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Research article

Rhamnogalacturonan I galactosyltransferase: Detection of enzyme activity and its hyperactivation

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ABSTRACT

Rhamnogalacturonan I (RG-I), one of the pectic components of the plant cell wall, is composed of a backbone of repeating disaccharide units of rhamnose and galacturonic acid, and side chains, such as galactans, arabinans, and arabinogalactans. The activity of RG-I galactosyltransferase, which transfers galactosyl residues to rhamnosyl residues in the RG-I backbone, has not been detected until now. Here, we detected galactosyltransferase activity in azuki bean epicotyls using fluorogenic RG-I oligosaccharide acceptors. This enzyme prefers oligosaccharides with a degree of polymerization more than 9. The enzyme activity was detected in the Golgi apparatus, which is the site of pectin synthesis. *In vitro* hyperactivation of this enzyme was also observed. Moreover, enzyme activity was increased up to 40-fold in the presence of cationic surfactants or polyelectrolytes.

1. Introduction

Approximately 30% of primary cell wall polysaccharides are comprised of pectin in eudicot and non-commelinid monocot plants (Ridley et al., 2001). Commelinid plants contain approximately 10% pectin in the primary cell wall (Carpita, 1996). Rhamnogalacturonan I (RG-I), like homogalacturonan and RG-II, is a pectic domain polysaccharide. The specific structure of RG-I varies depending on the environment, tissue, and developmental stages of the plants (Ridley et al., 2001).

RG-I has diglycosyl repeating units (→4GalUAα1-2Rhaα1→)$_n$ as the polysaccharide backbone (Lau et al., 1985). Further, its backbone is branched at the C-4 position of the Rha residues by galactan (linear and branched 4- or 4,6-linked β-D-Gal residues), arabinan (linear and branched 5- or 3,5-linked α-L-Ara*f*), and arabinogalactan (branched polysaccharide consisting of both Gal and Ara*f* residues) (Mikshina et al., 2015). In some plants, the backbone Rha residues are also substituted by single Gal residues (Schols et al., 1995).

The galactan content in RG-I varies depending on the tissue and plant type. RG-I in seed mucilage of myxospermous plants contains small amounts of galactan (Western et al., 2000), whereas the galactose content of potato pulp RG-I reaches up to approximately 80% (Khodaei et al., 2016). Moreover, the G-layers of tension wood fibers in some plants have been shown to deposit RG-I with long galactan chains (Gorshkova et al., 2015). Galactan in the cell wall of pea cotyledons is biosynthesized during a specific developmental stage and the occurrence of galactan in the cell wall is related to the firmness of the pea cotyledons (McCartney et al., 2000). It was also reported that galactans along with arabinans bind cellulose during cell wall assembly (Zykwinska et al., 2005).

Investigations into the biosynthetic mechanisms of RG-I have been limited. More than 10 glycolsyltransferases are proposed to be involved in RG-I biosynthesis (Caffall and Mohnen, 2009) and the RG-I backbone-synthetic rhamnosyltransferase has been previously characterized (Uehara et al., 2017) and its encoding genes were identified (Takenaka et al., 2018). Nevertheless, the galacturonosyltransferase-encoding genes responsible for the synthesis of RG-I backbones have not been identified, although candidates have been characterized (Kong et al., 2013; Voiniciuc et al., 2018). Several galactosyltransferase- and arabinosyltransferase-encoding genes involved in the elongation of galactan and arabinan, respectively, have been identified (Harholt et al., 2006; Liwanag et al., 2012; Laursen et al., 2018). However, the activity of the galactosyltransferase that transfers Gal residue to the Rha residue in the RG-I backbone (RG-GalT) has not been detected to date. One of the reasons for this is that RG-I backbone oligosaccharides, the acceptor

Abbreviations: BAC, benzalkonium chloride; CTAB, cetyltrimethylammonium bromide; CPC, cetylpyridinium chloride; ER, endoplasmic reticulum; RG-I, rhamnogalacturonan I; RG-GalT, rhamnogalacturonan I galactosyltransferase; PAA, polyallylamine

