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Study on antidiabetic activity of wheat and barley starch using asymmetrical flow field-flow fractionation coupled with multiangle light scattering

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ABSTRACT

The ability of asymmetrical flow field-flow fractionation (AF4) coupled online with multiangle light scattering (MALS) and refractive index detector (RI) (AF4-MALS–RI) for monitoring of change in molecular conformation of wheat and barley starch during germination process was evaluated. AF4 provides separation of starch molecules based on their hydrodynamic sizes, and MALS yields the molar mass and molecular size (radius of gyration, R_g). In vitro and in vivo anti-hyperglycemic effect of germinated wheat and barley was studied. The relationship between antidiabetic activity and molecular conformation was, for the first time, investigated. The ratio of R_g to the hydrodynamic radius (R_h) and the apparent density were proven to be important parameters as they offer an insight into molecular conformation. Results showed that, when geminated, the apparent density and the antidiabetic activity of barley were significantly increased, suggesting germination makes the molecules more compact which could contribute to enhancement of their antidiabetic activity. The information obtained by AF4-MALS–RI is valuable for understanding of germination mechanism, and thus for developing functional foods.

1. Introduction

The structure and degradation of biomacromolecules, such as starch, have a bearing on some human diseases such as obesity, diabetes, and some colo-rectal cancers, which are reaching epidemic proportions in developed countries, and are growing rapidly in developing countries [1–3]. In 2011, approximately 366 million people of the global population aged 20–79 years were estimated to have diabetes [4]. There is an increasing demand for healthy diet, and the use of functional food and directed nutrition is nowadays presented as a way to stay fit and healthy.

Wheat and barley are two of the most abundant and popular cereal materials and common sources of digestible carbohydrate in human diet. They provide minerals, dietary fiber and bioactive compounds [5]. The major carbohydrate in wheat and barley is starch, supplying from 20 to 50% of food energy, with higher fraction for "Asian" diet and also for diets of those in many developing countries.

Starch consists of two types of polydisperse polysaccharides, amylose and amylopectin. The former consists of linear chains of (1 → 4)-α-glucose linked residues having molar masses of 10^5 to 10^6 g/mol. The latter has a highly branched structure containing a mixture of (1 → 4) and (1 → 6)-α-glucose linkages with molar mass reaching up to about 10^8 g/mol [6]. Wheat and barley starch and starch–derivatives have been widely used in a variety of industrial applications including food, beverage, pharmaceuticals, household products, cosmetics, paper and packaging [7,8].

It is known that germination significantly changes the nutritional quality of the cereal seeds, including the starch content [9,10]. On germination, protein, carbohydrates and minerals become more bio-available and bio-accessible [11]. Low-processed food (i.e. germinated seed) has become more popular for functional foods [12,13].

Although some researchers postulated that the enhancement in bioactivity of germinated seeds is also linked with modification of the starch structure and content, little is known about variation in physicochemical properties of starch (such as molar mass, molar mass distribution, conformation, and so on) during seed germination [14,15]. The major reason for a scarcity of information may be related to difficulties in studying the molar mass distribution of ultrahigh molar mass polymer by size exclusion chromatography

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(SEC). While SEC is a well-established method for analysis of polymers, difficulties in its application to large biomacromolecules such as amylopectin include exceeding the exclusion limit of the column, irreversible interaction of the sample with column packing and subsequently low recovery, and shear degradation [16–18].

During the last decade, an alternative separation technique, asymmetrical flow field-flow fractionation (AF4) coupled with multiangle light scattering (MALS) and refractive index detector (RI) has shown its applicability to the determination of the molar mass distribution of various ultrahigh molar mass polymers [19–21]. Unlike SEC, AF4 uses an open channel that requires no stationary phase or packing material. Thus in AF4, the shear forces during separation are minimized [22].

The viscosity of polysaccharide solution depends on the molar mass and conformation of the molecules and the ability to form aggregates [23]. Thus, characterization of the distributions of molar mass and related parameters (such as apparent density) of wheat and barley starch in aqueous solution is important for improvements in the human and animal nutrition. However, so far, little has been reported on the effect of germination on physicochemical properties of cereal starch.

In this work, the capacity of AF4-MALS–RI to monitor the variation in conformation of wheat and barley starch during germination was evaluated. The aim of this work is to study the correlation between conformational properties of strach in wheat and barley and their antidiabetic activities.

2. Materials and methods

2.1. Materials

Wheat and barley seeds were obtained from farms in Gongeum and Chuncheon, Korea, respectively. Five week-old male C57BLks/J db/db mice were purchased from Joongang Experimental Animal Co., (Seoul, Korea). Deionized water was obtained from a Milli-Q Plus Ultra-Pure Water system (Millipore, MA, USA). All chemicals were used without further purification. Rat intestinal acetone powder, *p*-nitrophenyl-α-D-glucopyranoside, sodium nitrate ($NaNO_3$), sodium hydroxide (NaOH), and sodium azide (NaN_3) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydrochloride acid (35–37%) was purchased from Samchun Chemical (Pyeongtaek, Korea). Tea catechin was purchased from General Foods & Flavors Inc. (Seoul, Korea). Chitooligosaccharides was obtained from Kunpoong Bio Co., Ltd. (Seoul, Korea).

