Suppressive effects of whey protein hydrolysate on sucrose-induced hyperglycemia in silkworms

Yasuhiko Matsumoto, Miki Takahashi, Masahiro Umehara, Masato Asano, Hiroko Maruki-Uchida, Minoru Morita, Kazuhisa Sekimizu

1 Department of Microbiology, Meiji Pharmaceutical University, Tokyo, Japan; 2 Teikyo University Institute of Medical Mycology, Tokyo, Japan; 3 Genome Pharmaceuticals Institute Co., Ltd., Tokyo, Japan; 4 Health Science Research Center, Research and Development Institute, Morinaga and Company Limited, Kanagawa, Japan.

1. Introduction

Sucrose is one of the main sweeteners added to various foods (1). Increased blood glucose levels due to excessive intake of sucrose induces the development of lifestyle-related diseases (2, 3). Therefore, the development of strategies to suppress postprandial hyperglycemia induced by sucrose intake will contribute to the maintenance of human health (4).

Blood glucose levels are regulated at various stages by the functions of several organs: enzymatic decomposition of sucrose in the intestine, absorption from the intestine, distribution and metabolism in various organs, and excretion outside the body (5). Therefore, experiments to accurately evaluate active substances that suppress sucrose-induced hyperglycemia must be performed using whole animals.

We previously reported diabetic silkworm models for evaluating substances that suppress postprandial hyperglycemia by oral administration. In this study, orally administered whey protein hydrolysate (WPH), obtained by enzymatic treatment of whey protein, suppressed sucrose-induced hyperglycemia in silkworms in a dose-dependent manner. WPH also inhibited glucose-induced hyperglycemia in silkworms. These findings suggest that WPH contains a bioactive peptide that inhibits glucose uptake from the intestinal tract and thereby suppresses sucrose-induced hyperglycemia.

Keywords: Hyperglycemia, silkworm, sucrose, whey protein hydrolysate

Summary

Silkworms are useful for evaluating substances that suppress postprandial hyperglycemia by oral administration. In this study, orally administered whey protein hydrolysate (WPH), obtained by enzymatic treatment of whey protein, suppressed sucrose-induced hyperglycemia in silkworms in a dose-dependent manner. WPH also inhibited glucose-induced hyperglycemia in silkworms. These findings suggest that WPH contains a bioactive peptide that inhibits glucose uptake from the intestinal tract and thereby suppresses sucrose-induced hyperglycemia.

2. Materials and Methods

2.1. Reagents

Whey protein and WPH, prepared by enzymatic
hydrolysis of whey protein, were purchased from Fonterra Japan (Tokyo, Japan). The protein content of the whey protein was 80% and that of the WPH was 83%. High-performance liquid chromatography (HPLC) grade acetonitrile (> 99.8%) and special grade trifluoroacetic acid (> 98.0%) were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan).

2.2. Size exclusion chromatography

Whey protein (10.0 mg) and WPH (9.6 mg) were suspended in ultrapure water (2.0 mL). The mixture was centrifuged (20,400g, 20°C, 10 min), and the supernatant was subjected to size exclusion chromatography using a TSKgel® G2000SWXL HPLC column (300 × 7.8 mm i.d., particle size: 5 μm, Tosoh Corporation, Tokyo, Japan) with a TSKgel® SW guard column (40 × 6.0 mm i.d., particle size: 7 μm, Tosoh Corporation). The analysis was performed with an HPLC system (autosampler: AS-2057, pump: PU-2080, column oven: CO-2065, PDA detector: MD-2018, JASCO Corporation, Tokyo, Japan). The mobile phase was acetonitrile/water/trifluoroacetic acid (25/75/0.1 v/v/v). The column oven temperature was set to 30°C and the flow rate was 0.6 mL/min. Absorbance at 280 nm was monitored. The data was analyzed using ChromNAV (version 1.18.04, JASCO Corporation).

2.3. Silkworm rearing conditions and sucrose tolerance test

Silkworms were reared as reported previously (6,18). Sucrose or glucose tolerance tests using the silkworms were performed according to a previous report (10). Briefly, test samples were mixed with artificial diet containing 10% glucose or 10% sucrose. The diet was fed to the silkworms for 1 h and the glucose level in the silkworm hemolymph was measured using a glucometer (Accu-Chek, Roche, Basel, Switzerland).