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substrates of this enzyme, are not commercially available. Recently, RG-I oligosaccharides reductively aminated with 2-aminopyridine, a fluorescent labeling reagent, have been prepared from commercial RG-I (Uehara et al., 2017). Since they are highly sensitive to detection due to their fluorescence, these oligosaccharides are useful for the quantitative detection of enzymes that act on them. Using these oligosaccharides, the development of an assay for RG-GalT is reported in the present study. Azuki bean epicotyls were used as the enzyme source because they grow very rapidly with a growth rate up to 5 cm per day, and it is proposed that this plant is a rich source of pectin biosynthetic enzymes. The biochemical characterization of RG-GalT from azuki bean epicotyls is also described.

2. Materials and methods

2.1. Materials

N,*N*-dimethylcyclohexylamine, triethylamine, betaine, Triton X-100, and Brij35 were obtained from Wako Pure Chemicals (Osaka, Japan). Cetyltrimethylammonium bromide (CTAB), cetylpyridinium chloride (CPC), and UDP-Gal were purchased from Sigma (St. Louis, MO). Benzalkonium chloride (BAC) was from MP Biomedicals (Santa Ana, CA). n-Octyl-β-D-thioglucoside, n-dodecyl-β-D-maltoside, Zwittergents, and CYMAL6 were from Dojindo (Kumamoto, Japan). Polyallylamine (PAA) with an average molecular weight of 5,000 was from Nittobo medical (Tokyo, Japan). *Aspergillus niger* β-galactosidase was from Megazyme (Wicklow, Ireland). The TSKgel-DEAE-5PW (7.5 × 75 mm) column was obtained from Tosoh (Tokyo, Japan). The fluorescent-labeled RG-I oligosaccharides were prepared as described previously (Uehara et al., 2017). The structures and abbreviations of the oligosaccharides used in this study are listed in Table 1. All other chemicals used were of the highest grade commercially available.

2.2. Plant materials

Seeds of azuki bean (*Vigna angularis* cv. Erimo-wase) were purchased from Watanabe Seed (Miyagi, Japan). The seeds were soaked in water for 12 h, kept on a wet filter paper, and left for germination under dark conditions at 25 °C for 1 day. Then, the germinated seeds were sowed on moist vermiculite and cultivated under dark conditions at 25 °C for 5 days. The upper 5 cm of the epicotyls were used as the plant materials.

Table 1
The structures and abbreviations of RG-I oligosaccharides used in this study.

Abbreviation	Structure
RG_3-PA	
GR_4-PA	
RG_5-PA	
GR_6-PA	
RG_7-PA	
GR_8-PA	
RG_9-PA	
GR_{10}-PA	
GR_{12}-PA	
GR_{14}-PA	
GR_{10}(Gal)-PA	

Rha, GalUA, and Gal residues are represented by green triangles, yellow-divided diamonds, and yellow circles, respectively. PA, pyridylamino group, is covalently linked to the reducing-end of the oligosaccharides.

2.3. Preparation of the solubilized microsomal membrane enzyme from azuki bean epicotyls

Azuki bean epicotyls (30 g) were homogenized with a mortar and a pestle under liquid nitrogen, and proteins were extracted using 30 mL extraction buffer [50 mM HEPES-KOH buffer (pH 7.0), 25 mM KCl, 50% glycerol (v/v)] by stirring gently at 4 °C for 20 min. The suspension was centrifuged at 10,000 × *g* at 4 °C for 30 min. The supernatant was centrifuged at 100,000 × *g* at 4 °C for 60 min. The resultant microsomal pellet was solubilized in 30 mL solubilization buffer [20 mM HEPES-KOH buffer (pH 7.0), 2 mM EDTA, and 0.5% triton X-100] by stirring gently at 4 °C for 10 min. The supernatant obtained by re-centrifugation (30,000 × *g*, 4 °C, 10 min) was used as the solubilized microsomal membrane. The protein concentration was determined using the Pierce (Rockford, USA) BCA protein assay reagent kit according to the manufacturer's instructions.

