JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 3621–3626 3621
DOI:10.1021/jf903843f

Structural Investigations on Arabinogalactan-Protein from Wheat, Isolated with Yariv Reagent

Esther M. Göllner,* Wolfgang Blaschek, and Birgit Classen

Pharmaceutical Institute, Department of Pharmaceutical Biology, Christian-Albrechts-University of Kiel, Gutenbergstrasse 76, 24118 Kiel, Germany

For the first time a pure arabinogalactan-protein (AGP) could be isolated from whole grain of wheat (*Triticum aestivum* L.) by performing a double precipitation with β-glucosyl Yariv reagent. The putative bioactive AGP has been characterized with regard to its polysaccharide and protein parts. Analytical investigations by GLC-MS and ^{13}C NMR revealed a carbohydrate moiety consisting of a 1,3-Gal*p* backbone, linked in position 6 to short 1,6-Gal*p*-chains, terminating in Ara*f*. In the protein part, a high content of hydroxyproline has been found, probably responsible for linkage between protein and polysaccharide moieties. The molecular mass of AGP has been determined by size exclusion chromatography with laser light scattering detection and found to be 125 kDa. Alkaline hydrolysis of the protein resulted in single carbohydrate moieties with a molecular mass of about 20 kDa, indicating that AGP from whole grain of wheat belongs to the wattle blossom type of AGPs.

KEYWORDS: Arabinogalactan-protein; Poaceae; structural analysis; *Triticum aestivum*; wheat; whole grain; Yariv

INTRODUCTION

Arabinogalactan-proteins (AGPs) are macromolecular glycoproteins of the extracellular matrix containing high proportions of carbohydrate. They occur in all higher plants (*1*−*5*) and are assumed to play important roles in vegetative growth, cellular development, and programmed cell death and thus are presumably involved in molecular interactions of intercellular signaling (*2*, *5*−*8*). AGPs from gum arabic have been shown to induce embryogenesis in microspore culture from wheat (*9*). For AGPs from *Echinacea*, different immunomodulating properties such as activation of complement system, binding to human leucocytes, and enhancement of release of cytokines have been shown in vitro (*10*−*12*). As wheat is an important source of human nutrition, isolation and characterization of AGPs with potential immunomodulating activities from this plant are tasks of interest. Furthermore, arabinogalactans are regarded as prebiotic sources for the gut flora (*13*).

β-Glucosyl-Yariv reagent (βGlcY) is a synthetic phenylglycoside that specifically binds to all typical AGPs and is a very useful tool for AGP purification (*14*, *15*). In the past it has been claimed that extracts from *Triticum* species show no reaction with βGlcY (*16*). Therefore, it is common practice to isolate AGPs from wheat using other methods, mainly precipitation with ethanol, sometimes followed by gel filtration (*17*−*21*) including the disadvantage of possible contamination of AGP preparations with polysaccharides and other glycoproteins. Up to now, mainly AG-peptides with a molecular mass of about 25 kDa have been isolated from wheat, always from endosperm or flour. These AG-peptides have been shown to play a role in the fabrication of bakery products (*19*, *22*, *23*).

The aim of our work was isolation of pure AGPs from whole grain of wheat by precipitation with βGlcY. Analytical characterization of AGPs from this source is an important contribution to enhance knowledge on potential beneficial constituents of whole grain from *Triticum aestivum*.

MATERIALS AND METHODS

Isolation. Whole grain from *T. aestivum* L. (cultivar RITMO) (obtained from Institute of Phytopathology, Christian-Albrechts-University of Kiel, Germany) was washed with ethanol (96% v/v) and, after drying and grinding, extracted with demineralized water (1:10) by maceration for 24 h at 4 °C. Insoluble material was filtered off. Starch was removed by centrifugation (20000*g*, 10 min) as well as by treatment with α-amylase (20 °C, pH 6.9, Sigma-Aldrich, Munich, Germany). Proteins were precipitated by heat denaturation (90 °C, 10 min) and removed afterward by centrifugation (5000*g*, 10 min). Subsequently, the extract was concentrated by evaporation and dialyzed (MWCO = 12000−14000). The high molecular weight fraction was analyzed with regard to monosaccharide composition and used for Yariv precipitation. The Yariv reagent (1,3,5-tris[4-β-glucopyranosyl-oxyphenylazo]-2,4,6-trihydroxybenzene) was synthesized according to the method of Yariv et al. (*15*). The aqueous solution of the high molecular weight fraction was precipitated with βGlcY according to the method of Kreuger and van Holst (*24*). Subsequently, the obtained freeze-dried crude AGP preparation was dissolved in demineralized water and centrifuged at 20000*g* (15 min, 20 °C). The supernatant was used for a second precipitation with βGlcY to obtain pure AGP.

Size Exclusion Chromatography. The molecular weights of the AGP and its hydrolysis products have been determined by size exclusion chromatography on three PL aquagel−OH MIXED 8 μm columns in series (column temperature = 35 °C, Polymer Laboratories, Darmstadt, Germany) with online multiangle laser light scattering (MALLS) detection (mini DAWN, Wyatt Technology Europe Ltd, Dernbach, Germany). The samples were eluted with 0.1 mol/L $NaNO_3$ at a flow rate of 1 mL/min.

*Author to whom correspondence should be addressed (telephone +49 431 8801144; fax +49 431 8801102; e-mail mgoellner@pharmazie.uni-kiel.de).

