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Demethylation of a model homogalacturonan with the salt-independent pectin methylesterase from citrus: Part II. Structure–function analysis

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Abstract

Non-calcium sensitive pectin (NCSP), with a 94% degree of methylation (DM) was demethylated at pH 4.5 and 7.5 using monocomponent citrus salt-independent pectin methylesterase (PME). In a companion publication [Cameron, R. G., Luzio, G. A., Goodner, K., & Williams, M. A. K. (2008). Demethylation of a model homogalacturonan with the salt-independent pectin methylesterase from citrus: I. Effect of pH on demethylated block size and distribution. *Carbohydrate Polymers*], average demethylated blocks size ($\overline{BS}$), average number of demethylated blocks per molecule ($\overline{BN}$) and enzyme mode of action were characterized for a series of pectins using a *limited* digest with endo-polygalacturonase (EPG) to release demethylated blocks (DMBs) from each demethylated pectin. Rheology and calcium sensitivity measurements, on the same series of pectins, are presented herein to relate $\overline{BS}$ and $\overline{BN}$ values to functional properties. For the pH 4.5 – 50% DM sample with an excised $\overline{BS} = 8.6$ and $\overline{BN} = 3.9$, greater than 95% of the molecules appeared to be crosslinked with calcium ion and a storage modulus (G') value of 7.31 Pa was observed. For the pH 4.5 – 70% DM. The data indicated that for the pH 7.5 series, block number could be a limiting factor. The excised block size for the pH 7.5–60% DM sample was comparatively large at 12.2 compared to the pH 4.5 series. In contrast, block number of at least 2.0 appeared to be required before a significant level of calcium sensitivity was observed in the pH 7.5 series. Mixing the pH 4.5 – 70% DM sample with narrow-range size-classes of oligogalacturonic acids (OGalA) showed that small galacturonic acid oligomers with a degree of polymerization (DP) less than 13 could lower yield point (YP) and affect gelation, suggesting that even small excised DMBs need to be considered when relating pectin block size to functional properties. Published by Elsevier Ltd.

Keywords: Endo-polygalacturonase; Polysaccharide; Homogalacturonan; Rheology; Yield point; Storage modulus; G'; Loss modulus: G''; Mapping; Block size; Block number

1. Introduction

Citrus pectin, a complex polysaccharide, is composed of at least five different sugar moieties but 80–90% of its dry weight is galacturonic acid (GalA). The majority of the GalA is found in homogalacturonan (HG) regions of pectin, unbranched polymers of GalA in which a variable proportion of the GalA residues contain a methyl ester at their C6 position (Ridley, O'Neill, & Mohnen, 2001; Vincken et al., 2003). Pectin's functional properties and reactivity toward calcium and other cations is largely dependent on the amount of methylated GalAs and their distribution pattern within the HG stretches (Powell, Morris, Gidley, & Rees, 1982; Willats et al., 2001). Two general patterns of methyl ester distribution are recognized, random or ordered (Willats et al., 2001). Demethylation of pectin can be accomplished by enzymatic (PME) or chemical (alkaline demethylation) means. For enzymatic demethylation three different modes of action have been hypothesized (Denes, Baron, Renard, Pean, & Drilleau, 2000), two of which lead to blockwise (ordered) removal of esters.

Previous studies have demonstrated that plant PMEs can demethylate pectin in an apparent ordered process,

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described as processive hydrolysis of the methyl esters. The details of this catalytic process are not well understood. Catalysis by plant PMEs creates DMBs (Daas, Meyer-Hansen, Schols, De Ruiter, & Voragen, 1999; Denes et al., 2000; Duvetter et al., 2006; Hotchkiss et al., 2002; Kim, Teng, & Wicker, 2005; Limberg et al., 2000a; Limberg et al., 2000b; Savary, Hotchkiss, & Cameron, 2002). The presence or absence of these blocks and how these blocks are produced is important since it affects the reactivity of pectin with cations such as calcium. The presence of blocks and the nature of calcium ion crosslinks of blocks is believed to follow an egg box type interaction, or junction zone, in which two HG chains containing a sequence (or DMB) of adjacent non-esterified GalA units are coordinated to a middle layer of calcium ions (Grant, Morris, Rees, Smith, & Thom, 1973; Kohn, 1975; Limberg et al., 2000a; Rees, 1972; Rees, 1981). A minimum number of 9 consecutive non-esterified units was determined for an interaction to occur (Liners, Thibault, & Van Cutsem, 1992). Other determinations have been made where the minimum number was estimated to be 8 to 12 GalA units (Powell et al., 1982). In separate work threshold values of 15–20 residues were estimated for cooperative binding of Ca^{2+} by OGalA in an egg box-like system (Kohn & Furda, 1967; Kohn & Luknar, 1977) and in modeling for junction zones interactions (Braccini & Perez, 2001).