2.4. Assay for RG-GalT

The enzyme assay was carried out in a reaction mixture (total volume, 30 μL) containing the solubilized microsomal membrane, a reaction buffer [30 mM HEPES-KOH buffer (pH 7.0), 5 mM $MnCl_2$, 0.1 M sucrose, 0.03% BSA, 0.15% triton X-100], 1 mM UDP-Gal, and 50 μM RG-I oligosaccharide at 30 °C for 0.5–24 h. The reaction mixture was centrifuged at 2,000 × *g* at 25 °C for 1 min and the supernatant was analyzed by HPLC using a TSKgel-DEAE-5PW (7.5 × 75 mm) column and a fluorescence detector (excitation wavelength, 320 nm; emission wavelength, 400 nm). The elution of each oligosaccharide was performed under isocratic conditions with various concentrations of ammonium acetate buffer (pH 4.8), at a flow rate of 1 mL min^{-1}. The product was quantified based on the peak area on a chromatogram. One unit of enzyme activity was defined as the amount of the enzyme that transferred 1 nmol of Gal residue from UDP-Gal to GR_{10}-PA per min. The K_m and V_{max} values of the enzyme were determined by assaying various concentrations of GR_{10}-PA (14–1,000 μM) or UDP-Gal (16–5,000 μM) with 0.10 μg of the solubilized microsomal membrane. The kinetic parameters were calculated based on a Lineweaver-Burk plot. The enzyme product was treated with *A. niger* β-galactosidase at 40 °C for 12 h. The digest was analyzed using a TSKgel-DEAE-5PW (7.5 × 75 mm) column as described previously.

The effects of surfactants, polyelectrolytes, and osmolytes on RG-GalT activity were also examined. These additives at appropriate concentrations were used in the enzyme reaction in the presence of 0.15% triton X-100 in reaction buffer.

2.5. Structural analysis of oligosaccharides by mass spectrometry (MS)

MS/MS analysis was carried out by FT-ICR MS. The oligosaccharides were dissolved in 50% tetrahydrofuran and 1% acetic acid for infusion electrospray ionization mass spectrometry (EDI-MS) using an FT-ICR-MS SolariX 7.0 T (Bruker Daltonics). A syringe pump was used at a speed of 250 μL h^{-1} to load the sample solution onto the ESI source, with a capillary potential of 4,500 V, nebulizer gas (N_2) flow rate of 1.8 bar, dry gas (N_2) at 220 °C, and a flow rate of 3.7 L min^{-1}. In-source fragmentation was used for MS/MS analysis by applying additional skimmer potential of 96 V in an ESI source.

2.6. Subcellular localization of RG-GalT

Azuki bean epicotyls (10 g) chopped with a box-cutter knife were homogenized in buffer [10 mM HEPES-KOH buffer (pH 7.5), 1 mM EDTA, and 1 mM dithiothreitol] and centrifuged at 1,000 × *g* at 4 °C for 10 min. The resultant supernatant (3 mL) was overlaid onto a 20–55% (w/w) linear density sucrose gradient (30 mL) in the same buffer. The gradient was centrifuged at 4 °C for 3 h at 100,000 × *g* using the SW 28 rotor (Beckman, Brea, CA). Fractions (1.5 mL) were collected and

