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Note

Oxidative cross-linking of corn bran hemicellulose: formation of ferulic acid dehydrodimers

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Abstract

The aim of this study was to investigate the nature and extent of ferulate dehydrodimer cross-links formed during peroxide and peroxidase-catalysed gelation of an arabinoxylan ferulate polysaccharide from American Corn Bran (*Zea mays* L.). Alkali-soluble phenolics from the arabinoxylan (AXF) and its gel (AXFG), were identified and quantified by HPLC. AXF was rich in *trans*-ferulic acid and contained small quantities of 8-0-4′-diferulic acid ((*Z*)-β-(4-((*E*)-2-carboxyvinyl)-2-methoxyphenoxy)-4-hydroxy-3-methoxycinnamic acid; 8-0-4′-DiFA); 8,5′-diferulic acid benzofuran form (*trans*-5-((*E*)-2-carboxyvinyl)-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran-3-carboxylic acid; 8,5′-DiFA benzofuran form); and 5,5′-diferulic acid ((*E,E*)-4,4′-dihydroxy-5,5′dimethoxy-3′-bicinnamic acid; 5,5′-DiFA) in addition to vanillin and *trans*-coumaric acid. Gel formation resulted in a considerable increase in the levels of 8-0-4′-DiFA, 8,5′-DiFA benzofuran form and the appearance of significant quantities of 8,8′-diferulic acid open form (4,4′-dihydroxy-3,3′-dimethoxy-β,β′-bicinnamic acid; 8,8′-DiFA); 8,5′-diferulic acid open form ((*E,E*)-4,4′dihydroxy-3,5′-dimethoxy-β,3′-bicinnamic acid; 8,5′-DiFA) and 8,8′-diferulic acid aryltetralin form (*trans*-7-hydroxy-1-(4-hydroxy-3-methoxy-phenyl)-6-methoxy-1,2-dihydronaphthalene-2,3-dicarboxylic acid; 8,8′-DiFA aryl form). The results confirm that peroxidase/peroxide-induced gelation results from oxidative cross-linking of ferulic acid moieties. © 1997 Elsevier Science Ltd.

Keywords: Gel; Arabinoxylan; Cross-linking; Diferulic acid; Cell walls

1. Introduction

In recent years, the potential for phenolic-ester cross-linking of polysaccharides in-vitro has stimulated keen interest because of its possible importance in gelation [1,2]. Ferulic acid-rich pectic polysaccharides have been shown to undergo gelation in the presence of peroxidase and H_2O_2 [2]. Similarly, GB Biotechnology Ltd. 'Supergel' hemicellulose powder from American Corn Bran (*Zea mays* L.), substantially an arabinoxylan ferulate polysaccharide (AXF),

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can be converted with peroxidase and hydrogen-peroxide under controlled conditions to thermostable (sterilisable) cold-setting clear, brittle gels (AXFG) [3]. The properties of these natural biogels are such that they are of interest to the pharmaceutical and food industries in development of new and diverse products. However, there is no direct information on the nature of the cross-links formed during the gelling process. The aim of this study was to use recently-developed analytical techniques [4] to investigate the nature and extent of ferulate cross-links formed during gelation.

2. Results and discussion

The gel AXFG was found to be cold-setting and the viscosity could be controlled from a soft gel through to a firm, vibrant brittle gel; soft gels as low as 0.05% w/v or into a strong ringing gel at 1% w/v and up to 3% w/v could be made [3]. The gel was also thermostable (autoclavable, 121 °C, 115 psi) and quite stable in the semi- and fully-set condition with no apparent change over several months at elevated (25, 35, 45 and 50 °C) temperature. Thus the gel behaves as a 'perfect' gel with properties similar to those of synthetic gels [3].

AXF was analysed for carbohydrate composition after hydrolysis in 72% sulfuric acid [5] as described [6]. The absence of starch was confirmed by negative staining with I/I_2. AXF was rich in Ara*f*, Xyl*p* and UA (Glc*p*A) in ratios indicative of a highly-branched arabinoglucuronoxylan polymer (Table 1).