Published on Web 02/17/2010

Partial Acid Hydrolysis. Twenty milligrams of AGP was dissolved in 2 mL of 12.5 mM oxalic acid and heated at 100 °C for 5 h (*25*). The hydrolysate was cooled to room temperature, precipitated by the addition of ethanol (final concentration = 80% v/v), left overnight at 4 °C, and centrifuged (20000*g*, 10 min). After two washing steps with 80% ethanol, the precipitated residual polysaccharide was freeze-dried.

Alkaline Hydrolysis. Freeze-dried AGP was hydrolyzed in 0.44 N NaOH (105 °C, 18 h) (*26*), neutralized with HCl, dialyzed extensively against demineralized water (MWCO = 6000−8000, Spectra/Por, Houston, TX), and finally freeze-dried.

Monosaccharide Composition. To produce alditol acetates, the βGlcY-precipitated AGPs were hydrolyzed with trifluoroacetic acid (TFA, 2 mol/L) at 121 °C. After evaporation of TFA, monosaccharides were converted to alditol acetates by reduction and acetylation according to the method of Blakeney et al. (*27*) and analyzed by gas−liquid chromatography (GLC) on a fused silica capillary column (Optima-OV 225, 0.25 μm, L = 25 m, i.d. = 0.25 mm, Macherey-Nagel, Düren, Germany) using a gas chromatograph (HP 6890 Plus series; Hewlett-Packard, Nürnberg, Germany) with flame ionization detector. The helium flow rate was 1 mL/min and the oven temperature isothermal (230 °C); temperature of injector and detector was 240 °C. For quantitative analysis, a defined amount of *myo*-inositol was added to the samples as internal standard. For detection of uronic acids, monosaccharides of the AGP were converted into the corresponding trimethylsilyl ethers (TMS-derivates) and analyzed by GLC according to the method of Inngjerdingen et al. (*28*).

Linkage Analyses. Methylation was performed with potassium methylsulfinyl carbanion and methyl iodide in dimethyl sulfoxide as described by Harris et al. (*29*) Gas−liquid chromatography−mass spectrometry (GLC-MS) of partially methylated alditol acetates was done on a fused silica capillary column (0.25 i.d. × 25 m, OV-1701, Macherey-Nagel, Düren, Germany) using a gas chromatograph (HP 5890 series II, Hewlett-Packard, Nürnberg, Germany) with the following temperature program: 2 min at 170 °C, increase of 1 °C/min to 210 °C, 10 min at 210 °C. Helium flow was 0.7 mL/min. Mass spectra were recorded on a HP MS Engine 5898 A (Hewlett-Packard) instrument.

^{13}C Nuclear Magnetic Resonance Spectroscopy. The ^{13}C NMR spectrum of wheat AGP was recorded on a 75.47 MHz Fourier transformation spectrometer (Bruker, Bremen, Germany) at 300 K. Samples were dissolved in D_2O (10 mg/mL) with dioxane as standard (0.01%).

Amino Acid Composition of the Protein Part. The AGP was hydrolyzed with 6 N HCl at 110 °C for 22 h. After centrifugation, the supernatant was freeze-dried and separated by high-performance liquid chromatography (HPLC, column FGO369) in a buffer of lithium acetate with drifting pH from 2.95 to 10.3 and after-column derivatization of amino acids with ninhydrin. Amino acids were detected at 440 and 570 nm (Biochrom 30, Biochrom, Berlin, Germany).

Additionally, hydroxyproline was determined according to the method of Stegemann and Stalder (*30*) by oxidation of the imino acid by chloramine-T and coupling of the chromogen formed with Ehrlich's aldehyde in strong perchloric acid. The colored product was measured photometrically at 558 nm.

RESULTS AND DISCUSSION

Isolation and Monosaccharide Composition. During the isolation process of AGP, accompanying proteins were removed by heat denaturation. It is known that AGPs are generally stable against high temperatures, probably because their protein backbone is surrounded and protected by the polysaccharide moieties. After removal of proteins and starch, a high molecular weight fraction containing polysaccharides and arabinogalactan-proteins was isolated from an aqueous extract, which accounted for 0.9% w/w of the mass of the fruits. Isolation of this fraction was repeated four times. This high molecular weight fraction containing water-soluble polysaccharides and arabinogalactan-proteins is composed mainly of xylose (Xyl, 34.9%), arabinose (Ara, 30.3%), and galactose (Gal, 22.6%), together with minor amounts of glucose (Glc, 10.7%) and traces of mannose (Man), rhamnose (Rha), and fucose (Fuc, together 1.5%) (**Figure 1**). According to the literature, Xyl, Ara, and Glc had to be expected,

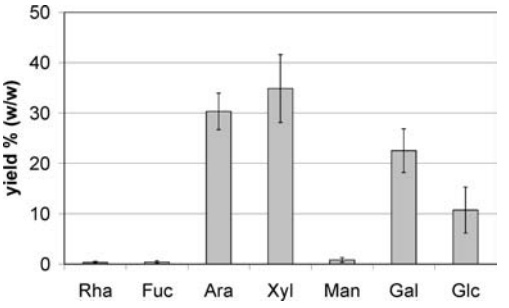

Figure 1. Monosaccharide composition of high molecular weight fraction (n = 4).

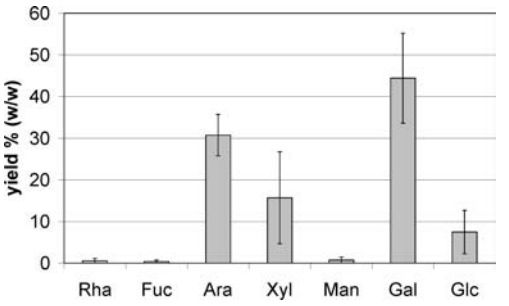

Figure 2. Monosaccharide composition of crude AGP (n = 10).