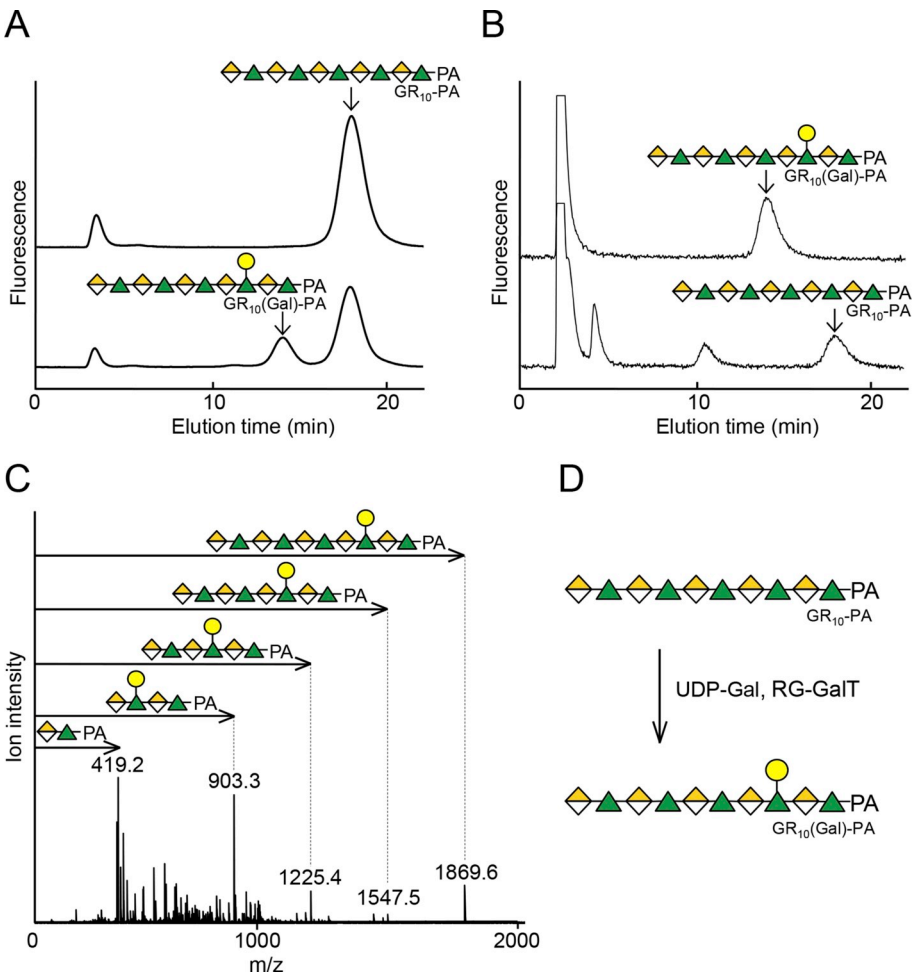

Fig. 1. RG-GalT activity of microsomal proteins from azuki bean epicotyls. (A) The enzyme was reacted with GR_{10}-PA as a substrate for 0 (upper panel) or 20 h (lower panel). The substrate, GR_{10}-PA, and the product, GR_{10}(Gal)-PA, were separated and quantified by DEAE-HPLC. The arrows indicate their elution positions. (B) The enzyme product, GR_{10}(Gal)-PA, (upper panel) was digested with *A. niger* β-galactosidase (lower panel) at 40 °C for 12 h. The peak that appeared at approximately 11 min was derived from β-galactosidase solution. (C) MS/MS spectrum of the enzyme product, GR_{10}(Gal)-PA. The fragmentation pattern is depicted above. (D) Reaction scheme of RG-GalT using GR_{10}-PA as an acceptor substrate. A Gal residue was transferred from UDP-Gal to the fourth Rha residue from the non-reducing end of GR_{10}-PA. Green triangles, yellow-divided diamonds, and yellow circles represent Rha, GalUA, and Gal residues, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

assayed for RG-GalT and organelle marker enzymes as described previously (Ohashi et al., 2017).

