Molecular structure of large-scale extracted β-glucan from barley and oat: Identification of a significantly changed block structure in a high β-glucan barley mutant

Mette S. Mikkelsen [a,*], Birthe M. Jespersen [a], Flemming H. Larsen [a], Andreas Blennow [b], Søren B. Engelsen [a]

[a] Quality & Technology, Department of Food Science, Faculty of Science, University of Copenhagen, Rolighedsvej 30, 1958 Frederiksberg C, Denmark
[b] VKR Research Centre Pro-Active Plants, Department of Plant Biology and Biotechnology, Faculty of Science, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark

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ABSTRACT

Health effects of β-glucan are typically related to dose, size and viscosity without taking the specific molecular structure into account. High β-glucan mutant barley, mother barley and oat β-glucans were large-scale extracted by comparable protocols using hot water, enzyme assisted hydrolysis and ethanol precipitation leading to similar molecular masses (200–300 kDa). Multivariate data analysis on all compositional, structural and functional features demonstrated that the main variance among the samples was primarily explained by block structural differences as determined by HPSEC–PAD. In particular the barley high β-glucan mutant proved to exhibit a unique block structure with DP3 and DP4 contributions of: 78.9% and 16.7% as compared to the barley mother (72.1% and 21.4%) and oat (66.1% and 29.1%). This unique block structure was further confirmed by the ^{1}H NMR determination of the β-1,4 to β-1,3 linkage ratio. Low solubility of the barley samples was potentially an effect of substructures consisting of longer repetitive cellotriosyl sequences. FT-Raman and NMR spectroscopy were useful in measuring sample impurities of α-glucans and prediction of β-linkage characteristics.

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1. Introduction

Viscous dietary fibres, such as mixed-linkage (1 → 3, 1 → 4)-β-D-glucan, a polysaccharide particularly occurring in oats and barley, account for the majority of the clinical benefits observed with dietary fibres (Kendall, Esfahani, & Jenkins, 2010). The most widely documented nutritional benefits of β-glucan in food are the flattering of the postprandial blood glucose and insulin rises as well as the reduction of serum cholesterol levels. Both barley and oat β-glucans give rise to these responses and effectiveness is strongly related to dose, size and viscosity (Lazaridou & Biliaderis, 2007; Wood, 2007). However, the bare amount of β-glucan is insufficient to determine the health effects of the fibre as also the molecular structure and physical properties directly related to these parameters are potentially important for the health promoting effects. Normally, these more detailed data are not taken into account in human intervention studies which is partially the reason why the results often remain controversial (Wood, 2004). In addition, there is sparse evidence for parallel studies comparing the health effects

of barley and oat β-glucans (Biorklund, van Rees, Mensink, & Onning, 2005; Delaney et al., 2003).

For this reason we intended to conduct a thorough comparison of barley and oat β-glucan structure and functionality all the way from raw plant material to health effects. The present work includes large-scale extraction of barley and oat β-glucans, molecular structure and physico-chemical functionality elucidation and multivariate data analysis for exploitation of significant differences and inner relations between β-glucan properties.

Due to the complexity of the raw plant material, β-glucan extraction and purification typically involve several steps: (i) inactivation of endogenous enzymes in the grain, (ii) extraction with water or alkali solutions, (iii) removal of protein and starch using hydrolytic enzymes and/or selective adsorption, (iv) precipitation of β-glucan from the purified solution with alcohol and freeze-, drum- or spray drying of the extract (Izydorczyk & Dexter, 2008). The extraction method affects the purity of the product, the fundamental molecular structure and the molecular mass of the β-glucan polymer (Beer, Wood, & Weisz, 1997; Burkus & Temelli, 1998; Roubroeks, Mastromauro, Andersson, Christensen, & Aman, 2000; Temelli, 1997). Kvist and Lawther (2005) previously extracted soluble barley and oat β-glucan in large-scale for food applications

* Corresponding author. Tel.: +45 35 33 35 07; fax: +45 35 33 32 45.
E-mail address: skau@life.ku.dk (M.S. Mikkelsen).

using hot (95 °C) water, heat-stable α-amylase and centrifugal separation of the solution to provide a soluble fibre complex and an aqueous pellet mostly comprising of the protein and oil together with the insoluble fibre material from the milled grain. This method was modified for the large-scale extraction of β-glucans investigated in the present study.