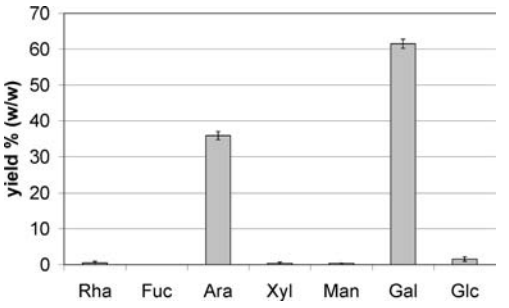

Figure 3. Monosaccharide composition of pure AGP (n = 2).

because arabinoxylans and (1→3)(1→4)-β-D-glucans are the important polysaccharides of wheat besides cellulose and starch (*31*, *32*).

From this fraction, an AGP has been isolated by precipitation with Yariv reagent. This experiment has been repeated 10 times. β-GlcY is known to precipitate most AGPs. The mechanism of this interaction is still not fully understood. It seems that self-association of Yariv phenylglycosides results in a higher order structure that is necessary for interaction with AGPs (*33*), but there is still no consensus on whether the carbohydrate or the protein part of AGP interacts with the βGlcY (*1*). Thus, structural details of AGPs necessary for binding remain to be elucidated. In the past it has been claimed that AGPs are absent in *Triticum* or show at least no reaction with βGlcY (*16*). This paper shows for the first time that isolation of AGPs from whole grain of wheat is possible by precipitation with βGlcY. As already mentioned, interaction of AGPs with βGlcY is regarded to be specific and should result in pure AGPs. Many AGPs isolated in the past consist of Gal and Ara and minor amounts of accompanying neutral or acidic monosaccharides (*34*, *35*). As these accompanying monosaccharides such as Rha, Xyl, Glc, GalA, or GlcA are often located at the periphery of AGPs, they might be important for the biological activities of these molecules. After a first Yariv precipitation, crude AGP from wheat consisted of 75.2% Ara and Gal in a ratio of 1:1.4 with the main accompanying monosaccharides

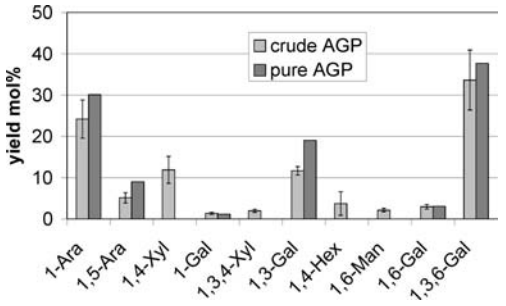

Figure 4. Linkage types of the monosaccharides of the carbohydrate moiety of crude and pure AGP.

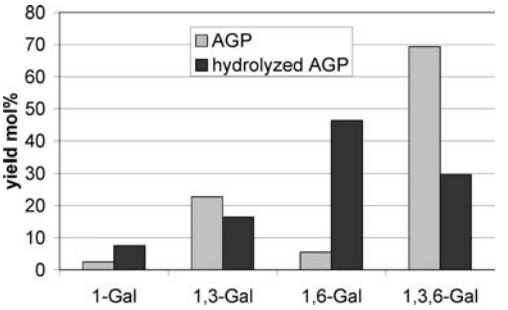

Figure 5. Linkage types of the monosaccharides of the carbohydrate moiety of AGP and hydrolyzed AGP regarding only the galactose part.

Xyl (15.7%) and Glc (7.5%) and traces of Man, Rha, and Fuc (together = 1.6%; **Figure 2**). Yield of this crude AGP was 0.01% w/w of the fruits.

Crude AGP was used for a second Yariv precipitation and yield of AGP decreased to 0.001% w/w of the fruits, but the content of Ara and Gal raised to 97.4% with Ara and Gal in a ratio of 1:1.7 and only traces of other monosaccharides (**Figure 3**). Silylation analysis showed that no uronic acids are present in this purified AGP.

In the plant, the function of AGPs might be related to binding of AGPs to other cell wall polysaccharides. Probably, after a single Yariv precipitation, small amounts of β-glucans and arabinoxylans remain attached to AGPs. According to Neukom and Markwalder (*36*), separation of AGPs from arabinoxylans is possible by precipitation of arabinoxylans with ammonium sulfate. With the help of a second Yariv precipition step, purification of AGPs from arabinoxylans and also other polysaccharides can be achieved. Although interaction of βGlcY with AGPs is specific, precipitation might lead to isolation of accompanying macromolecules which have been attached to the AGP before. This might be the case not only for polysaccharides but also for lectins present in plant tissue. For *Viscum album* it has been shown that a single Yariv precipitation leads to an AGP accompanied by traces of lectins (*37*). This leads to the conclusion that − depending on the plant material used for βGlcY precipitation − it has to be carefully verified whether small amounts of monosaccharides and amino acids are really part of the native AGP or might belong to accompanying molecules. In case of any doubt, a second Yariv precipitation should be state of the art.

Size Exclusion Chromatography. For wheat AGP a molecular mass of about 125 kDa has been determined by size exclusion chromatography with laser light scattering (LLS) detection. AG-peptides isolated from wheat endosperm show lower molecular masses between 22 and 31 kDa (*17*, *21*, *38*). On the other hand, higher molecular masses between 50 and 100 kDa have been determined for AG-peptides from wheat flour in comparison to pullulan standards (*20*, *39*). Alkaline degradation of our AGP, which hydrolyzes only the protein part of the AGP, resulted in polysaccharide moieties with a molecular mass of about 20 kDa, indicating that six polysaccharide moieties are attached to the protein core in AGP from whole grain of wheat.

