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Sweet potato (*Ipomoea batatas* (L.) Lam. 'Tainong 57') storage root mucilage exhibited angiotensin converting enzyme inhibitory activity *in vitro*

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ABSTRACT. Sweet potato (*Ipomoea batatas* (L.) Lam. 'Tainong 57') storage root mucilage was extracted (crude mucilage) and further purified by SDS and heating treatment (purified mucilage). Purified mucilage treated with 2-mercaptoethanol moved as a single band in SDS-PAGE. This purified mucilage was active to inhibit angiotensin converting enzyme (ACE) as shown by spectrophotometric method in a dose-dependent manner (28.7 to 59.8% ACE inhibition, respectively, by 50 to 400 μg/mL mucilage) using (*N*-(3-[2-furyl] acryloyl)-Phe-Gly-Gly) (FAPGG) as a substrate. The concentration of mucilage required for 50% inhibition (IC_{50}) of ACE activity was 364.5 μg/mL while that of captopril was 10 nM (8.68 μg/mL). The commercial polysaccharide pectin (50 to 400 μg/mL) showed no inhibitory activity against ACE. When using fluorescent silica TLC to detect FAPGG and FAP, the results also showed that mucilage inhibited ACE. The mucilage showed mixed type inhibition against ACE, and the Michaelis constant in the presence of mucilage was 12 mM. We suggest that consumption of sweet potato storage root mucilage may benefit people's health.

Keywords: Angiotensin converting enzyme (ACE); Mucilage; Sweet potato.

INTRODUCTION

Many bioactive peptides have common structural properties that include a relatively short length of peptide residues (e.g. 2-9 amino acids), possessing hydrophobic amino acid residues in addition to proline, lysine or arginine groups. Bioactive peptides are among the many functional components identified in foods. These are small protein fragments that have biological effects once they are released during gastrointestinal digestion in the organism or by previous in vitro protein hydrolysis. Bioactive peptides with immunostimulating (Parker et al., 1984; Fiat et al., 1993), opioid (Zioudrou et al., 1979), antithrombotic (Scarborough et al., 1991), caseino-phosphopeptic (Maubois and Leonil, 1989; Fox and Mulvihill, 1993), bactericidal (Bellamy et al., 1993), antioxidant or angiotensin-converting enzyme inhibitor (Ehlers and Riordan, 1989; Ariyoshi, 1993; Huang et al., 2004a) functions have been the focus of research in recent years.

ACE (peptidyldipeptide hydrolyase EC 3.4.15.1) is a glycoprotein and a dipeptide-liberating exopeptidase classically associated with the renin-angiotensin system regulating peripheral blood pressure (Mullally et al., 1996). ACE removes a dipeptide from the C-terminus of angiotensin I to form angiotensin II, a very hypertensive compound. Several endogenous peptides, such as enkephalins, β-endorphin, and substance P, were reported to be competitive substrates and inhibitors of ACE (Mullally et al., 1996). Several food-derived peptides also inhibited ACE, including α-lactalbumin and β-lactoglobulin (Pihlanto-Leppälä et al., 1998), casein (Maruyama et al., 1987), zein (Yano et al., 1996), and gelatin (Chen et al., 1999; Kim et al., 2001). Several antioxidant peptides (reduced glutathione and carnosine-related peptides) (Hou et al., 2003) and synthetic peptides also exhibited ACE I activities (Chen et al., 2003).

The major components of plant mucilage are pectins. Pectins are largely acidic polysaccharides that form gels in the extracellular matrix and are present in all cell walls. The two most common pectins found in dicotyledonous plants are polygalacturonic acid (PGA) and rhamnogalacturonan I (RG I) (Brett and Waldron, 1990; Carpita and Gibeaut, 1993; Cosgrove, 1997). PGA is an unbranched chain of α-1,4-linked galacturonic acid (GalUA) residues, while RG I is a highly substituted, branched polysaccharide with a backbone of alternating α-1,4-linked GalUA and α-1,2-linked rhamnose (Brett and Waldron, 1990). There are reports concerning the

