/*v*) SDS, 2% (*w*/*v*) DTT), then was equilibrated for another 15 min with the same equilibration buffer without DTT containing 2.5% (*w*/*v*) iodoacetamide. Equilibrated strip was sealed on the top of 12% SDS–PAGE gel for electrophoresis. The gel was visualized with 0.1% Coomassie brilliant blue (CBB) R-250 and scanned with a high precision scanner (ScanMaker 9700XL, Microtek) at a resolution of 600 dpi. Spot analysis was performed using PDQuest (version 8.0.1, Bio-Rad). Triplicate experiments were carried out for each sample. The intensity ratio of the corresponding spots in different gels was calculated, and the spots with a ratio of ≥2 or ≤0.5 were defined as quantitative different spots.

2.4. In-gel digestion and mass spectrometry analysis

In-gel digestion was performed as reported by Zhou et al. [16]. The protein spots were excised from the stained gels, washed twice in milli-Q water, destained by sonication in 25 mM