Alkali-labile phenolics were analysed by HPLC with diode-array detection [4]. Ferulic acid was identified as the major wall phenolic in AXF, however vanillin and *p*-coumaric acid were also present (Table 2). In addition, approximately 13% of the ferulic acid present was in the form of three ferulic acid dehydrodimers (Fig. 1). These were (1) 8-0-4′-diferulic acid ((*Z*)-β-(4-((*E*)-2-carboxyvinyl)-2-methoxyphenoxy)-4-hydroxy-3-methoxycinnamic acid; 8-0-4′-DiFA); (2) 8,5′-diferulic acid benzofuran form

Table 1
Carbohydrate compositions of AXF and AXFG

	Carbohydrate (mol%)								Total μg/mg
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA	
AXF	0.2	–	28.2	47.7	–	5.7	2.5	16.0	480.5
AXFG	0.3	–	27.5	49.0	–	5.9	2.2	14.3	491.9

Table 2
Total esterified phenolic acids content of AXF and AXFG (μg/g)

Phenolic	AXF	AXFG
Vanillic acid	–	61
p-Hydroxybenzaldehyde	–	11
Vanillin	40	94
trans-Coumaric acid	342	332
8-8′-DiFA (Aryltetralyn form)	–	406
trans-Ferulic acid	6815	1174
8-8′-DiFA	–	359
8-5′-DiFA	–	604
cis-Ferulic acid	406	–
5-5′-DiFA	389	396
Unknown A	56	94
8-0-4′-DiFA	331	1109
8-5′-DiFA (Benzofuran form)	346	2260

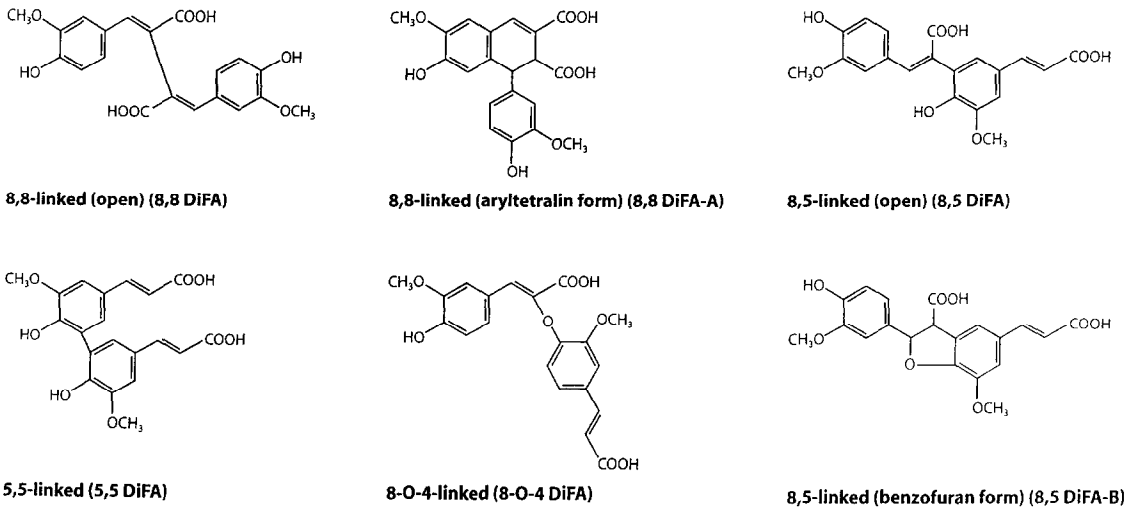

Fig. 1. Ferulic acid dimers detected in AXF and AXFG.

(*trans*-5-((*E*)-2-carboxyvinyl)-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran-3-carboxylic acid; 8,5′-DiFA benzofuran form); and (3) 5,5′-diferulic acid ((*E*,*E*)-4,4′-dihydroxy-5,5′dimethoxy-3′-bicinnamic acid; 5,5′DiFA). The 8-0-4′-DiFA has now been shown to be the major dehydrodimer in several species [4,7,8]. However, in the extracted AXF, it was at lower levels than both of the other dehydrodimers. One other unidentified component ('A', Table 2) with spectral properties similar, but not identical, to those of 5-5′ DiFA was also detected.

