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Review

Biocatalytic cross-linking of pectic polysaccharides for designed food functionality: Structures, mechanisms, and reactions

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ABSTRACT

Recent research has demonstrated how cross-linking of pectic polysaccharides to obtain gel formation can be promoted by enzymatic catalysis reactions, and provide opportunities for functional upgrading of pectic polysaccharides present in agro-industrial sidestreams. This review highlights the mechanisms of formation of functional pectic polysaccharide cross-links, including covalent cross-links (notably phenolic esters and uronyl ester linkages) and non-covalent, ionic cross-links (which involve calcium and borate ester links). The treatise examines how such cross-links can be designed *via* specific enzymatic reactions, and highlights the most recent data concerning enzyme catalyzed engineering of cross-links for in situ structural design of functional properties of foods.

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1. Introduction

There is a large body of research data available that has aimed at unraveling the structures of plant cell wall polysaccharides, including pectin and various pectic polysaccharide elements, in order to understand their biosynthesis and cell wall functionality *in planta*

Abbreviations: AX, arabinoxylan; DE, degree of methoxylation; DP, degree of polymerization; diFA, dehydrodimer of ferulic acid; FA, ferulic acid; Gal*p*A, galacturonic acid; HG, homogalacturonan; PME, pectin methyl esterase; RG, rhamnogalacturonan; SBP, sugar beet pectin

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(Cosgrove, 2000; Ridley et al., 2001; Caffall and Mohnen, 2009). Plant cell walls consist of complex matrices of polysaccharides, which (particularly in the primary cell walls) include cellulose, hemicelluloses, and pectin, and, depending on the tissue and type of cell wall, may include various levels of lignin. Each of these components have different structural complexities and play different physiological roles in plants (Caffall and Mohnen, 2009). In addition to the distinct significance of each type of polysaccharide in plant cell walls, various covalent and non-covalent cross-links exist between the polysaccharides within the cell wall matrices. These cross-links and conjunctions include ionic bridges, borate-diol ester bonds, hydrophobic interactions, di-ferulic acid structures (i.e. dehydrodiferulic acid conjugates), and presumably also some other types of bonds including other covalent bonds (Sila et al., 2009). These different types of polysaccharide cross-links appear to exert crucially significant functions in the plant during cell wall growth and development, and they also have a distinct impact on the physical and macromolecular properties of plant materials and in turn on plant food functionality, processing, and quality (Parr et al., 1996; Waldron et al., 2003; Singh et al., 2010). Hence, the control of and/or the controlled manipulation of these polysaccharide cross-links can be used to design certain functionalities in foods and provide a way for utilization of pectic polysaccharide functionalities in various other applications. Pectins or pectic polysaccharides are already widely used as food ingredients in various applications, e.g. as gelling agents and emulsion stabilizers (Willats et al., 2006), and pectic polysaccharide structures have also recently been proposed to be potentially suitable for use in non-food applications such as for biomedical and biopharmaceutical purposes (Itoh et al., 2011; Munarin et al., 2011; Takei et al., 2011) or as new components in (bio)plastic manufacture (Liu et al., 2011).

Significant progress has recently been achieved within exploitation of enzymatic reactions for improving pectic polysaccharide functionality for these applications (Ngouémazong et al., 2012a,b; Zaidel et al., 2011, 2012; Zeeb et al., 2012), and the most recent direction of this research includes the use of enzyme catalyzed modifications for valorization of agro-industrial byproduct streams *via* controlled improvement of textural and macromolecular properties of specific structural elements of pectins (Zaidel et al., 2011, 2012; Min et al., 2011; Fissore et al., 2012). A crucially important prerequisite for the further development of these novel applications is the understanding of the chemical structures, reactions, and mechanisms of the different cross-links that determine the macromolecular properties of the cross-linked materials. The purpose of this review is (i) to highlight the different types of naturally occurring cross-links in various polysaccharide matrices, focusing mainly on pectin polysaccharides, and (ii) to examine the chemical structures and the mechanisms of cross-linking that can be induced directly or indirectly by enzyme catalyzed reactions, and in turn be used to design specific rheological or other functional traits. The treatise will also include an overview of the application of enzymatically cross-linked polysaccharides for structural design of food macromolecular properties, and discuss the functional assessment methodologies used to evaluate these properties.