Total β-glucan content of barley grain generally ranges between 2.5% and 11.3% by weight, whereas the range for oat is 2.2–7.8%. Munck, Moller, Jacobsen, and Sondergaard (2004) reported β-glucan levels as high as 15–20% in a barley low starch mutant line *lys5f* and explained the overall constant production of polysaccharides as a pleitropic effect of the mutation (Patron et al., 2004). This high β-glucan mutant barley was included in the present study along with its barley mother line. β-Glucan from different genera of cereals share the same general linear molecular structure, but exhibit variations in molecular mass, linkage pattern (ratio of β-1,4 to β-1,3 linkages), block structure (ratio of cellotriosyl/cellotetraosyl units; DP3/DP4) and amount of longer cellulose-like fragments (DP $\geqslant$ 5) (Izydorczyk, Biliaderis, & Lazaridou, 2006). The oligomer block structure of β-glucan can be analysed using digestion with endo-1,3-β-D-glucanase (lichenase) that releases the β-1,4-linked segments of DP3-DP$_n$ (Blennow, Bay-Smidt, Wischmann, Olsen, & Moller, 1998). The differences in the molar ratio of DP3/DP4 units can be regarded as a fingerprint of the structure of cereal β-glucans and generally follows the order of wheat (3.0–4.5), barley (1.8–3.5), rye (1.9–3.0) and oat (1.5–2.3) (Fincher, 2009; Izydorczyk et al., 2006; Lazaridou, Biliaderis, Micha-Screttas, & Steele, 2004; Wood, Weisz, & Blackwell, 1991, 1994).

Raman and ^{1}H liquid-state NMR spectroscopy are widely applied methods in compositional and structural investigations of polysaccharides (Mikkelsen et al., 2010; Salomonsen, Jensen, Stenbaek, & Engelsen, 2008; Synytsya, Copikova, Matejka, & Machovic, 2003). The advantage of using spectroscopic techniques is the high throughput and exploratory character of the measurements that enables fast and simultaneous detection of several different components, and in combination with multivariate data analysis spectroscopy makes a powerful approach to screen for variation in large sample sets (Munck et al., 2010). Multivariate data analysis can also be used to predict structural and compositional features of polysaccharides and cereal grains from spectral data (Jacobsen, Sondergaard, Moller, Desler, & Munck, 2005; Salomonsen et al., 2008). Earlier we have found β-glucan specific structural information in Raman (the anomeric β-configuration adsorption band at ~890 cm^{-1}) and ^{1}H NMR spectra (the anomeric β-1,3 and β-1,4 resonances at 4.75 and 4.55 ppm, respectively) (Mikkelsen et al., 2010). In this work we demonstrate how Raman and ^{1}H NMR spectroscopy can be used to predict β-glucan compositional and structural features. These features were further linked to variation in physical characteristics important for determining health related assets of β-glucans.

2. Materials and methods

Barley materials for large-scale β-glucan extraction were high β-glucan-low starch mutant line in BOMI *lys5f* (β-glucan: 16.5–19.8%, starch: 30.0%, protein: 16.0%) and mother line BOMI (β-glucan: 6.0%, starch: 52.3%, protein: 12.8%) (Munck et al., 2004). Extracted barley mutant samples were denoted: Bm1, Bm2, Bm3, Bm4 and barley mother samples were denoted: B1, B2, B3, B4. Oat β-glucan (O1, O2) was purified from oat β-glucan concentrate (Promoat™, β-glucan: ~35%, starch: ~45%, protein: ~4.5%) obtained from Biovelop (Kimstad, Sweden) using the same large-scale procedure as for the barley raw materials. For the structural and functional comparison of β-glucan isolates, pure low, medium and high viscosity barley (BL, BM, BH) and medium and

high viscosity oat (OM, OH) β-glucans from Megazyme International Ltd. (Bray, Ireland) were used as references along with crude and purified barley (BC, BP) and oat (OC, OP) β-glucan extracts earlier studied by Mikkelsen et al. (2010). Lichenan$_{1,3/1,4-BG}$ (Lich), cellulose$_{1,4-BG}$ (Cell) and curdlan$_{1,3-BG}$ (Curd) (Sigma–Aldrich, Brøndby, Denmark) were included as structural reference samples (Table 1).

