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Identification and characterization of a dipeptidyl peptidase IV inhibitor from aronia juice

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ABSTRACT

Aronia berries have many potential effects on health, including an antioxidant effect, effect for antimutagenesis, hepatoprotection and cardioprotection, an antidiabetic effect and inhibition of cancer cell proliferation. Previous human studies have shown that aronia juice may be useful for treatment of obesity disorders. In this study, we found that aronia juice has an inhibitory effect against dipeptidyl peptidase IV (DPP IV) (EC 3.4.14.5). DPP IV is a peptidase that cleaves the N-terminal region of incretins such as glucagon-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). Inactivation of incretins by DPP IV induces reduction of insulin secretion. Furthermore, we identified that cyanidin 3, 5-diglucoside as the DPP IV inhibitor in aronia juice. DPP IV was inhibited more strongly by cyanidin 3, 5-diglucoside than by cyanidin and cyanidin 3-glucoside. The results suggest that DPP IV is inhibited by cyanidin 3, 5-diglucoside present in aronia juice. The antidiabetic effect of aronia juice may be mediated through DPP IV inhibition by cyanidin 3, 5-diglucoside.

1. Introduction

Aronia berries have various potential health effects, including an antioxidant effect by radical scavenging activity, antimutagenesis by phenolic compounds, hepatoprotection by anthocyanins, which decrease the toxicity and accumulation of cadmium, cardioprotection in men with mild hypercholesterolaemia, antidiabetic effect, and inhibition of colon cancer cell proliferation [1]. Aronia juice has been shown to have a beneficial effect on plasma glucose level in diabetic humans [2] and rats [3]. However, its mechanism is unknown.

Dipeptidyl peptidase IV (DPP IV) (EC 3.4.14.5) is a serine peptidase [4] that cleaves the N-terminal region of incretins such as glucagon-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), and reduction of insulin secretion is induced by inactivation of incretin by DPP IV [5–8]. DPP IV inhibitors have beneficial effects on plasma glucose level in diabetic patients [9]. DPP IV inhibitors have also been found in several plants [10].

In this study, we found that aronia juice has an inhibitory effect on DPP IV. Furthermore, we identified cyanidin 3, 5-diglucoside as the DPP IV inhibitor in aronia juice. DPP IV was inhibited more strongly by cyanidin 3, 5-diglucoside than by cyanidin and cyanidin 3-glucoside. The antidiabetic effect of aronia juice may be mediated through DPP IV inhibition by cyanidin 3, 5-diglucoside.

2. Materials and methods

2.1. Materials

Aronia juice was kindly provided by Nakagaki Consulting Engineer (Osaka, Japan). Gly-Pro-MCA was purchased from Peptide Institute (Osaka, Japan). DPP IV was purified from porcine seminal

Abbreviations: DPP IV, dipeptidyl peptidase IV; GIP, glucagon-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; MCA, methylcumarin amide.

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plasma [4]. Supel Sphere Carbon/NH_2 SPE Cartridge, InertSustain C18 column and ACQUITY UPLC M-Class HSS T3 column were obtained from SUPELCO (PA, USA), GL Sciences (Tokyo, Japan) and Waters (MA, USA), respectively. Cyanidin and cyaniding 3-glucoside were purchased from TOKIWA PHYTOCHEMICAL (Chiba, Japan) and EXTRASYNTHESE (Cedex, France), respectively. Cyanidin 3, 5-diglucoside was obtained from Sigma–Aldrich (MO, USA). All other chemicals were of analytical grade and purchased from Wako Pure Chemicals (Osaka, Japan).

2.2. Assay of proteolytic activity

Enzyme activity was measured by fluorometrical determination (excitation, 380 nm; emission, 440 nm) of the liberation of AMC at 37 °C in a mixture containing 10 μl of 10 mM substrate, 100 μl of 0.5 M Tris–HCl buffer (pH 9.0), 5 μl of enzyme solution, and Milli Q water (18 mΩ) in a total volume of 1 ml. After incubation for 30 min, 2 ml of 0.2 M acetic acid was added to the mixture to terminate the reaction.

2.3. Identification of a DPP IV inhibitor

All fractionation steps were performed at room temperature unless otherwise specified. At each step, the inhibitory activity of DPP IV was measured in 50 mM Tris–HCl buffer (pH 9.0) using Gly-Pro-MCA as a substrate.

Step 1 Supel Sphere Carbon/NH_2 SPE chromatography

Aronia juice was applied at a flow rate of 5 ml/h to a Supel Sphere Carbon/NH_2 SPE Cartridge (bed volume: 6 ml) that had been previously equilibrated with 50 mM phosphate buffer (pH 7.0). After pass-through fractions had been collected, the column was washed extensively with ethanol and then eluted with a stepwise gradient of 50 mM phosphate buffer (pH 7.0) containing 2.0 M NaCl. Fractions with DPP IV inhibitory activity were subjected to the next step.