3. Results

3.1. Detection of RG-GalT activity in azuki bean epicotyls

The RG-GalT assay was performed by incubating the reaction mixture containing 50 μM GR_{10}-PA (Table 1), 1 mM UDP-Gal, and the solubilized microsomal membrane from azuki bean epicotyls. The microsomal pellet solubilized in buffer containing 0.5% triton X-100 was used as the solubilized microsomal membrane. Incubation of the reaction mixture at 30 °C for 20 h generated a new peak on a chromatogram at the elution time of 14 min (Fig. 1A). The time course of the RG-GalT reaction showed that this product increased linearly for at least the first 24 h. Linearity between the protein concentration and the amount of the product was also observed for 0–1.0 μg of the solubilized microsomal membrane in the 22-h reaction. These results indicated that the new peak eluted at 14 min, as shown in Fig. 1A, was a product of the enzyme reaction.

The RG-GalT reaction was expected to produce GR_{10}(Gal)-PA (Table 1). The enzyme product was digested by *A. niger* β-galactosidase to give an original substrate oligosaccharide, GR_{10}-PA (Fig. 1B). The enzyme product was then analyzed by mass spectrometry. Its mass (1869.6) corresponded with the calculated mass value of 1869.5 for GR_{10}(Gal)-PA. MS/MS analysis of this product detected the daughter ions such as the presumed GR_4(Gal)-PA (mass of 903.3) and GR_2-PA (mass of 419.2) (Fig. 1C) showing that RG-GalT transfers the Gal residue preferentially to the fourth Rha residue from the non-reducing end of RG_{10}-PA (Fig. 1D). Taken together, these results suggest that the enzyme transfers a Gal residue from UDP-Gal to the inner Rha residue of a GR_{10}-PA through β-linkage.

3.2. Characterization of RG-GalT from azuki bean epicotyls

The acceptor substrate specificity of the enzyme was investigated using oligosaccharides with a degree of polymerization (DP) of 3–14. The longer oligosaccharides (DP > 9), rather than the shorter oligosaccharides (DP < 8), functioned as acceptor substrates (Fig. 2A). GR_{12}-PA and GR_{14}-PA were the best among tested substrates. Because of the low yield of GR_{12}-PA and GR_{14}-PA during the preparation of RG-I oligosaccharides (Uehara et al., 2017), GR_{10}-PA was used as a substrate for subsequent enzyme characterization. The optimum pH for the enzyme activity was 6.5–7.0. The optimum temperature of the enzyme was approximately 30 °C under the conditions used in this study. The RG-GalT was slightly activated by manganese ion (Fig. 2B). The optimum Mn^{2+} concentration for enzyme activation was 3 mM, whereas Zn^{2+} and Cu^{2+} ions inactivated the enzyme. Activation of the enzyme by monovalent cations was not observed. Detergents were needed to solubilize the enzyme from the microsomal fraction of azuki bean, suggesting that RG-GalT is a membrane-bound enzyme. Moreover, some detergents, such as Zwittergent 3–12, Cymal-6, and TritonX-100, are appropriate for solubilizing the enzyme from the microsomal fraction. The optimum concentration of Triton X-100 for solubilization of the enzyme from the microsomal fraction was 0.5%.

The homogenate of azuki bean epicotyls was divided into soluble and microsomal fractions. RG-GalT activity was predominantly detected in the solubilized microsomal fraction, and not in the soluble