Structural Characterization of the Polysaccharide Moiety of AGP. Linkage analysis of the permethylated alditol acetates gave the following overview of the linkage types of the monosaccharides of crude and pure AGP from wheat (**Figure 4**). In crude AGP higher amounts of 1,4-Xyl*p* and 1,3,4-Xyl*p* have been detected, indicating an arabinoxylan is still present in crude AGP preparation. Arabinoxylans consist of a linear 1,4-linked xylopyranosyl backbone. The xylose units can be unsubstituted or monosubstituted at position 3 or also disubstituted at positions 2 and 3 with arabinose residues. Structural variations of arabinoxylans can be indicated by the arabinose to xylose ratio with typical average values of 0.5−0.6; also, extreme values of 0.31 or 1.06 have been reported for arabinoxylans from wheat (*31*). It should be mentioned that GLC-MS mass spectra and retention times for permethylated alditol acetates of 1,2-Xyl*p* and 1,4-Xyl*p* are identical. In contrast to pure AGP, crude AGP also contains a small amount of 1,4-linked hexoses, indicating that some starch or some (1→3)(1→4)-β-D-glucans are still present.

In pure AGP, terminally linked Ara (30.1 mol %) and 1,5-linked Ara (9.0 mol %) have been found to be in the furanose form. Gal is present in the pyranose form, and the linkage types are 1,3,6-Gal*p* (37.7 mol %), 1,3-Gal*p* (19.0 mol %), 1,6-Gal*p* (3.0 mol %), and terminally linked Gal*p* (1.2 mol %). Carbohydrate moieties of wheat AGP can be classified as type-II arabinogalactans with a 1,3- and 1,3,6-Gal*p* backbone and side chains originating at O-6 of the backbone composed of 1,6-Gal*p* and terminal Ara*f* residues (*40*). Special features of wheat AGP are appreciable amounts of 1,5-linked Ara*f* residues as well as complete lack of uronic acids. High levels of 1,5-linked Ara*f* residues are associated with arabinans in the rhamnogalacturonan 1 pectic fraction (*41*) or with neutral arabinans (*42*) but have also been detected in different AGPs, for example, from *Echinacea* (*42*, *43*), *Acacia* (*44*), *Baptisia* (*45*), or *Pinus* (*35*). 1,5-Ara*f* might be part of the AG-polysaccharide. Another possibility would be that besides arabinogalactan polysaccharides, small 1,5-Ara*f* oligosaccharides are attached to the protein backbone in wheat AGP.

Lack of uronic acids has also been described for arabinogalactan-peptides from wheat (*17*, *18*, *20*, *21*), but in an AGP from maize, minor amounts of glucuronic acids have been detected (*46*).

Partial acid hydrolysis by mild oxalic acid caused Ara bonds to be cleaved preferentially, whereas Gal bonds are more or less stable under these conditions and resulted in a polysaccharide mainly composed of Gal*p*, leading to the conclusion that Ara*f* residues are located at the periphery of the molecule. After mild acid hydrolysis 1-Ara*f* and 1,5-Ara*f* decrease significantly from 24 to 6 mol % and from 5 to 0 mol %, respectively. **Figure 5** shows only the galactose linkage types of AGP before and after partial hydrolysis, without regard to arabinose residues. Oxalic acid treatment leads to a large increase of the relative proportion of 1,6-Gal*p* and a decrease in the 1,3,6-Gal*p* residues, concluding arabinose was linked mainly to position 3 of 1,3,6-Gal*p*. An increase of 1-Gal*p* residues and the missing increase of 1,3-Gal*p* also support the proposed structural model of the carbohydrate moiety described below.

The signals in the ^{13}C NMR spectrum for wheat AGP could be ascribed to different anomeric C-atoms of the sugar components of the AGP (**Table 1**). The presented chemical shifts are in close agreement with data for other AGPs (*47*−*50*). Thereby, it is also possible to distinguish between α- and β-configurations. All arabinose residues could be assigned to be in the α-configuration and all galactose units in the β-glycosidic configuration.

Table 1. Chemical Shifts in the ^{13}C NMR Spectra and Assignment to C-Atoms of Single Components of Wheat AGP

δ	C-atom	component
107.652	C_1	α-L-Ara*f*
101.633	C_1	β-D-Gal*p*, 1,3-β-D-Gal*p*, 1,6-β-D-Gal*p*, 1,3,6-β-D-Gal*p*
82.329	C_4	α-L-Ara*f*
79.765	C_2	α-L-Ara*f*
	C_4	1,5-α-L-Ara*f*
75.035	C_3	α-L-Ara*f*
71.876	C_5	β-D-Gal*p*, 1,3-β-D-Gal*p*, 1,6-β-D-Gal*p*, 1,3,6-β-D-Gal*p*
68.323	C_2	β-D-Gal*p*, 1,3-β-D-Gal*p*, 1,6-β-D-Gal*p*, 1,3,6-β-D-Gal*p*
66.932	C_4	β-D-Gal*p*, 1,3-β-D-Gal*p*, 1,3,6-β-D-Gal*p*
59.736	C_5	α-L-Ara*f*

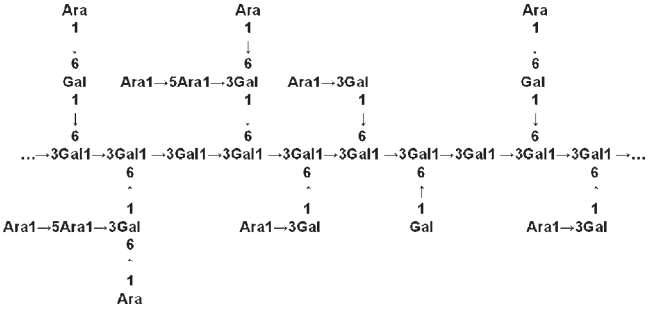

Figure 6. Structural proposal for the polysaccharide moiety of wheat AGP.