Extensive analyses for the presence of DMBs in pectin have been published. Lengths of the DMBs have been estimated with indirect statistical methods on GalA oligomers with varying DMs (Catoire, Pierron, Morvan, du Penhoat, & Goldberg, 1998; Denes et al., 2000). DMB length also has been estimated by enzymatic methods using exo-polygalacturonase (exo-PG) and EPG (Limberg et al., 2000a). Lengths of partially esterified blocks were estimated by (Limberg et al., 2000b) using pectin lyase (cleaves the HG in highly methylated regions), although since the enzyme cleaves within a fully methylated stretch, these fragments would contain a portion of a methylated fragment. Proton NMR resonances for H-4 and H-5 protons of $GalA_1$ have different chemical shifts depending on whether the GalA unit and its next neighbors are methylated (Andersen, Larsen, & Grasdalen, 1995; Grasdalen, Einar Bakoy, & Larsen, 1988), have been used to determine the ratio of an unmethylated GalA triad sequence relative to mixed or fully methylated triads. Enzymatic fingerprinting using endopectin lyase and EPG II (Limberg et al., 2000b) and characterization using pectin-specific antibodies (Willats et al., 2000) revealed discernible differences between the methyl-esterification patterns on the model pectins produced by the action of plant PME (producing blockwise demethylation) and fungal PME or base catalysis (producing random demethylation). EPG has been used for analyzing methylation patterns with special focus on the presence of DMBs in the analyzed pectins (Daas et al., 1999). Another approach (Limberg et al., 2000a) was used with a combination of EPG and exo-PG to quantify the amount of GalA units located in DMBs by measuring the amount of liberated GalA after combined digestion.

PME action patterns and the presence of DMBs have been shown to have significant effects on the rheological properties of pectin (Luzio, 2003; Powell et al., 1982; Schmelter, Wientjes, Vreeker, & Klaffke, 2002; Willats et al., 2001). Yield stress measurements for pectins with a blockwise distribution of unmethylated GalAs, indicate that interchain associations in the presence of calcium ion are stronger than those involving pectins with random distributions for DM values greater than 45% (Powell et al., 1982). Rheological properties of pectins have been related to measurements of degree of blockiness (DB), calculated by measuring $GalA_1$, $GalA_2$ and $GalA_3$ released from pectins treated with EPG (Lofgren, Guillotin, Evenbratt, Schols, & Hermansson, 2005). A high DB value can be related to the blockwise distribution of unmethylated GalA residues in pectin (Daas, Voragen, & Schols, 2000; Daas et al., 1999).

Since DM and DMB size are related to pectin functionality, detailed knowledge of pectin fine-structure, block sizes and their numbers could aid in understanding functional properties obtained from rheological measurements. Chromatography has allowed for separation and detection of GalA oligomers (Cameron, Luzio, Kauffman, & Grohmann, 2004; Hotchkiss, Lecrinier, & Hicks, 2001). Now it is also possible to quantify individual GalA oligomer fractions by HPLC using an evaporative light scattering detector (Cameron & Grohmann, 2005; Cameron, Luzio, Goodner, & Williams, 2008). As reported in a companion publication (Cameron et al., 2008), a demethylated pectin series was produced by a salt-independent citrus PME from high DM pectin that originally contained no DMBs and was not calcium sensitive. For this demethylated pectin series, average demethylated blocks size ($\overline{BS}$), average number of demethylated blocks per molecule ($\overline{BN}$) and enzyme mode of action were characterized using a *limited* digest with endo-polygalacturonase (EPG) to release demethylated blocks (DMBs) from each demethylated pectin.

The work reported herein focuses on determining the functional properties of the demethylated pectin series prepared and structurally analyzed in the companion study. The functional properties measured were: the relative amount of calcium sensitive pectin as measured by the calcium sensitive pectin ratio (CSPR), yield point (YP), storage modulus (G') and loss modulus (G'') values in the presence of calcium ion. The purpose is to relate structural determinations with functional properties. Resulting rheological and calcium sensitivity properties are discussed with regards to results from block size and number characterization of the demethylated pectin series.