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physiological activities of pectins on the interactions between fibroblast growth factors and receptors (Liu et al., 2001), on the modulation of lung colonization of B16-F1 melanoma cell (Platt and Raz, 1992), and on the inhibition of human cancer cell growth and metastasis in nude mice (Nangia-Makker et al., 2002). Pectin diets could also reduce the incidence of colon cancer in rats (Hardman and Cameron, 1995). Yam (*Dioscorea batatas*, Dioscoreaceae) is a major tuber crop and its mucilages are mainly composed of mannan-protein macromolecules with antioxidant activities and angiotensin converting enzyme inhibitory activities (Tsai, and Tai, 1984; Hou et al., 2001; Hou et al., 2002; Hou et al., 2003, Lee et al., 2003). The mucilage from sweet potato storage roots has antioxidant activities against both hydroxyl and peroxyl radicals (Huang et al., 2005).

No report concerning mucilage of sweet potato on the ACE activities is presently available. In this work we report for the first time that purified mucilage from sweet potato displayed ACE inhibitor activity in comparison with captopril serving as a positive control and commercial pectin as a negative control in a series of *in vitro* tests.

MATERIALS AND METHODS

Materials

Tris, electrophoretic reagents, and silica gel 60 F254 were purchased from E. Merck Inc. (Darmstadt, Germany); Captopril was purchased from Calbiochem Co. (CA, USA); Seeblue prestained markers for SDS-PAGE including myosin (250 kDa), BSA (98 kDa), glutamic dehydrogenase (64 kDa), alcohol dehydrogenase (50 kDa), carbonic anhydrase (36 kDa), myoglobin (30 kDa), and lysozyme (16 kDa) were from Invitrogen (Groningen, The Netherlands); FAPGG, ACE (I unit/mL, rabbit lung), nonylamine, pectin (from citrus fruit, degree of esterification 94%), coomassie brilliant blue R-250, and other chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Mucilage extraction and purification

Mucilage extraction and purification were done according to the method of Hou et al. (2002). Fresh sweet potato (*Ipomoea batatas* (L.) Lam. 'Tainong 57') storage roots were purchased from a local market. After cleaned with water, the storage roots were cut into strips for crude mucilage extraction. Sweet potato strips were homogenized with two volumes (w/v) of 50 mM Tris-HCl buffer (pH 8.3) containing 1% vitamin C. After centrifugation at 14,000 xg for 30 min, the supernatants were mixed with isopropanol to a final concentration of 70 %, and stirred quickly at 4°C overnight. The precipitates were filtered and dehydrated with 100% isopropanol, then rinsed with acetone. After drying at 40°C in an oven, the crude mucilage was ground and collected for further purification by both SDS and heating procedures. About 1.0 g crude mucilage powder was dissolved in 200 mL distilled water and kept in a 50°C water bath. Forty mL of 5% SDS solution (dissolved in 45% ethanol) were added to the crude mucilage solution. The mixture was kept with gentle stirring at 50°C for 30 min, then at room temperature for another 2 h. After that, the mucilage solution was placed in an ice bath to quickly lower the temperature in order to precipitate the SDS-protein complex. After centrifugation at 14,000 xg at 0 °C for 30 min, the supernatants were precipitated with isopropanol and dried at 40°C in an oven as described earlier. The semi-purified mucilage was again ground, dissolved, and then heated in boiling water for 20 min. After centrifugation at 14,000 xg at 0°C for 30 min, the supernatants were mixed with isopropanol to a final concentration of 70%. The purified mucilage was filtered, dehydrated, rinsed with acetone, dried, and then collected for further uses.

Protein and PAS stainings on 10% SDS-PAGE gels

Samples were mixed with sample buffer, namely 60 mM Tris-HCl buffer (pH 6.8) containing 2% SDS, 25% glycerol, and 0.1% bromophenol blue with 2-mercaptoethanol, and subjected to electrophoresis according to the method of Laemmli (1970). Coomassie brilliant blue G-250 was used for protein staining (Huang et al., 2004b). Periodic acid-Schiff (PAS) staining was used for oligosaccharide staining. After electrophoresis the gel was placed in fixative (7.5% acetic acid), shaken gently for 60 min. To oxidize the oligosaccharides the gel was treated with 0.2% periodic acid for 45 min at 4°C. The gel was washed with distilled water and stained with Schiff's reagent in dark at 4°C (Deepak et al., 2003).

