PEPTIDES 29 (2008) 891–897

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journal homepage: www.elsevier.com/locate/peptides

The effect of pea albumin 1F on glucose metabolism in mice

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ARTICLE INFO

Article history:
Received 16 November 2007
Received in revised form
21 January 2008
Accepted 25 January 2008
Published on line 3 February 2008

Keywords:
Pea albumin 1F
Isoforms
Glucose metabolism
Voltage-dependent anion channel 1 (VDAC-1)

ABSTRACT

Pea albumin 1F (PA1F), a plant peptide isolated from pea seeds, can dramatically increase blood glucose concentration by subcutaneous injection with a dosage of 5 or 10 μg/g (body weight) in normal and type II diabetic mice (KK/upj-Ay). The voltage-dependent anion channel 1 (VDAC-1) has been identified as the PA1F binding protein from mice pancreatic cell membrane, which may be involved in the regulation of enhancing blood glucose in response to PA1F binding. The results clearly show that peptide-signaling molecules from plants can affect mammalian physiological functions, especially, in association with glucose metabolism.

1. Introduction

Extracts from various parts of the plant, such as roots, stems, leaves, flowers, fruits and seeds have been widely used in traditional and modern medicine for thousands of years. Most of the active extracts are alkaloids, flavonoids and saponins, but none of them have been characterized as plant peptide hormones due to the short history of plant signaling peptides, beginning from the discovery of systemin in 1991 [13]. In the past 15 years, several other plant peptidergic signal molecules have been reported, such as leginsulin, rapid alkalinization factor, phytosulfokines and ENOD40 [14]. However, all of the studies were focused on their functions in plant development and growth, and so far, no physiological effects of plant derived signal peptides have been reported in mammals.

Previously [3], we have described the isolation of a bioactive peptide from porcine intestine named Aglycin. Subcutaneous administration of this small peptide increases blood glucose concentration in mice. Binding of Aglycin to voltage-dependent anion channel 1 (VDAC-1) in the porcine pancreas is believed to mediate this biological response. Recent attempts, using bioinformatics and the available genome information, have not resulted in the identification of the gene(s) coding for Aglycin. However, a search for the Aglycin amino acid

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Abbreviations: MALDI-TOF-MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; PA1A-F, pea albumin 1A-F; PMSF, phenylmethylsulphonylfluoride; RP-HPLC, reversed phase-high-performance liquid chromatography; 7S Bg, basic 7S globulin; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TFA, trifluoroacetic acid; VDAC-1, voltage-dependent anion-selective channel protein 1.

0196-9781/$ – see front matter
doi:10.1016/j.peptides.2008.01.013

sequence in the Swiss-Prot database revealed that it is identical to one of the pea albumin isoforms named PA1B [7] and that PA1B has high stability to resist protease hydrolysis [16]. This raised the possibility that Aglycin isolated from porcine intestine is of exogenous origin from legume food sources. As expected, purified PA1B was found to have the same effect on enhancing blood glucose concentration in mice [3]. So far, six PA1B isoforms have been reported (PA1A-F, Swiss-Prot entries P62926–62931, respectively). To investigate whether other isoforms have the same function as Aglycin/PA1B do in mammals, we took advantage of PA1B resisting protease hydrolysis to identify all the isoforms, and one isoform PA1F, was purified for investigating its activity in mice.

2. Materials and methods

2.1. Polypeptides extraction

2 kg of pea seeds (*Pisum sativum* L.) were soaked in water for 12 h at room temperature and subsequently homogenized and incubated in 25 l precooled 0.5 M acetic acid (containing 0.1% beta-mercaptoethanol, v/v) for 12 h at 4 °C. After centrifugation at 7000 rpm for 25 min at 4 °C, the peptides contained in the supernatant was adsorbed to alginic acid at pH 2.7, followed by elution of the peptides with 0.2 M HCl from the alginic acid. With the above eluate, after adjusting the pH to 3.5 with sodium acetate, the peptides were precipitated by saturated NaCl (320 g/l) at 4 °C for 12 h and a precipitate was collected by suction filtration. The precipitate was dissolved in water (0.1 g/ml), after disposing of the water insoluble fraction by centrifugation at 12,000 rpm for 15 min at 4 °C, the supernatant was adjusted to water:acetone to 1:4 (v/v) and further extracted at −20 °C for 12 h, followed by centrifugation at 12,000 rpm for 15 min at −4 °C. Finally, the supernatant was concentrated with rotary devaporizer and lyophilized.