2.4. Statistical analysis

All experiments were performed at least twice. The significance of differences was calculated using a two-tailed Student’s t-test. A p value of less than 0.05 was considered significant.

3. Results

3.1. Characterization of whey protein and WPH by size exclusion chromatography

We compared the size exclusion chromatography patterns between whey protein and WPH. Each sample was subjected to size exclusion chromatography. The whey protein produced major peaks at elution times ranging from 11.5 to 13.5 min (Figure 1). The WPH, in contrast, did not have these two major peaks, and instead multiple peaks were observed at elution times ranging from 14 to 20 min (Figure 1). This finding suggests that the WPH comprised whey proteins digested into low molecular mass peptides.

3.2. Suppressive effect of WPH on sucrose-induced hyperglycemia in silkworms

When an artificial diet containing 10% sucrose is fed to silkworms for 1 h, the glucose level in the silkworm hemolymph increases to 300-400 mg/dL (10). In the present study, we investigated the effects of whey protein and WPH on the sucrose-induced hyperglycemia in silkworms. The blood glucose level was lower in silkworms fed the 10% sucrose diet supplemented with 10% WPH than in control silkworms (Figure 2). Supplementation with acarbose, an α-glycosidase inhibitor used as a positive control, also exhibited a suppressive effect (Figure 2). In contrast, when whey protein was added to the 10% sucrose diet, the blood glucose level of the silkworms did not decrease (Figure 2). We next examined the dose dependence of the inhibitory effect of WPH on sucrose-induced hyperglycemia in silkworms in a sucrose tolerance test. WPH in the range of 0-10% of the diet suppressed the sucrose-induced hyperglycemia in silkworms in a dose-dependent manner (Figure 3).

3.3. Suppressive effect of WPH on glucose-induced hyperglycemia in silkworms

To clarify the mechanism of the suppression of the
4. Discussion

The findings of the present study demonstrated that WPH inhibited sucrose-induced hyperglycemia in an in vivo evaluation system using silkworms, whereas whey protein did not have the same effect. Further, WPH suppressed glucose-induced hyperglycemia. This finding indicates that the anti-hyperglycemic effect of WPH cannot be explained by the inhibition of α-glycosidase. Sucrose in the intestine is degraded to glucose and fructose by α-glycosidase, and these monosaccharides are transferred into the blood via sugar transporters on the intestinal cells. WPH may inhibit the activity of sugar transporters responsible for the uptake of monosaccharides from the intestine into the blood. Another possibility is that WPH promotes the uptake of monosaccharides into the organs from the bloodstream.

Whey protein has an inhibitory effect on sweetened beverage-induced hyperglycemia in young adult females (15). We demonstrated that whey protein did not suppress sucrose-induced hyperglycemia in silkworms. In humans, whey protein may be degraded to WPH by proteases in the stomach and small intestine. In silkworms, whey protein may not be fully degraded to active WPH. Otherwise WPH may have a different hypoglycemic effect compared to whey protein, because it is hydrolyzed by enzyme and may have special peptides.

In conclusion, WPH has the potential to suppress postprandial hyperglycemia, but inhibition of α-glycosidase does not explain the suppressive activity of WPH. We suggest that the use of WPH in combination with α-glycosidase inhibitors might prevent the onset of life-related diseases such as diabetes and obesity.
Acknowledgements

We thank Kana Hashimoto and Mari Maeda (Genome Pharmaceuticals Institute Co., Ltd, Tokyo, Japan) for technical assistance in rearing the silkworms. The project was supported by JSPS KAKENHI grant number JP15H05783 (Scientific Research (S) to KS), JSPS KAKENHI grant number JP17K08288 (Scientific Research (C) to YM), and the Supporting Industry Program by Ministry of Economy, Trade and Industry. The project was also supported by Genome Pharmaceuticals Institute Co., Ltd. (Tokyo, Japan).

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(Received September 21, 2019; Accepted September 29, 2019)