Determination of ACE inhibitory activity by spectrophotometry

The ACE inhibitory activity was measured according to the method of Holmquist et al. (1979) with some modifications. Four microliters (4 microunits) of commercial ACE (1 unit/mL, rabbit lung; Sigma Chemical Co.) was mixed with 50 μL of different amounts of mucilage and commercial pectin (50, 100, 200 and 400 μg), and then 200 μL of 5×10^{-4} M *N*-[3-(2-*f*uryl) acryloyl]-*P*he-*G*ly-*G*ly [FAPGG, dissolved in 50 mM Tris-HCl buffer (pH 7.5) containing 0.3 M NaCl] was added. The decreased absorbance at 345 nm (ΔA inhibitor) was recorded during 5 min at room temperature. Deionized water was used instead of sample solution for blank experiments (ΔA blank). Captopril (molecular mass 217.3 Da) was used as a positive control for ACE inhibitor (1.25, 2.5, 5, 10, 20, 40, and 80 nM). The ACE activity was expressed as ΔA 345 nm, and the ACE inhibition (percent) was calculated as follows: [1 - (ΔA inhibitor /ΔA control)] × 100. Means of triplicates were determined. The 50% inhibition (IC50) of ACE activity was calculated as the concentrations of samples that inhibited 50% of ACE activity under these conditions.

Determination of ACE inhibitory activity by TLC

The ACE inhibitory activity of mucilage was determined by the TLC method (Holmquist et al., 1979). The reactions between mucilage and ACE or pectin and ACE were according to the methods of Anzenbacherova et al. (2001) with some modifications. Each 100 µL of mucilage and pectin (250 µg) was premixed with 15 microunits ACE for 1 min, and then 200 µL of 5 × 10^{-4} M FAPGG was added and allowed to react at room temperature for 10 min. Then 800 µL of methanol was added to stop the reaction. The blank experiment contained FAPGG only; in the control experiment, ACE reacted with FAPGG under the same conditions. Each was dried under reduced pressure and redissolved with 400 µL of methanol, and 50 µL was spotted on a silica gel 60 F254. The FAPGG and FAP (ACE hydrolyzed products) were separated by TLC in butanol - acetic acid-water, 4:1:1 (v/v/v), and observed under UV light.

Determination of the kinetic properties of ACE inhibition by mucilage

The kinetic properties of ACE (4 mU) without or with purified mucilage (200 µg) in total volume of 250 µL were determined using different concentrations of FAPGG as substrates (0.1 mM to 0.5 mM). The Michaelis constants Km (without mucilage) and Km′ (with 200 µg/mL mucilage) were calculated from Lineweaver-Burk plots.

RESULTS AND DISCUSSION

Extraction and purification of mucilage from sweet potato storage roots

The crude mucilage was obtained by isopropanol precipitation and the yield was about 1.5%. For further purification, it was first treated by SDS, which binds to proteins, and the complexes formed were removed by centrifugation. The semi-purified mucilage was then heated in boiling water for 20 min. The total recovery of purified mucilage after the two purification steps was about 24% that of the crude one. After drying at 40°C in an oven, the purified mucilage was ground and then collected for further uses (Figure 1).

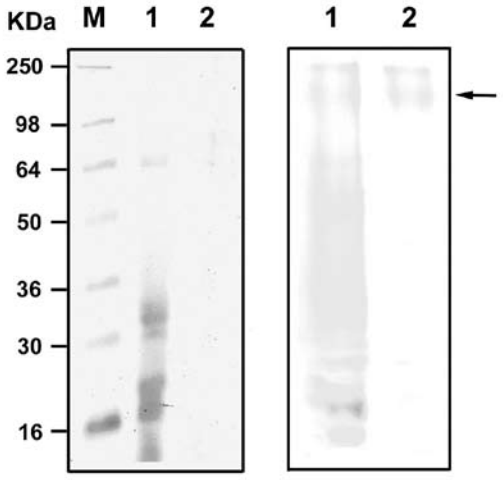

Figure 1. The protein (A) and PAS (B) stainings of the crude (lane 1) and purified (lane 2) mucilage from sweet potato storage root on SDS-PAGE gels after 2-mercaptoethanol treatment. The gel system contained 2.5 cm, 4% stacking gel and 4.5 cm, 15% separating gel. M indicates the Seeblue™ prestained markers of SDS-PAGE. The mucilage of 10 µg was loaded in each well. Arrow indicates the position of the mucilage.