2.1. Large-scale extraction of β-glucans

β-Glucans were isolated from barley grains by hot water and enzymatic hydrolysis treatment using a modified procedure of Kvist and Lawther (2005). A flow diagram outlining the steps during β-glucan extraction is shown in Fig. 1. The Application Pilot Plant at Novozymes A/S (Bagsværd, Denmark) operating with 600 L tanks, pipe connections and mono pumps was used for the following extraction steps except for the ethanol precipitation which was conducted at the University of Copenhagen. Barley grains were ground on a Brabender disc mill twice before dispersion using different concentrations of the raw materials (10% w/v mother line, 4% w/v mutant line, 5% w/v oat β-glucan concentrate) into 450 L, 95 °C, tap water. Termamyl SC (EC 3.2.1.1, Novozymes A/S, Bagsværd, Denmark) thermostable α-amylase (2% w/w, enzyme/starch) was added and the solution held with mixing for 30 min, pH 6. The mixture was then passed through a FrymaKoruma Toothed Colloid MZ 130 wetmill (600 L/h, Romaco FrymaKoruma, Neuenburg, Germany) and circulated back to the tank for a further 30 min of starch dextrification, 95 °C and pH 6. Large particles and insoluble fibres were decanted away using a Westfalia CA 225-110 separator (600 L/h, GEA Westfalia Separator Group GmbH, Oelde, Germany) and amylase activity was stopped in a Hydroheather M101MG (400 L/h, 2 bar, Hydro-Thermal Corporation, Waukesha, WI, USA) at 125 °C for 4 min. The solution was centrifuged using a Westfalia SB7 centrifuge (300 L/h, GEA Westfalia Separator Group GmbH, Oelde, Germany) thereby removing most of the denatured protein fraction prior to addition of Alcalase AF 2.4L (EC 3.4.21.62, Novozymes A/S, Bagsværd, Denmark) protease (2.5% w/w, enzyme/protein) at 60 °C for 30 min, pH 7. Protease activity was terminated at 85 °C for 15 min and the solution cooled to 25 °C before precipitation of β-glucan in 50–80% food grade ethanol. The gum material was filtered, freeze-dried and ground into fine β-glucan powder. Barley β-glucans were extracted in two true replicate processes corresponding to samples: B1, B2, Bm1, Bm2 in repetition 1 and B3, B4, Bm3, Bm4 in repetition 2, whereas oat β-glucan (O1, O2) was purified in one single process. Batches 1 and 3 are β-glucans immediately precipitating out in 80–50% ethanol whereas batches 2 and 4 are β-glucans precipitating out in the remaining 50:50% β-glucan-ethanol solution overnight. However, as no significant differences were found for isolates of the same origin, Bm1–4, B1–4 and O1–2 were treated as replicate samples, respectively.

2.2. Compositional analyses

Samples were analysed for their β-glucan, starch, and protein contents in duplicates. Dietary fibre analysis was performed on four replicate samples. Total β-glucan content was determined by the method of McCleary and Mugford (1997) using the Megazyme (Megazyme International, Wicklow, Ireland) mixed linkage β-glucan assay kit and same conditions as Mikkelsen et al. (2010). The content of total starch along with total (TDF), soluble (SDF) and insoluble dietary fibres (IDF) was determined according to the Megazyme procedures provided with the test kits (Approved Methods 76–13 and 32–07, AACC 2000). Protein was analysed using the Kjeldahl method (Approved Method 46–12, AACC 2000).