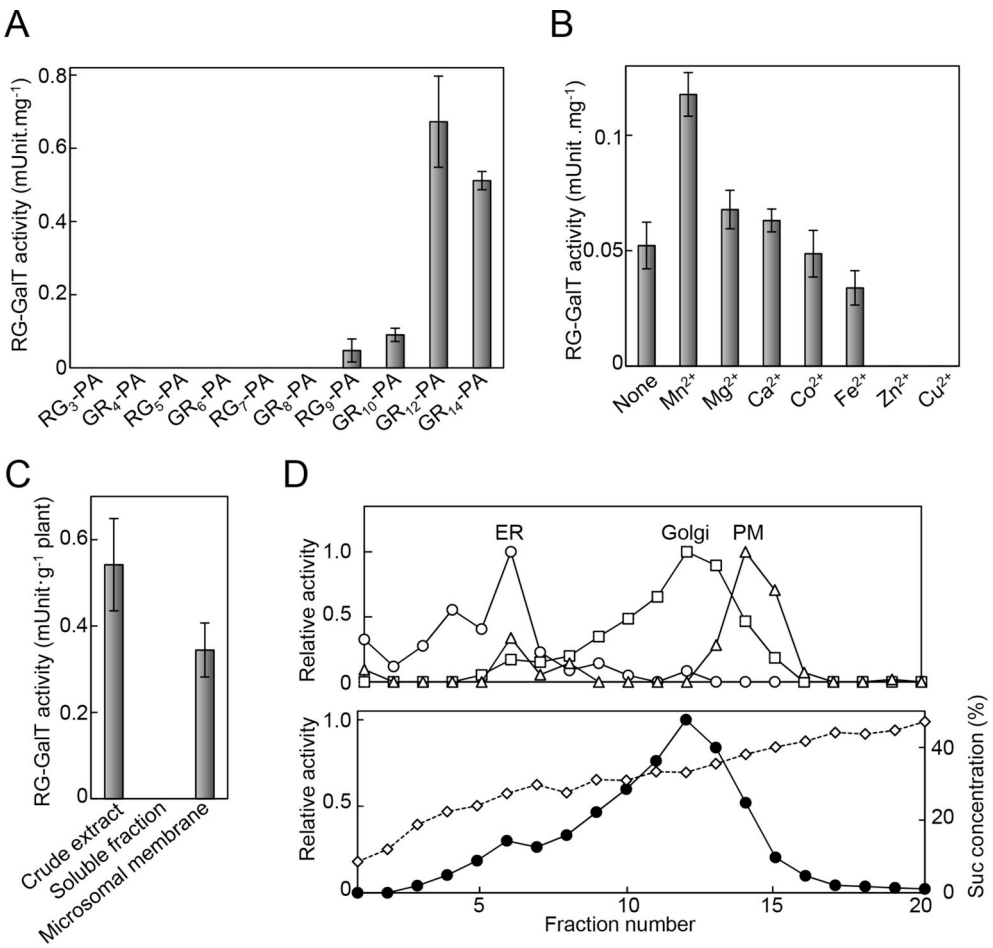

Fig. 2. Biochemical characterization of RG-GalT from azuki bean epicotyls. (A) Acceptor substrate specificity of RG-GalT. The solubilized microsomal membrane was reacted with 5 μM RG-I oligosaccharides. Structures of the substrates are shown in Table 1 (B) Metal ion dependence of RG-GalT. The concentration of each metal ion was 3 mM. (C) Distribution of RG-GalT activity in the soluble fraction and the solubilized microsomal membrane per gram of plant material. For A–C, the activities are presented as mean values with standard errors of three independent biological samples. (D) Subcellular localization of RG-GalT. The particulate membrane preparation from azuki bean epicotyl was fractionated by 20–55% sucrose (Suc) linear density gradient centrifugation. The upper panel shows the profiles of organelle marker enzymes. NADH-dependent cytochrome *c* reductase (open circles), homogalacturonan galacturonosyltransferase (open squares), and vanadate-sensitive H^+-ATPase (open triangles) were used as endoplasmic reticulum (ER), Golgi, and plasma membrane (PM) marker enzymes, respectively. The lower panel shows the profile of RG-GalT activity (closed circle) and Suc density (open rhomboid) in each fraction. RG-GalT activity co-eluted with a Golgi maker enzyme.

fraction (Fig. 2C). The organelles of azuki bean epicotyls were separated by sucrose density gradient ultracentrifugation and each fraction was assayed for RG-GalT. The sedimentation position of RG-GalT (approximately 33% sucrose, fraction 12) exclusively coincided with that of the Golgi membrane marker protein, and was separated from that of the ER and plasma membrane marker proteins (Fig. 2D). This sedimentation pattern shows that RG-GalT localized to the Golgi apparatus where pectin is biosynthesized.