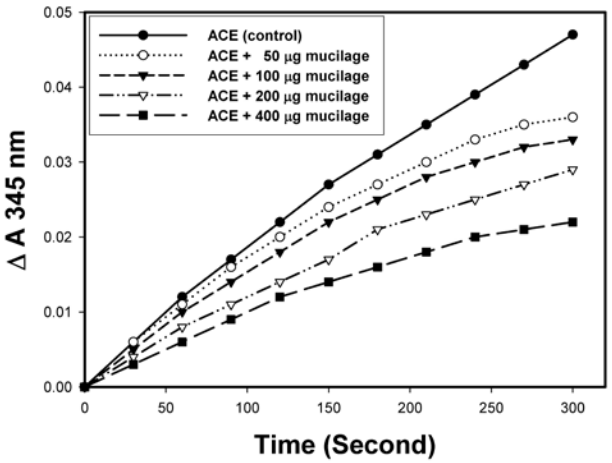

Figure 2. Dosage effect of the mucilage (50, 100, 200, and 400 µg) from sweet potato storage root on the ACE activity (Δ*A* 345 nm).

Determination of ACE inhibitory activity of mucilage by spectrophotometry

The purified mucilage was used for determinations of ACE inhibitory activity. Figure 2 shows the dose effect of mucilage (50, 100, 200, and 400 µg) on the ACE activity (Δ*A* 345 nm). Each line shows the time course of mucilage effect in 5 min. Compared with the ACE only (control), it was found that the higher the amount of mucilage added, the lower the Δ*A* 345 nm found during 300 sec ACE inhibitory reactions (Figure 2).

Effects of mucilage, pectin and captopril on ACE activity by spectrophotometry

From Figure 2, it was found that mucilage exhibited ACE inhibitory activity. It was interesting to know whether commercial pectin also exhibited the ACE inhibitory activity. Figure 3A shows the effects of mucilage (50, 100, 200, and 400 µg), commercial pectin (50, 100, 200, and 400 µg) and captopril (Figure 3B; 1.25, 2.5, 5, 10, 20, 40 and 80 nM) on ACE activity. It was found that commercial pectin showed less ACE inhibitory activity (less than 10% inhibition) and no dose-dependent inhibition patterns. However, mucilage exhibited dose-dependent ACE inhibitory activities (50~400 µg respectively, 23.4~53.2% inhibitions). From calculations, the 50% inhibition (IC_{50}) of ACE activity was 364.5 µg/mL mucilage compared to that of 10 nM (8.68 µg/mL) for captopril, which was similar to the report

(7 nM) of Pihlanto-Leppälä et al. (1998). And the IC_{50} of yam mucilage in inhibiting ACE activity was 256.2 μg/mL (Lee et al., 2003). Both pectin and purified mucilage were macromolecules, but only the purified mucilage showed special dose-dependent ACE inhibitory activity. In the literature, the protein hydrolysates were used as sources for purification of peptides as ACE inhibitors (Maruyama et al., 1987; Mullally et al., 1996; Yano et al., 1996; Chen et al., 1999; Kim et al., 2001; Chen et al., 2003). From calculations, the IC_{50} of mucilage for inhibiting ACE activity was 364.5 μg/mL, which was close to those of the synthetic peptides α-lactorphin (YGLF, 322.7 μg/mL) and *β*-lactoglobulin hydrolysates (GLDIQK, 391 μg/mL).