Step 2 Reversed-phase column chromatography

The sample solutions were subjected to reversed-phase HPLC on an InertSustain C18 column (4.6 × 150 mm) using a 0–100% acetonitrile/0.1% TFA gradient at a flow rate of 1.0 ml/min. Each peak was evaporated, and peaks containing DPP IV inhibitory activity were subjected to the next step.

Step 3 LC–MS/MS analysis

The sample solutions were subjected to an ACQUITY UPLC M-Class HSS T3 column (75 μm × 150 mm) using an ACQUITY UPLC M-Class system at a flow rate of 5 μl/min. MS/MS experiments were performed using a Xevo G2 QTof (Waters, MA, USA) with an ESI source. MassLynx v4.1 software was used for instrument control and data acquisition. The capillary temperature was 120 °C, and the capillary voltage was 3 kV. Mass spectra were recorded between m/z 100 and 1000 in the positive ion mode.

2.4. Statistical analysis

Data are expressed as means ± S.E. Statistical analyses were performed using analysis of variance (one-way ANOVA) followed

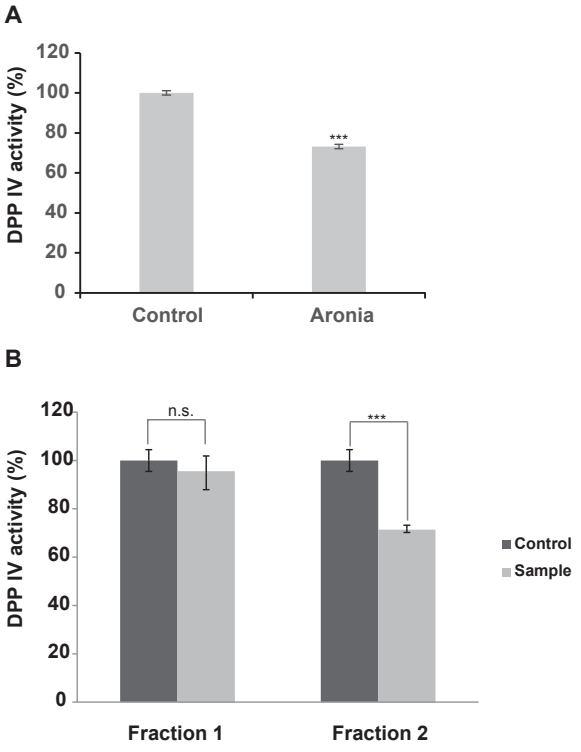

Fig. 1. Inhibition of DPP IV by aronia juice. A. DPP IV inhibitory activity by aronia juice was measured using a synthetic substrate, Gly-Pro-MCA. Values are means ± S.E. n = 4 experiments. Statistically significant: $^{***}p < 0.001$. B. DPP IV inhibitory activities in fractions from Spelco column chromatography were measured using a synthetic substrate, Gly-Pro-MCA. Values are means ± S.E. n = 4 experiments. Statistically significant: $^{***}p < 0.001$. Not significant: n.s.

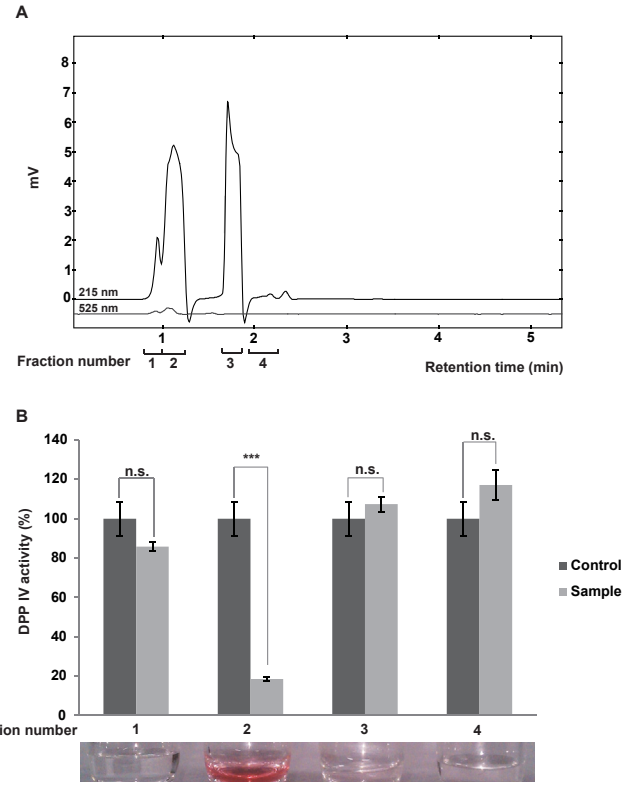

Fig. 2. Separation of the DPP IV inhibitory fraction through column chromatography. A. Separation of a DPP IV inhibitor using RP-HPLC. Four fractions were obtained. B. Enzyme activities the fractions were measured. Fraction number 2 had inhibitory activity against DPP IV, and its visible colour was shown to be red. Values are means ± S.E. n = 4 experiments. Statistically significant: $^{***}p < 0.001$. Not significant: n.s.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)