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Isolation of cereal arabinogalactan-peptides and structural comparison of their carbohydrate and peptide moieties

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Abstract

Arabinogalactan (AG)-peptides were isolated and purified from wheat and durum wheat and for the first time from spelt, triticale, rye and barley using three dedicated isolation procedures. The AG-peptide molecules have molecular weights of approximately 23,500 with the exception of triticale (27,500) and rye (33,000). The fine structure of the carbohydrate parts revealed close resemblances among the purified cereal AG-peptide samples. They consist of a (1→6)-β-D-galactopyranosyl backbone substituted in the C(O)3-position with a single α-L-arabinofuranosyl or a single β-D-galactopyranosyl residue. The latter can also be substituted in its C(O)3-position with a single α-L-arabinofuranosyl residue. The AG-peptide peptide cores typically exist of 15 amino acids including three highly conserved hydroxyprolines (Hyp), each linked to a carbohydrate chain. The peptide amino acid sequence of spelt and durum wheat AG-peptides showed high similarity with the wheat AG-peptide peptide sequence while triticale, rye and barley AG-peptide peptide cores displayed less similarity. Homology with the N-terminal part of cereal grain softness protein (GSP) precursors indicates that the cereal AG-peptide peptides are a processing product of GSP synthesis. An overall structural model is proposed.

Keywords: Arabinogalactan-peptide; Cereals; Grain softness protein; Structure

1. Introduction

Non-starch polysaccharides (NSP) in cereal flours consist mainly of arabinoxylan (AX), arabinogalactan (AG)-peptides, and (1→3, 1→4)-β-glucans, the last being especially abundant in barley, oats and and rye (Henry, 1987; Preece and Hobkirk, 1953; Viëtor et al., 1991). Structural characteristics as well as functional properties of both water extractable and water unextractable AX from most cereals have been intensively studied. In contrast, research on cereal AG-peptides has concentrated mainly on wheat AG-peptides (Fincher and Stone, 1974; Fincher et al., 1974; Izydorczyk et al., 1991b; Loosveld et al., 1997, 1998; Neukom and Markwalder, 1975; Strahm et al., 1981; Van den Bulck et al., 2002; Westerlund et al. 1989a,b, 1990) and durum wheat (Ingelbrecht et al., 2002). The presence of galactose containing polysaccharides including AG-peptides in other cereals has been referred to in general terms (Burke et al., 1974; Clarke et al., 1979;

Abbreviations: AG, arabinogalactan; AG-peptide(s), arabinogalactan-peptide; A/G, arabinose to galactose ratio; AS, ammonium sulphate; AX, arabinoxylan(s); A/X, arabinose to xylose ratio; G-peptide(s), galactan-peptide(s); GSP, grain softness protein; ^{1}H-NMR, proton nuclear magnetic resonance; HPSEC, high-performance size exclusion chromatography; Hyp, hydroxyproline; Mr, molecular weight; NSP, non-starch polysaccharides.

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Fincher and Stone, 1974; Meuser and Suckow, 1986; Preece and Hobkirk, 1953; Schindler et al., 1995; Schopfer, 1990).

The wheat flour AG-peptides are small water extractable proteoglycans with reported apparent molecular weights (Mr) ranging from 22,000 to 27,000 (Fincher et al., 1974; Loosveld et al., 1998), over 30,000–32,000 (McNamara and Stone, 1981; Strahm et al., 1981) to 70,000 (Izydorczyk et al., 1991a). Apparent Mr of AG-peptides from different wheat cultivars are similar (Izydorczyk et al., 1991a; Loosveld et al., 1998) suggesting the somewhat broad range observed is probably due to differences in measurement techniques.

