Isolation and Characterization of Edestin from Cheungsam Hempseed

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Edestin, a major hempseed storage protein from Cheungsam, was isolated to apparent homogeneity by acid precipitation and gel filtration chromatography. The native molecular weight of purified edestin was approximately 300 kDa by Sephacryl S-300 gel filtration. Upon adding 2-mercaptoethanol, the isolated edestin of 56.7 kDa on the non-reduced sodium dodecyl sulfate polyacrylamide gel was converted into subunits, suggesting that the protein might be composed of subunits linked by disulfide bond. Cheungsam edestin was rich in essential amino acids and it has 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity. The results suggest that Cheungsam edestin might be utilized as a superior antioxidative nutrient.

Key words: characterization, Cheungsam, edestin, isolation

Hemp, non-drug varieties of Cannabis sativa L., has provided an important source of industrial fiber. Hemp fiber has been used in the production of specialty paper, ropes, fabrics, automotive and building insulation, construction materials, and many other durable goods. The use of hemp as a source of food, fiber and medicine is widespread in the old world, and the whole seed continues to be used as important agricultural commodities in Canada, USA, and China [Tang et al., 2006; Wang et al., 2009; Lua et al., 2010]. A variety of hempseed has been known to contain high quality oil and protein, with considerable amounts of dietary fiber, vitamins, and minerals [Callaway, 2004]. The hempseed is recognized as an important source of essential fatty acids [Lua et al., 2010]. The hempseed oil showed significant health benefits, such as reducing Low-density lipoprotein (LDL)-cholesterol, platelet aggregation and high blood pressure, wound healing, and alleviating atopic dermatitis [Callaway et al., 2005]. Hempseed and hempseed meal are known to be excellent sources of digestible protein, especially hempseed lacks the anti-nutritional trypsin-inhibiting factors that are found in soy and most other vegetable products. Thus, a greater proportion of the protein in hempseed seems to be digested and available for absorption.

Hempseed protein was found to mainly consist of high-quality storage protein, named edestin, which accounts for about 60-80% of the total protein content, with albumin making up the balance. Edestin was easily digested and contained nutritionally significant amounts of all essential amino acids [Callaway, 2005]. As with the soy protein glycinin, edestin was a kind of hexameric legumin [St Angelo et al., 1968; Patel et al., 1994; Tang et al., 2006]. Edestin was reported to be similar to serum globulins, and the biologically active protein of edestin was metabolized in the human body to biosynthesize immunoglobulins, hormones, haemoglobin, and enzymes [Tombs, 1960].

In Korea, a new variety of industrial fiber hemp, named “Cheungsam”, was developed by crossing IH3 Netherland variety and Korea local variety [Moon et al., 2002]. The new variety of “Cheungsam” is regarded as non-drug type hemp, with a low level of δ-9 tetrahydrocannabinol (THC). However, the information on the edestin from Cheungsam is not available, although we characterized hempseed protein isolate of Cheungsam in our previous report [Kim and Lee, 2011]. In this investigation, we described the isolation and characterization of edestin from Cheungsam by using gel filtration chromatography following acid precipitation. Some neutrochemical properties of edestin were characterized.

Materials and Methods

Hempseed preparation. Dehulled hempseeds from industrial fiber hemp, named “Cheungsam”, were ground in liquid nitrogen and then dispersed in deionized water at room temperature.