Determinations of ACE inhibitory Activity of Mucilage by TLC

The FAPGG and FAP (product of ACE catalyzed hydrolysis reaction) were separated by TLC using water saturated 1-butanol:acetic acid:water, 4:1:1 (V/V/V) as developing solvents according to the methods of Holmquist et al. (1979). Figure 4 shows the qualitative results of TLC chromatograms of a silica gel 60 F254 showing the effects of 250 μg of commercial pectin

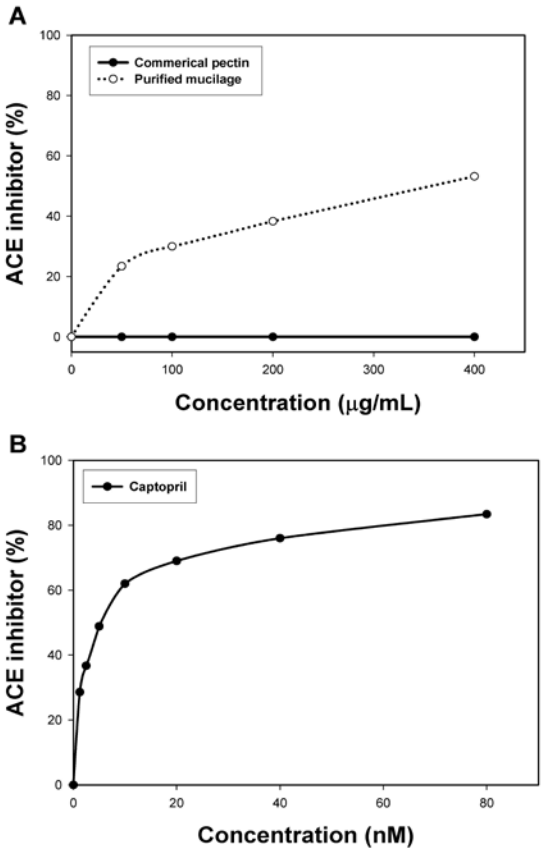

Figure 3. The effects of the purified mucilage, commercial pectin, and captopril on ACE activity as determined by spectrophotometry. (A) Purified mucilage (50, 100, 200, and 400 μg/mL) or commercial pectin (50, 100, 200, and 400 μg/mL); (B) Captopril (1.25, 2.5, 5, 10, 20, 40, and 80 nM). The inhibition of ACE (%) was calculated according to the equation [(1-(ΔA inhibitor) ÷ ΔA control)] × 100 %.

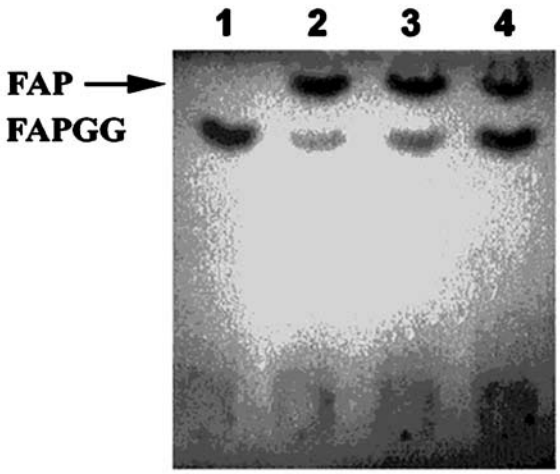

Figure 4. The TLC chromatograms of a silica gel 60 F254 showing the effects of the mucilage from sweet potato storage root or commercial pectin on ACE activity. Lane 1, blank test (FAPGG only); lane 2, control test (ACE reacted with FAPGG to produce FAP); lane 3, control test plus 250 μg commercial pectin; lane 4, control test plus 250 μg mucilage. Each solution was dried under reduced pressure and redissolved with 400 μL methanol. Each 50 μL was spotted on a silica gel 60 F254. The FAPGG and FAP were separated by water saturated 1-butanol : acetic acid : water, 4:1:1 (V/V/ V). Arrow indicated the position of FAP.

(lane 3) or mucilage (lane 4) on 15 microunits of ACE. When compared to the control test (lane 2), it was found that mucilage (lane 4) inhibited ACE activity (i.e. less FAP produced) as observed under UV light. However, similar FAP productions were found between the control test (lane 2) and commercial pectin (lane 3). This result demonstrated again that mucilage exhibited ACE inhibitory activity.