2.2. Starch preparation from germinated wheat and barley seeds

Wheat and barley seeds were germinated by soaking them in distilled water containing 0.5% (w/w) tea catechin and 1.0% (w/w) chitooligosaccharides at 25 °C for 24 and 48 h, respectively. After soaked germination, seeds were dried for 24 h at 50 °C and pulverized using a grinder (Hibell Co., Ltd., Hwaseong, Korea). Then, 10% (w/w) dispersion of sample was prepared by adding germinated sample into distilled water and autoclaved for 15 min at 121 °C. Finally, the suspension was centrifuged at 7000 rpm for 20 min at 30 °C. Powder samples were obtained by freeze–drying (see Table S1) the supernatants. Wheat powders obtained by 24 h and 48 h-germination were labeled "W-24" and "W-48", respectively, and the wheat powder obtained without germination was labeled "W-0". Barley powders were labeled likewise "B-0", "B-24", and "B-48", respectively.

2.3. Sample preparation for AF4 analysis of starch

Sample solutions for AF4-MALS–RI analysis were prepared by the following procedure: 10 mg of the powder sample was mixed with 1 mL of 1 M NaOH in a 20 mL vial, and stirred with a magnetic stirring bar at 400 rpm for 2 min at 70 °C in an oil bath (Daihan Scientific Co., Ltd., Seoul, Korea). Then, 8 mL of the AF4 carrier liquid (deionized water containing 50 mM $NaNO_3$ and 3 mM NaN_3) was added and stirred at 70 °C. Finally, the solution was neutralized by adding 1 mL of 1 M HCl, and stirred again. The sample solution was filtered through a 0.45 μm syringe filter prior to injection into AF4.

Table 1
Method used for AF4 analysis of starch.

Time (min)	Mode	$V_{c,initial}$ (mL/min)	$V_{c,final}$ (mL/min)	Focusing flow rate (mL/min)
3.7	Elution	0.9	0.9	–
2.0	Focusing	–	–	1.0
2.0	Focusing + injection	–	–	1.0
2.3	Focusing	–	–	1.0
8.0	Elution	0.9	0.1	–
30	Elution	0.1	0.1	–
1.0	Elution	0.1	0	–
20	Elution	0	0	–

2.4. AF4-MALS–RI analysis of starch

The AF4 system used in this work was an Eclipse 2 Separations System (Wyatt Technology Europe, Dernbach, Germany). It was connected to a DAWN EOS multiangle light scattering detector (Wyatt Technology, Santa Barbara, CA, USA) operating at the wavelength of 690 nm and a RID-10 differential refractive index detector (Shimadzu, Kyoto, Japan). An Agilent 1100 pump (Agilent Technologies, Waldbronn, Germany) with an in-line vacuum degasser delivered the carrier liquid into the AF4 channel. Between the pump and the AF4 channel was placed a 0.1 μm YYLP membrane filter (Millipore Corp. MA, USA) to ensure the carrier liquid entering AF4 channel is particle-free. The channel was assembled with a 350 μm-thick Mylar spacer and a regenerated ultrafiltration cellulose membrane with the cut-off of 10 kDa. The actual channel thickness was measured to be 295 μm from the elution time of ferritin (from horse spleen) based on the method in [24]. The channel geometry was trapezoidal with the tip-to-tip length of 26.5 cm and breadths at the inlet and the outlet of 2.2 and 0.6 cm, respectively. Injection of the sample into the channel was performed at the flow rate of 0.2 mL/min for 2 min. The concentration of the sample solution was 0.5–1.0 mg/mL, and the sample injection volume was 100 μL. In order to avoid excessive retention, a cross-flow programming was employed in this study, where the cross-flow was deceased linearly from 0.9 to 0.1 mL/min for 8 min, and then was maintained at 0.1 mL/min for 30 min as shown in Table 1. The thickness of the sample equilibrium layer increases with decreasing cross-flow rate, resulting in a reduction in retention time, especially for larger ones. The carrier liquid for AF4 was deionized water containing 50 mM $NaNO_3$ and 3 mM NaN_3, which was filtered through a 0.1 μm regenerated cellulose membrane filter.

2.5. Determination of in vitro α-glucosidase inhibition

In order to investigate the inhibition activity of wheat and barley starch, an in vitro α-glucosidase inhibition test was performed. 0.3 g of rat intestinal acetone powder was suspended in 9 mL of 0.9% (w/v) saline, and the suspension was sonicated for 30 s 12 times at 37 °C. Then the suspension was centrifuged at 13,000 rpm for 30 min at 4 °C and the supernatant was taken for assaying. Sample extracts were prepared by adding 20 mg of the sample powder into 1 mL of 0.1 M phosphate buffer (pH 6.9) followed by centrifugation at 5600 rpm for 5 min. In order to see whether the starch affect antidiabetic activity, suspensions of entire sample were also prepared by the same manner without centrifugation.