2.2. Identification and structural analysis of PA1B isoforms

250 μg lyophilized material was dissolved in 200 μl water (adjusted to pH 2.0 with 1 M HCl) and 50 μl pepsin (1 μg/μl) was added into the reaction mixtures, another 250 μg lyophilized material without pepsin acted as control. After 12 h incubation at 37 °C, the reaction mixtures were subjected to RP-HPLC analysis using an Agilent 1100 system (Agilent Technologies, Wilmington, DE, USA) fitted with a Zorbax C_{18} column (4.6 mm × 150 mm, 5 μm particles). Eluent A was 0.1% trifluoroacetic acid (TFA) in water, and eluent B, 0.1% TFA in acetonitrile. A linear gradient of 10–60% eluent B in 50 min (1 ml/min) was employed. The undigested peaks absorbing at 214 nm were collected and lyophilized, respectively.

Molecular masses of each undigested peak recovered from RP-HPLC were determined using MALDI-TOF-MS in an Applied Biosystems Voyager 4307 instrument (Foster City, CA, USA), using α-cyano-4-hydroxycinnamic acid at 10 mg/ml (dissolved in 70% acetonitrile, 0.1% TFA) as matrix. Edman degradation was carried out without prior reduction and alkylation of the sample in an Applied Biosystems Procise HT instrument. Cysteine residues were indirectly identified by the presence of gaps in the otherwise clearly interpretable sequence. Computer searches of peptide sequences were performed in the Swiss-Prot and TrEMBL databases and theoretical molecular mass were calculated with Antheprot 5.0.

For high throughput preparation of PA1F, the lyophilized materials were separated on a TSK ODS-120T column (7.8 mm × 300 mm, 10 μm particles), at a flow rate of 2.8 ml/min. The peak corresponding to PA1F was collected and lyophilized for bioactivity assay.

2.3. Detection of PA1F binding protein

The same procedure was used to detect the PA1F binding protein as described in Ref. [3] for Aglycin binding protein purification and characterization, except that 5 g fresh mice pancreas was used to instead of porcine pancreas and PA1F was coupled to CNBr-activated Sepharose 4B (Amersham Pharmacia Biotech, Uppsala, Sweden) instead of Aglycin. After affinity chromatography, SDS-PAGE separation and in gel tryptic digestion, peptide mass fingerprinting of PA1F binding protein was determine by MALDI-TOF-MS and submitted to database searches using the Mascot software (http://www.matrixscience.com).

2.4. Biosensor analysis

To verify the interaction between the affinity purified protein and PA1F, Spreeta biosensor and software (American TI Corp., Attleboro, MA, USA) was used immediately after the affinity chromatography. Briefly, PA1F was immobilized onto the surface plasmon resonance sensor chip according to the manufacturer's protocol. The efficiency of immobilization was evaluated by atomic force microscopy using a NanoIIIa instrument (Digital Instrument Company, Santa Barbara, CA, USA). 1 ml eluted fractions from each peak (Fig. 2) in the affinity chromatography were diluted one time with HEPES-buffered saline (HBS; 10 mM HEPES, pH 7.4, 0.15 M NaCl), then transferred them into the sample tubes and injected continuously over the sensor chip surface at a flow rate of 20 μl/min for 2–3 min at 25 °C. After each injection, the sensor chip was thoroughly washed with HBS containing 0.05% Triton X-100. BSA was used as control to show it cannot specifically bind with PA1F. Binding interactions were continuously monitored and plotted as refractive index vs. time and displayed in a sensorgram.

2.5. Bioactivity

C57BL/6 mice (18–20 g, $n = 200$), and type II diabetic mice (KK/upj-Ay, 30–35 g, $n = 80$, in diabetic condition) were obtained from the animal center of China Medical College (Beijing). 120 C57BL/6 mice were used to produce type I diabetes mouse model with streptozotocin as described in Ref. [17] and 80 mice in a diabetic situation were selected. All mice were fasted for 8 h and initial blood glucose concentrations were determined using Accu-Chek advantage blood glucose monitor (Roche Diagnostics, Basel, Switzerland; maximum limit: 33.3 mmol/l). Based on these basal glucose concentrations, each type of mouse ($n = 80$) model was equally divided into four subgroups