Isolation of edestin from Cheungsam. In order to isolate edestin from Cheungsam hempseeds, acid precipitation of hempseed protein isolate was followed by chromatographic separation. One gram of hempseed flour was mixed with 100 mL deionized water, and the mixture was adjusted to pH 10.0 with 1.0 N NaOH. After continuously stirring for 1 h, the
suspensions were centrifuged at 8,000 g for 30 min and the precipitates were discarded. Then, the pH of the supernatants was adjusted to pH 5.0 at 4°C with 1 N HCl, and the precipitates were collected by centrifugation at 6,500 g for 25 min. The obtained precipitates were washed with pre-cooled deionized water, and dispersed in the deionized water. The dispersions were subjected to dialysis with 10 mM Tris buffer (pH 10.0) at 4°C for overnight. The dialysate was applied to Sephacryl S-300 column pre-equilibrated with 10 mM Tris buffer (pH 10.0). After washing with buffer, hempseed protein isolate was then further purified through Sephacryl S-300 gel filtration chromatography to an electrophoretically homogeneous form [Downes and Hall, 1998; Barbarino and Lourenço, 2005]. The absorption spectrum and absorbances were recorded on a UV-visible spectrophotometer. All spectra were recorded at room temperature.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was performed with slight modifications of discontinuous buffer system of Laemmli [1970]. For SDS-PAGE under reduced condition, the protein samples were directly dissolved in the sample buffer, namely 0.125 M Tris-HCl buffer (pH 8.0) containing 0.1% (w/v) SDS, 0.05% (w/v) bromophenol blue, 30% (v/v) glycerol and 5% (v/v) β-mercaptoethanol (2-ME). The SDS-PAGE under non-reduced conditions was carried out as mentioned above, except using the samples dissolved in the sample buffer without 2-ME [Tang et al., 2006; Kim et al., 2011].

Periodic acid-Schiff (PAS) staining for carbohydrate. After finishing SDS-PAGE, gels were fixed overnight in 25% isopropanol, 10% acetic acid and 65% water. Gels were washed for 1 h in running water, soaked in 1% NaIO₄, 3% acetic acid for 1 h and then washed for 2 h in running water and twice for 0.5 h with distilled water. The gels were then stained for 2 h with Schiff's reagent prepared from Basic Fuchsin [Kim and Lee, 2011]. Excess Schiff's reagent was removed by soaking in 0.5% sodium metabisulphite. The gels were then soaked for two times 3 h in 7% acetic acid.

Amino acid analysis. The amino acid composition of edestin subunits, which were eluted from the SDS-PAGE gel, were determined by an automatic amino acid analyzer (Waters M510, Milford, MA), using PICO, TAG column [Wang et al., 2008]. The determination was carried out 38°C, and the detection wavelength 254 nm and flow rate 1.0 mL per min. The samples were hydrolyzed with 6 N HCl for 24 h at 110°C in sealed tube. The amino acid composition was calculated as relative peak area of high-pressure liquid chromatography (HPLC) chromatogram against peak area of standard amino acid.

N-terminal amino acid sequence analysis. After SDS-PAGE, the purified protein on the gel was transferred to a polyvinylidene difluoride (PVDF) membrane by electroblotting and stained with Coomassie blue. The stained material was excised and used for N-terminal sequencing by the automated Edman degradation method at the Korea Basic Science Institute (KBSI).

1. l-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. The DPPH radical scavenging activity was determined by the method described earlier [Seo et al., 2010]. 2 mL of the sample solution was mixed with freshly prepared 2 mL of 0.2 mM DPPH ethanolic solution and vortexed for about 10 s. The resulting solution was then left to stand for 30 min, prior to reading the absorbance at 517 nm. Ethanol was used as a blank. Reduction of absorbance at 517 nm indicates an increase in DPPH scavenging activity. Each experiment was performed at least three times in triplicate.

Results and Discussion

Isolation of edestin from Cheungsam hempseed. Cheungsam edestin was isolated by acid precipitation and Sephacryl S-300 gel filtration chromatography. Table 1 shows the purification profile of edestin from hempseeds of Cheungsam. The overall isolation yield of edestin was about 48.6% and total amount of edestin (142.9 mg) was isolated from 1 g of hempseeds.

The isolation profile of edestin on the SDS-polyacrylamide gel under reduced and non-reduced conditions were shown in Fig. 1. On the SDS-polyacrylamide gels under the reduced condition with 2-ME, the edestin was composed of

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total protein (mg)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>293.95</td>
<td>100</td>
</tr>
<tr>
<td>Acid precipitation</td>
<td>144.35</td>
<td>49.1</td>
</tr>
<tr>
<td>Gel-filtration (Edestin)</td>
<td>142.9</td>
<td>48.6</td>
</tr>
</tbody>
</table>

Fig. 1. SDS-polyacrylamide gel electrophoretic profile of edestin in the presence (A) and absence (B) of β-mercaptoethanol. M: Molecular weight markers, 1: Crude extract, 2: Acid precipitates, 3: Purified edestin through gel filtration, 4: Commercial edestin (MP Biomedicals), 5: Carbohydrate staining for purified edestin by periodic acid-Schiff staining.