2. Structures of pectic polysaccharides

Pectic polysaccharides can principally be divided into three main types of structures having different backbones of homogalacturonan (HG), rhamnogalacturonan I (RGI) and rhamnogalacturonan II (RGII), respectively, which are covalently linked to each other (Willats et al., 2006; Coenen et al., 2007; Holck et al., 2011) (Fig. 1A). HG mainly consists of linear α-(1,4)-linked-galacturonic acid residues, and the galacturonic acid (Gal*p*A) moieties within this backbone may be methyl esterified at C-6 and/or *O*-acetylated at the C-2 and/or C-3 position, and certain HG stretches may be extensively substituted with xylose (β-(1,3)-Xyl*p* substitutions) to form xylogalacturonan (Voragen et al., 2009). The RGI backbone is made up of consecutive repeating units of [→2)-α-L-Rha*p*–(1,4)-α-D-Gal*p*A-(1→], and the rhamnose moieties of the RGI may be substituted at the *O*-4 position with different glycan side chains including α-(1,5)-linked-arabinans, β-(1,4)-linked-galactan (Oosterveld et al., 2000) and/or arabinogalactan I (AGI) or arabinogalactan II (AGII) (Lerouge et al., 1993) (Fig. 1A). AGI is composed of a β-(1,4)-linked-galactan backbone with Ara*f* residues attached to *O*-3 of the galactosyl residues whereas AGII is substituted by short chains of α-(1,6)-linked-Ara*f*-[β-(1,6)-linked-Gal*p*]$_n$ (n=1, 2 or 3) (Ridley et al., 2001), and the galactosyl residues of the side chains can be substituted with α-(1,3)-linked-Ara*f*. On the RGI side chains, feruloyl groups, either as single ferulic acid (FA) moieties or in the form of ferulic acid dehydrodimers (diFAs), are esterified to the *O*-2 position of the Ara*f* residues in the α-(1,5)-linked-arabinan backbone, but may also be bound to the *O*-5 on the terminal arabinose (Levigne et al., 2004), or, to a much lesser extent, at the *O*-6 position of the galactopyranosyl (Gal*p*) residues in the β-(1,4)-galactan chains (Colquhoun et al., 1994). The exact abundance and distribution of the FA substitutions vary among different plants and among different plant tissues. In for example sugar beet pectin the arabinan side chains on RGI contain about 0.7–0.8 wt% of FA, with approximately 0.1 wt% diFAs (Micard et al., 1997; Zaidel et al., 2011) whereas in e.g. potato RGI side chains there is less than 0.05 wt% FA and hardly any diFAs (Singh et al., 2011). Rhamnogalacturonan II (RGII) contains eleven different glycosyl residues which are attached as side chains (assigned A to D) to the HG backbone built of α-(1,4)-linked-Gal*p*A residues, hence despite the name RGII there is no rhamnogalacturonan backbone made up of repeating units of [→2)-α-L-Rha*p*–(1,4)-α-D-Gal*p*A-(1→] and the side chains protrude directly from the C-2 and C-3 of the Gal*p*A moieties (Whitcombe et al., 1995; Ridley et al., 2001). In spite of its complexity and low amount in the cell wall (typically ~1–4 wt%) (O'Neill et al., 2004), RGII is thought to have a highly conserved structure and to play an important role in the plant cell wall functionality (Willats et al., 2006).

3. Covalent cross-linking of pectic polysaccharides

The physico/chemical properties of pectins and notably their gelation ability rest on different kinds of cross-linking mechanisms involving different structural entities of the pectin molecule(s). In addition to the ongoing research concerning the biosynthesis, structure and functionality of pectic polysaccharides during plant growth and development, a relatively large number of studies have been published recently concerning modification, including enzymatic modification, of polysaccharide cross-links in situ for food applications (Table 1).

As detailed further below, two main types of linkages can be promoted either directly or indirectly via enzymatic catalysis on pectic polysaccharides; these types include: (i) ionic cross-linking of HG taking place *via* divalent cation bridges, and (ii) phenolic ester oxidative cross-linking between feruloyl groups on the side chains of RGI. As discussed further, below, RGII cross-links *via* borate ester bonds, but it is uncertain if this cross-linking can be promoted by enzyme catalysis, e.g. if shorter RGII moieties resulting from enzyme catalyzed cleavage of adjacent backbone stretches of the RGII/HG chains increase the cross-linking propensity (Table 1).

3.1. Ionic cross-links

3.1.1. Cross-linking mechanism and reaction conditions

Ionic cross-linking mainly refers to the interaction *via* divalent cations between two non-esterified Gal*p*A moieties in