The small core peptide of the wheat AG-peptide (8.0–9.0%, w:w) is composed of 15–20 amino acids, with hydroxyproline (Hyp), Glu/Gln, Ala, Ser, Thr, and Val as the most important (Fincher et al., 1974; Meuser and Suckow, 1986; Strahm et al., 1981). The recently described complete 15 amino acid sequence (Van den Bulck et al., 2002) for the wheat AG-peptide is closely related to a sequence in the wheat grain softness protein (GSP), whose proposed role as a determinant of grain texture is increasingly debated (Giroux and Morris, 1998). The wheat AG-peptide peptide has a Mr of 1534. The carbohydrate moiety (91.0–92.0%, w:w) is *O*-glycosidically linked to the peptide chain through the Hyp residues (Fincher et al., 1974; McNamara and Stone, 1981; Strahm et al., 1981; Van den Bulck et al., 2002). It consists of a β-D-galactopyranosyl backbone, substituted with α-L-arabinofuranosyl residues (Fincher et al., 1974) with an arabinose to galactose (A/G) ratio of 0.66–0.73 (Fincher and Stone, 1974; Fincher et al., 1974; Loosveld et al., 1998; Neukom and Markwalder, 1975). Methylation analysis revealed that most galactose residues (75–80%) were linked at both C(O)3 and C(O)6, the remainder being either linked at C(O)3 (10–12%) or C(O)6 (4–13%). No terminal galactose was found (Loosveld et al., 1998; Neukom and Markwalder, 1975). Analyses of wheat AG-peptides and wheat AG-peptides dearabinosylated with an α-L-arabinofuranosidase indicated that arabinose residues are mostly attached as single residues at C(O)3 of (1→6)-β-D-galactopyranosyl residues and none are linked at the C(O)6 of (1→3)-β-D-galactopyranosyl residues. Dearabinosylation also decreased the proportion of (1→3)-linked and increased terminal galactose residues, further indicating that the galactose backbone is branched (Loosveld et al., 1998). The carbohydrate portions of durum and common wheat AG-peptides are quite similar although minor differences in linkage profiles exist (Ingelbrecht et al., 2002).

Here we report the isolation of AG-peptides from spelt, triticale, rye, and barley and the determination and comparison of the amino acid sequences of their peptides and the structure of their carbohydrate portions.

2. Experimental

2.1. Materials

All reagents were of at least analytical grade and obtained from Sigma-Aldrich (Bornem, Belgium). Specialty chemicals were *alpha*-amylase (Termamyl 120 LS, Novozymes, Bagsvaerd, Denmark), amyloglucosidase (Sigma, St Louis, MO, USA), Montmorillonite KS 10 clay (Aldrich, Bornem, Belgium), Shodex standard P-82 pullulans (Showa Denko K.K., Tokyo, Japan), and an *Aspergillus niger* α-L-arabinofuranosidase solution (Megazyme, Bray, Ireland). Xylanases from *Trichoderma viride* (Embl accession number AJ012718; Furman-Matarasso et al., unpublished), *Trichoderma longibrachiatum* (Swissprot accession number P36218; Törronen et al., 1992), and *Aspergillus niger* (Swissprot accession number P55329; Krengel and Dijkstra, 1996) were purchased from Megazyme. Xylanase from *Aspergillus aculeatus* (GenBank accession number AAE69552; Kofod et al., 2001) was obtained from Novozymes. A single anion exchange purification step for the latter resulted in an α-L-arabinofuranosidase free preparation.

Commercial wheat (*Triticum aestivum* L.) and spelt (*Triticum spelta* L.) flours were obtained from Vanden Bempt (St-Joris Weert, Belgium), barley (*Hordeum vulgare* L., cv. Majestic, harvest 2000), triticale (*Triticosecale* Wittmack, cv. Babor, harvest 2000), and rye (*Secale cereale* L., cv. Halo, harvest 2001) were from AVEVE (Landen, Belgium). Durum wheat semolina (*Triticum durum* Desf., cv. AC Avonlea, harvest 1999) was supplied by the 'Canadian Grain Commission' (Winnipeg, Canada). Triticale, barley, and rye were milled at a moisture level of 14.5% on a Bühler MLU-202 laboratory mill (Bühler, Uzwil, Switzerland) according to AACC method 26–31 (Anonymous, 2000).

2.2. Standard analyses

Moisture contents was determined using AACC-method 44-19 (Anonymous, 2000). Ash content was determined according to AACC-method 08-01 (Anonymous, 2000). Protein contents of flour samples and their aqueous extracts were determined using the Dumas method. The procedure was an adaptation of the AOAC Official Method (1995) to an automated Dumas protein analysis system (EAS varioMax N/CN, Elt, Gouda, The Netherlands). Protein content of AG-peptide fractions was determined according to Lowry et al. (1951) with bovine serum albumin as standard. Monosaccharide compositions of samples were estimated using gas–liquid chromatography following hydrolysis, reduction and acetylation as described by Loosveld et al. (1997). The apparent Mr of isolated AG-peptides was determined using High Performance Size Exclusion Chromatography (HPSEC) (Loosveld et al., 1998).