In summary, the analytical data led to the following structural proposal for the polysaccharide moiety of wheat AGP (**Figure 6**). Although the literature on AGP structures is abundant, reports on structural characterization of AGPs from monocotyledonous plants are very limited and mainly focus on arabinogalactan-peptides isolated from wheat endosperm by precipitation with ethanol (*17*, *18*, *20*, *38*, *51*−*53*). Structural models for these AG-peptides propose a peptide backbone with some Hyp residues being linked to the AG moieties (*17*, *38*, *51*). From endosperm of wheat, spelt, triticale, rye, and barley, van den Bulck et al. (*38*) isolated AG-peptides with molecular masses ranging between 22 and 33 kDa. The peptide core typically has three Hyp residues, each linked to a carbohydrate chain. The main monosaccharides are Gal and Ara, but minor amounts of Xyl and Glc have also been found. For the carbohydrate moieties, van den Bulck et al. (*38*) propose a 1,6-linked Gal*p* backbone, sometimes substituted in the C(O)3-position with a single Ara or Gal residue or a Gal-Ara disaccharide, respectively. These AG-peptides have been isolated only from flour gained from endosperm, whereas AGPs described here have been isolated from whole grain. It has to be elucidated whether smaller AG-peptides and larger AG-polysaccharides coexist in whole grain, maybe located in different tissues. Microscopic investigations revealed intense staining of cell walls of the aleurone layer of wheat grain by βGlcY, whereas the starchy endosperm remained unstained (*54*). It might be speculated that AG-peptides are located mainly in the starchy endosperm, whereas βGlcY-precipitable AGPs are presumably located in the outer regions of whole grain like the aleurone layer, testa, and pericarp, which are omitted as bran during flour production. Another possibility would be that AG-peptides are degradation products of AGPs, either due to enzymatic degradation processes in the plant or due to chemical degradation during isolation process, thereby losing their affinity to βGlcY (*19*). From *Lolium multiflorum*, an AGP of high molecular mass (220−280 kDa) has been isolated by precipitation with Yariv reagent and thoroughly characterized (*54*). There are great similarities to AGP from whole grain of wheat: an Ara to Gal ratio of approximately 1:2 with only trace amounts of neutral sugars and no uronic acids present, and a carbohydrate structure characterized by 1,3-, 1,6-, and 1,3,6-linked Gal*p* and terminal and 1,5-linked Ara*f* residues.

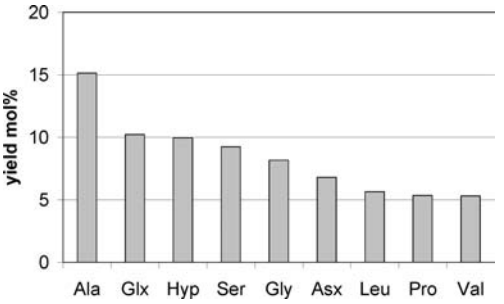

Figure 7. Composition of the protein fraction of pure AGP with amino acids >5 mol %.

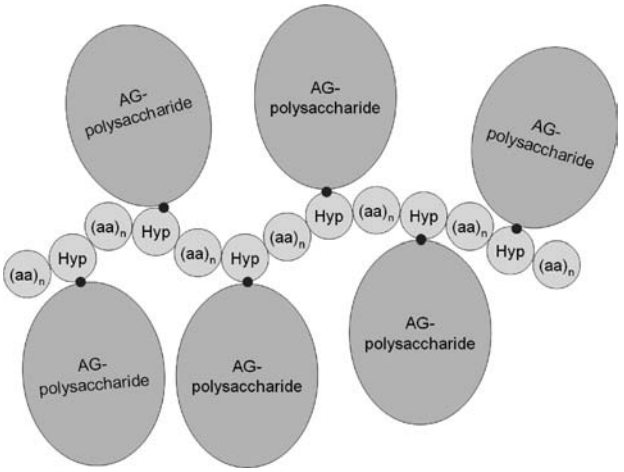

Figure 8. Structural proposal for wattle blossom model of wheat AGP (Hyp = hydroxyproline, aa = amino acid, AG = arabinogalactan).

Amino Acid Composition of the Core Protein of the AGP. Analyses of the amino acids of the pure AGP show the protein fraction to be characterized by high amounts of alanine (Ala, 15.1%), gutamine/glutamic acid (Glx, 10.2%), hydroxyproline (Hyp, 9.9%), serine (Ser, 9.2%), and glycine (Gly, 8.2%) as main components, accompanied by minor amounts of asparagine/aspartic acid (Asx, 6.8%), leucine (Leu, 5.6%), proline (Pro, 5.4%), and valine (Val, 5.3%) (**Figure 7**). Amino acids with molar concentration under 5% are not presented. Interestingly, there is great correspondence to amino acid data for AG-peptides isolated from wheat: Ala is the dominating amino acid, followed by Glx and Hyp and Ser in the fourth place (*17*, *51*). Hydroxyproline is known as the main amino acid responsible for O-glycosidic linkage between protein and polysaccharide moieties in AGPs. Strahm et al. (*51*) were able to exclude the possibility of *O*-glycosyl-serine or -threonine type of linkage for AG-peptides, because no β-elimination reaction products occurred after alkaline hydrolysis.