Determination of the kinetic properties of ACE inhibition by mucilage

The Lineweaver-Burk plots of ACE (4 mU) without or with purified mucilage (200 μg/mL) with different concentrations of FAPGG are shown in Figure 5. The results indicated that purified mucilage acted as a mixed type inhibitor with respect to the substrate (FAPGG). Without the purified mucilage, the calculated Km was 1 mM FAPGG for ACE. In the presence of purified mucilage (200 μg/ mL), the calculated Km´ was 12 mM. In conclusion, the mucilage exhibited dose-dependent ACE inhibitory activity and acted as a mixed type inhibitor with respect to the substrate (FAPGG). In the yam without the purified mucilage, the calculated Km was 0.255 mM FAPGG for ACE and in the presence of purified mucilage, the calculated Km´ was 0.3304 mM. The purified mucilage exhibited dose-dependent ACE inhibitory activity and acted as a mixed type inhibitor with respect to the substrate (FAPGG) in the yam too. The Lineweaver-Burk plots of ACE inhibitory activity were just the same for purified mucilage of both sweet potato and yam (Lee et al., 2003).

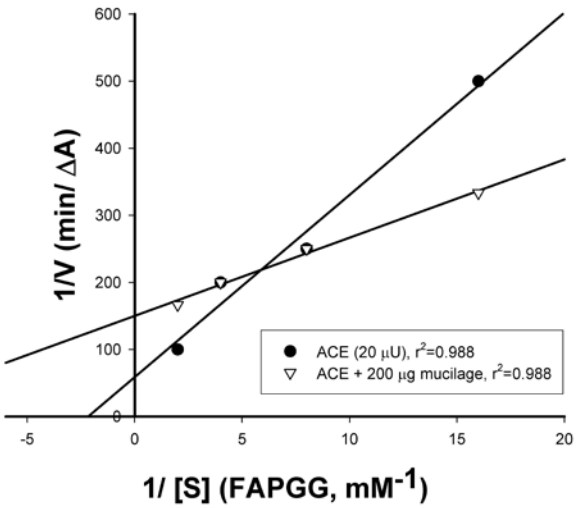

Figure 5. The Lineweaver-Burk plots of ACE (4 mU) without or with purified mucilage (200 μg/mL) from sweet potato storage root using different concentrations of FAPGG (0.1 to 0.5 mM).

In conclusion, the purified mucilage of sweet potato storage roots exhibited dose-dependent ACE inhibitory activity in vitro. The mucilage acted as a mixed type inhibitor toward ACE with an IC_{50} of 364.5 μg/mL, which is less than those of several peptides acting as ACE inhibitors. It might be a potential ingredient for hypertension control and deserves further investigations.

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甘藷黏質多醣具有血管收縮素轉化酶抑制活性

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甘藷 (*Ipomoea batatas* (L.) Lam. 'Tainong 57') 塊根黏質多醣經由初抽；及進一步 SDS 和熱處理之純化步驟，在電泳膠片顯示達到均質。以 (N-(3-[2-furyl] acryloyl)-Phe-Gly-Gly) (FAPGG) 為受質，利用分光光度計的方法分析此一均質的甘藷黏質多醣抑制血管收縮素轉化酶的能力 (50 到 400 μg/mL 黏質多醣，分別抑制 28.7 到 59.8% 血管收縮素轉化酶活性) 其效果隨劑量增加而增加。甘藷黏質多醣對於血管收縮素轉化酶之 50% 抑制濃度 (IC_{50}) 為 364.5 μg/mL，對照組 captopril 為 10 nM (8.68 μg/mL)。商品化的多糖類果膠 (50 到 400 μg/mL) 對於血管收縮素轉化酶並沒有類似的抑制效果。另外利用螢光 silica TLC 偵測 FAPGG 及其水解產物 FAP，結果也顯示甘藷黏質多醣對於血管收縮素轉化酶有抑制的效果。甘藷黏質多醣對於血管收縮素轉化酶是屬於混合型抑制，而 Michaelis 常數為 12 mM.

關鍵詞：甘藷；血管收縮素轉化酶；黏質多醣。