Supergel made with 2% (w/w) AXF (AXFG) was analysed for its carbohydrate composition. The carbohydrate composition of AXFG was essentially the same as the parent AXF (Table 1). However, the phenolic moieties exhibited marked differences. Gelation was accompanied by considerable increases in 8-0-4′-DiFA and 8-5′-DiFA (benzofuran form) although there was little change in 5,5′-DiFA. In addition, gelation resulted in the appearance of significant levels of three other dimers (Fig. 1): (1) 8,8′-diferulic acid open form (4,4′-dihydroxy-3,3′dimethoxy-β,β′-bicinnamic acid; 8,8′-DiFA); (2) 8,5′-diferulic acid open form ((*E*,*E*)-4,4′-dihydroxy-3,5′dimethoxy-β,3′-bicinnamic acid; 8,5′-DiFA); and (3) 8,8′diferulic acid aryltetralin form (*trans*-7-hydroxy-1-(4-hy- droxy-3-methoxy-phenyl)-6-methoxy-1,2-dihydro- naphthalene-2,3-dicarboxylic acid; 8,8′-DiFA aryl form). The unknown component 'A' also increased by a factor of 2.

The gelling-related increase in the ferulic acid dimers was consistent with the peroxidase-catalysed oxidative cross-linking of the ferulic acid moieties. The average molecular weight of AXF was approximately 1×10^6 [3], hence a 2% (w/w) solution would be approximately 20 μM. Since the peroxide-induced increase in diferulic acid moieties was of the order of 212 μM, it appears that gelation was accompanied by an increase in cross-links in the order of 10 per AXF molecule. Interestingly, the decrease in monomeric ferulic acid was greater than the relative increase in diferulic acids. This may be due to degradation or, more probably, polymerisation of the ferulic acid to forms undetectable by the methodology used. We have noted a similar situation in peroxide-catalysed cross-linking of FA in cell walls of sugarbeet (Ng et al., unpublished). Interestingly, Grabber et al. [9], who investigated hydrogen-peroxide and peroxidase-catalysed cross-linking of ferulates in maize suspension culture cell walls, reported that only a fraction of the ferulates cross-linked into lignin were releasable by solvolysis, highlighting the abundance of normally underestimated cross-linked structures. Hence, it is likely that AXF is more highly cross-linked in the final gel, than our results indicate.

The thermal stability of the gel was due to the cross-linking of thermally-stable arabinoxylan hemicellulose by thermally-stable phenolic acid dehydrodimers. The thermal stability of such phenolic cross-links has also been implicated in the thermal stability of cell-adhesion and therefore texture in Chinese water chestnut [7] and sugarbeet [10]. The ratios of the phenolic dehydrodimers in AXFG is significantly different to that in the cell walls of Chinese water chestnut. This suggests that the cross-linking mechanism may be affected by the specificity of the peroxidase enzymes and nature of the polysaccharides to which the ferulic acid moieties are esterified.

3. Experimental

Gelation.—GB Biotechnology Ltd. (GB) have developed a patented process [3] to produce hemicellulose extracts from which a dry powder can be made called 'SuperGel'. Gel (AXFG) was prepared by sprinkling the SuperGel hemicellulose powder (AXF) prepared from American Corn Bran (*Zea mays* L.) onto the surface of the aqueous solvent at room temperature to a concentration of 2% w/v. The pH was adjusted to 7.1 with dilute (0.5–1% w/v) KOH and stirring continued for at least 30 min. When dissolution was complete, the pH was adjusted to between 5.5 and 7.5 as required. To 100 ml of the solution, peroxidase (horse-radish, 6 drops of 1 mg/ml) was then added, followed by H_2O_2 (0.1 ml of 3.5% w/v). Gelation occurred within 1–2 min, and was complete within 30–60 min.

Analysis.—Phenolic acids were extracted for 24h in alkali (KOH, 2M), and then identified and quantified by HPLC with diode-array detection as described [4]. Cell-wall neutral sugars and uronic acids were analyzed as described [6]. All data presented are means of duplicate analyses, with standard deviations of less than 4%.

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