A high content of 0.59% w/w Hyp in the whole AGP was confirmed by photometric quantification of Hyp according to the method of Stegemann and Stalder (*30*). The protein content of AGP was 6.0% w/w calculated from amino acid analyses in combination with Hyp content found in the AGP.

Structural Model for Wheat AGP. On the basis of the results of structural analyses and alkaline hydrolysis of AGP, we propose that wheat AGP belongs to the wattle blossom type model, where large AG blocks are attached to the protein chain resulting in an overall globular structure (*55*, *56*). In wheat AGP, six polysaccharide moieties of about 20 kDa each should be attached to the protein core (**Figure 8**). For other AGPs, glycan chain sizes from

30 to 150 sugar residues have been estimated (*50*, *57*, *58*). On the basis of an Ara:Gal ratio of approximately 1:1.7 in wheat AGP, an arabinogalactan of 20 kDa has a glycan chain size of about 120 monosaccharides. Our investigations have confirmed that Yariv-precipitable AGPs are present in fruits of *T. aestivum*. Further work will be required to elucidate whether AGPs belong to the beneficial, especially immunostimulating, constituents of whole grain of wheat.

ACKNOWLEDGMENT

Silylation analysis was kindly performed by Prof. B. Smestad Paulsen (University of Oslo, Norway).

LITERATURE CITED

(1) Seifert, G. J.; Roberts, K. The biology of arabinogalactan proteins. *Annu. Rev. Plant Biol.* **2007**, *58*, 137–161.
(2) Showalter, A. M. Arabinogalactan-proteins: structure, expression and function. *Cell. Mol. Life Sci.* **2001**, *58* (10), 1399–1417.
(3) Gaspar, Y.; Johnson, K. L.; McKenna, J. A.; Bacic, A.; Schultz, C. J. The complex structures of arabinogalactan-proteins and the journey towards understanding function. *Plant Mol. Biol.* **2001**, *47*, 161–176.
(4) Bacic, A.; Currie, G.; Gilson, P.; Mau, S. L.; Oxley, D.; Schultz, C.; Sommer-Knudsen, J.; Clarke, A. E. Structural classes of arabinogalactan−proteins. In *Cell and Developmental Biology of Arabinogalactan−Proteins*; Nothnagel, E. A., Bacic, A., Clarke, A. E., Eds.; Kluwer Academic/Plenum Publishers: New York, 2000; pp 11−23.
(5) Majewska-Sawka, A.; Nothnagel, E. A. The multiple roles of arabinogalactan proteins in plant development. *Plant Physiol.* **2000**, *122*, 3–9.
(6) Gao, M.; Showalter, A. M. Yariv reagent treatment induces programmed cell death in Arabidopsis cell cultures and implicates arabinogalactan protein involvement. *Plant J.* **1999**, *19*, 321–331.
(7) Schultz, C.; Gilson, P.; Oxley, D.; Youl, J.; Bacic, A. GPI-anchors on arabinogalactan-proteins: implications for signalling in plants. *Trends Plant Sci.* **1998**, *3* (11), 426–431.
(8) Ding, L.; Zhu, J. K. A role for arabinogalactan-proteins in root epidermal cell expansion. *Planta* **1997**, *203* (3), 289–294.
(9) Letarte, J.; Simion, E.; Miner, M.; Kasha, K. J. Arabinogalactans and arabinogalactan-proteins induce embryogenesis in wheat (*Triticum aestivum* L.) microspore culture. *Plant Cell Rep.* **2006**, *24* (12), 691–698.
(10) Alban, S.; Classen, B.; Brunner, G.; Blaschek, W. Differentiation between the complement modulating effects of an arabinogalactan-protein from *Echinacea purpurea* and heparin. *Planta Med.* **2002**, *86*, 1118–1124.
(11) Thude, S.; Classen, B.; Blaschek, W.; Barz, D.; Thude, D. Binding studies of an arabinogalactan-protein from *Echinacea purpurea* to leucocytes. *Phytomedicine* **2006**, *13*, 425–427.
(12) Classen, B.; Thude, S.; Blaschek, W.; Wack, M.; Bodinet, C. Immunmodulatory effects of arabinogalactan-proteins from *Baptisia* and *Echinacea*. *Phytomedicine* **2006**, *13*, 688–694.
(13) Robinson, R. R; Swennen, K.; Feirtag, J.; Slavin, J. L. Effects of dietry arabinogalactan on gastrointestinal and blood parameters in healthy human subjects. *J. Am. Coll. Nutr.* **2001**, *20* (4), 279–285.
(14) Yariv, J.; Lis, H.; Katchalski, E. Precipitation of arabic acid and some seed polysaccharides by glycosylphenylazo dyes. *Biochem. J.* **1967**, *105*, 1C–2C.
(15) Yariv, J.; Rapport, M. M.; Graf, L. The interaction of glycosides and saccharides with antibody to the corresponding phenylazo glycosides. *Biochem. J.* **1962**, *85*, 383–388.
(16) Jermyn, M. A.; Yeow, Y. M.; Woods, E. F. Class of lectins present in the tissues of seed plants. *Aust. J. Plant Physiol.* **1975**, *2* (4), 501–531.
(17) Fincher, G. B.; Stone, B. A. A water-soluble arabinogalactan-peptide from wheat endosperm. *Aust. J. Biol. Sci.* **1974**, *27* (2), 117–132.
(18) Ingelbrecht, J. A.; Loosveld, A. M. A.; Grobet, P. J.; Schols, H.; Bakx, E.; Delcour, J. A. Characterization of the carbohydrate part of arabinogalactan peptides in *Triticum durum* Desf. *semolina*. *Cereal Chem.* **2002**, *79* (2), 322–325.
(19) Loosveld, A. M. A.; Delcour, J. A. The significance of arabinogalactan-peptide for wheat flour bread-making. *J. Cereal Sci.* **2000**, *32* (2), 147–157.
(20) Loosveld, A. M. A.; Maes, C.; Van Casteren, W. H. M.; Schols, H. A.; Grobet, P. J.; Delcour, J. A. Structural variation and levels of water-extractable arabinogalactan-peptide in European wheat flours. *Cereal Chem.* **1998**, *75* (6), 815–819.
(21) McNamara, M. K.; Stone, B. A. Isolation, characterization and chemical synthesis of a galactosyl-hydroxyproline linkage compound from wheat endosperm arabinogalactan-peptide. *Lebensm. -Wiss. -Technol.* **1981**, *14* (4), 182–187.
(22) Schroder, M.; Soe, J. B.; Zargahi, M. R.; Rouau, X. Production of substrate for galactose oxidase by depolymerisation of an arabinogalactan-peptide from wheat flour. *J. Agric. Food Chem.* **1999**, *47*, 1483–1488.
(23) Guttieri, M. J.; Souza, E. J.; Sneller, C. Nonstarch polysaccharides in wheat flour wire-cut cookie making. *J. Agric. Food Chem.* **2008**, *56*, 10927–10932.
(24) Kreuger, M.; van Holst, G. J. Arabinogalactan-protein epitopes in somatic embryogenesis of *Daucus carota* L. *Planta* **1995**, *197*, 135–141.
(25) Gleeson, P. A.; Clarke, A. E. Structural studies on the major component of *Gladiolus* style mucilage, an arabinogalactan-protein. *Biochem. J.* **1979**, *181* (3), 607–621.
(26) Tan, L.; Qiu, F.; Lamport, D. T. A.; Kieliszewski, M. J. Structure of a hydroxyproline (Hyp)-arabinogalactan polysaccharide from repetitive Ala-Hyp expressed in transgenic *Nicotiana tabacum*. *J. Biol. Chem.* **2004**, *279* (13), 13156–13165.
(27) Blakeney, A. B.; Harris, P. J.; Henry, R. J.; Stone, B. A. A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydr. Res.* **1983**, *113*, 291–299.
(28) Inngjerdingen, K. T.; Debes, S. C.; Inngjerdingen, M.; Hokputsa, S.; Harding, S. E.; Rolstad, B.; Michaelsen, T. E.; Diallo, D.; Paulsen, B. S. Bioactive pectic polysaccharides from *Glinus oppositifolius* (L.) Aug. DC., a Malian medicinal plant, isolation and partial characterization. *J. Ethnopharmacol.* **2005**, *101*, 204–214.
(29) Harris, P. J.; Henry, R. J.; Blakeney, A. B.; Stone, B. A. An improved procedure for the methylation analysis of oligosaccharides and polysaccharides. *Carbohydr. Res.* **1984**, *127*, 59–73.
(30) Stegemann, H.; Stalder, K. H. Determination of hydroxyproline. *Clin. Chim. Acta* **1967**, *18*, 267–273.
(31) Ordaz-Ortiz, J. J.; Saulnier, L. Structural variability of arabinoxylans from wheat flour. Comparison of water-extractable and xylanase-extractable arabinoxylans. *J. Cereal Sci.* **2005**, *42*, 119–125.
(32) Wood, P. J.; Beer, M. U. Functional oat products. In *Functional Food: Biochemical and Processing Aspects 1*; Mazza, G., Ed.; Technomic Puplishing: Lancaster, PA, 1998; pp 1−37.
(33) Nothnagel, E. A. Proteoglycans and related components in plant cells. *Int. Rev. Cytol.* **1997**, *174*, 195–291.
(34) Nunes, F. M.; Reis, A.; Silva, A. M. S.; Domingues, M. R. M.; Coimbra, A. M. Rhamnoarabinosyl and rhamnoarabinoarabinosyl side chains as structural features of coffee arabinogalactans. *Phytochemistry* **2008**, *69*, 1573–1585.
(35) Putoczki, T. L.; Pettolino, F.; Griffin, M. D. W.; Moeller, R.; Gerrard, J. A.; Bacic, A.; Jackson, S. L. Characterization of the structure, expression and function of *Pinus radiata* D. Don arabinogalactan-proteins. *Planta* **2007**, *226*, 1131–1142.
(36) Neukom, H.; Markwalder, H. Isolation and characterization of an arabinogalactan from wheat flour. *Carbohydr. Res.* **1975**, *39*, 387–389.
(37) Herbst, B.; Classen, B.; Blaschek, W. Characterisation of arabinogalactan-proteins from *Viscum album* L. berries and herb. In *Misteltoe in Therapy of Tumor 2*; Scheer, R., Alban, S., Becker, H., Holzgrabe, U., Kemper, F. H., Kreis, W., Matthes, H., Schilcher, H., Eds.; KVC Publisher: Essen, Germany, 2009; pp 121−132.
(38) Van den Bulck, K.; Swennen, K.; Loosveld, A. M. A.; Courtin, C. M.; Brijs, K.; Proost, P.; Van Damme, J.; Van Campenhout, S.; Mort, A.; Delcour, J. A. Isolation of cereal arabinogalactan-peptides and structural comparison of their carbohydrate and peptide moieties. *J. Cereal Sci.* **2005**, *41*, 59–67.

(39) Izydorczyk, M.; Biliaderis, C. G.; Bushuk, W. Comparison of the structure and composition of water-soluble pentosans from different wheat varieties. *Cereal Chem*. **1991**, *68* (2), 139–144.
(40) Aspinall, G. O. Chemical characterization and structure determination of polysaccharides. In *The Polysaccharides 1*; Aspinall, G. O., Ed.; Academic Press: NewYork, 1982; pp 35−131.
(41) Willats, W.; McCartney, L.; Mackie, W.; Knox, J. P. Pectin: cell biology and prospects for functional analysis. *Plant Mol. Biol*. **2001**, *47*, 9–27.
(42) Thude, S.; Classen, B. High molecular weight constituents from roots of *Echinacea pallida*: an arabinogalactan-protein and an arabinan. *Phytochemistry* **2005**, *66* (9), 1026–1032.
(43) Classen, B.; Witthohn, K.; Blaschek, W. Characterization of an arabinogalactan-protein isolated from pressed juice of *Echinacea purpurea* by precipitation with the β-glucosyl Yariv reagent. *Carbohydr. Res*. **2000**, *327*, 497–504.
(44) Mollard, M.; Basile, D. *Acacia senegal* cells cultured in suspension secrete a hydroxyproline-deficient arabinogalactan protein. *Plant Physiol. Biochem*. **2000**, *32*, 703–709.
(45) Wack, M.; Classen, B.; Blaschek, W. An acidic arabinogalactan-protein from the roots of *Baptisia tinctoria*. *Planta Med*. **2005**, *71*, 814–818.
(46) Kieliszewski, M. J.; Kamyab, A.; Leykam, J. F.; Lamport, D. T. A. A histidine-rich extensin from *Zea mays* is an arabinogalactan protein. *Plant Physiol*. **1992**, *99* (2), 538–547.
(47) Wack, M. Isolation and characterization of arabinogalactan-proteins from *Baptisia tinctoria* and *Baptisia australis*. *Dissertation*, Christian-Albrechts-University of Kiel, Germany, **2004**.
(48) Joseleau, J. P.; Chambat, G. ^{13}C-NMR spectroscopy to identify complex polysaccharide fractions from the plant cell wall. *Physiol. Veg*. **1984**, *22* (4), 461–470.
(49) Samuelsen, A.; Paulsen, B. S.; Wold, J. K.; Knutsen, S. H.; Yamada, H. Characteruzation of a biologically active arabinogalactan from the leaves of *Plantago major* L. *Carbohydr. Polym*. **1998**, *35*, 145–153.
(50) Gane, A. M.; Craik, D.; Munro, S. L. A.; Howlett, G. J.; Clarke, A. E.; Bacic, A. Structural analysis of the carbohydrate moiety of arabinogalactan-proteins from stigmas and styles of *Nicotiana alata*. *Carbohydr. Res*. **1995**, *277*, 67–85.
(51) Strahm, A.; Amado, R.; Neukom, H. Hydroxyproline-galactoside as a protein−polysaccharide linkage in a water soluble arabinogalactan-peptide from wheat endosperm. *Phytochemistry* **1981**, *20* (5), 1061–1063.
(52) Loosveld, A. M. A.; Grobet, P. J.; Delcour, J. A. Contents and structural features of water-extractable arabinogalactan in wheat flour fractions. *J. Agric. Food Chem*. **1997**, *45*, 1998–2002.
(53) Van den Bulck, K.; Loosveld, A. M. A.; Courtin, C. M.; Proost, P.; Van Damme, J.; Robben, J.; Mort, A.; Delcour, J. A. Amino acid sequence of wheat flour arabinogalactan-peptide, identical to part of grain softness protein GSP-1, leads to improved structural model. *Cereal Chem*. **2002**, *79* (3), 329–331.
(54) Anderson, R. L.; Clarke, A. E.; Jermyn, M. A.; Knox, R. B.; Stone, B. A. A carbohydrate-binding arabinogalactan-protein from liquid cultures of endosperm from *Lolium multiflorum*. *Aust. J. Plant Physiol*. **1977**, *4*, 143–158.
(55) Bacic, A.; Currie, G.; Gilson, P.; Mau, S. L.; Oxley, D.; Schultz, C.; Sommer-Knudsen, J.; Clarke, A. E. Structural classes of arabinogalactan−proteins. In *Cell and Developmental Biology of Arabinogalactan-Proteins*; Nothnagel, E. A., Bacic, A, Clarke, A. E., Eds.; Kluwer Academic/Plenum Publishers: New York, 2000; pp 11−23.
(56) Fincher, G. B.; Stone, B. A.; Clarke, A. E. Arabinogalactan-proteins: structure, biosynthesis, and function, Poaceae. *Annu. Rev. Plant Physiol*. **1983**, *34*, 47–70.
(57) Qi, W.; Fong, C.; Lamport, D. T. A. Gum arabic glycoprotein is a twisted hairy rope: a new model based on *O*-galactosylhydroxyproline as the polysaccharide attachment site. *Plant Physiol*. **1991**, *96* (3), 848–855.
(58) Tsumuraya, Y.; Hasimoto, Y.; Yamamoto, S.; Shibuya, N. Structure of L-arabino-D-galactan-containing glycoproteins from radish leaves. *Carbohydr. Res*. **1984**, *134* (2), 215–228.

Received for review November 11, 2009. Revised manuscript received February 11, 2010. Accepted February 11, 2010.