3.3. Hyperactivation of RG-GalT by additives

During the course of biochemical characterization, some additives were found to induce enzyme hyperactivation. When quaternary ammonium surfactants with a long alkyl chain such as BAC, CTAB, and CPC were added to the enzyme reaction mixture, RG-GalT activity increased 14 to 29-fold (Fig. 3A). The optimum concentration of these compounds was approximately 3 mM. PAA, a cationic polyelectrolyte prepared through the polymerization of allylamine, was also a useful additive for RG-GalT hyperactivation. This additive increased enzyme activity up to 40-fold (Fig. 3A), and its optimum concentration was approximately 10 μM. Other quaternary or tertiary amines such as betaine, triethylamine, *N*,*N*-dimethylcyclohexylamine failed to function as additives for enzyme hyperactivation. When the concentration of NaCl in the reaction mixture was increased, the enzyme activity in the presence of these additives decreased compared to that without additives (Fig. 3B).

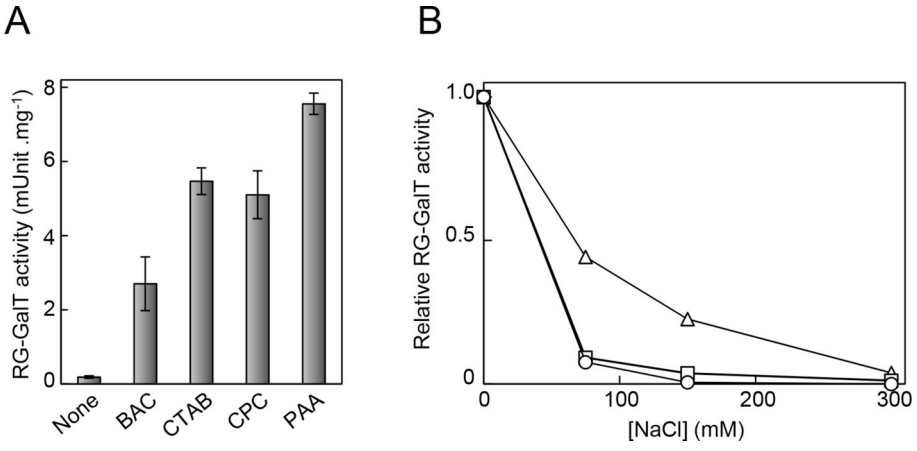

Fig. 3. Hyperactivation of RG-GalT from azuki bean epicotyls. (A) Hyperactivation of the RG-GalT with additives benzalkonium chloride (BAC; 3 mM), cetyltrimethylammonium bromide (CTAB; 3 mM), cetylpyridinium chloride (CPC; 3 mM), polyallylamine (PAA; 10 μM). The activities are presented as mean values with standard errors of three biological independent samples. (B) RG-GalT activity in the presence of additives at various NaCl concentrations. Reaction mixtures containing 3 mM CTAB or 10 μM PAA were mixed with various concentrations of NaCl. Circles and squares show the reaction in the presence of PAA and CTAB, respectively. Triangles indicate reactions in the absence of an additive.

Table 2
Kinetic parameters of the RG-GalT from azuki bean epicotyls.

	Acceptor substrate		Donor substrate	
	K_m (mM)	V_{max} (mUnit/mg)	K_m (mM)	V_{max} (mUnit/mg)
RG-GalT	0.78	0.18	4.0	0.030
RG-GalT hyperactivated with BAC	0.12	0.56	0.2	0.57

GR_{10}-PA was used as the acceptor substrate. The RG-GalT was hyperactivated using 3 mM benzalkonium chloride (BAC).

The apparent K_m and V_{max} values of RG-GalT were subsequently determined (Table 2). A decrease in K_m and increase in V_{max} were observed in the enzyme hyperactivated with 3 mM BAC, suggesting that enzyme hyperactivation was conferred by both an increase in the affinity between the enzyme and the substrate and the enzyme turnover for catalysis.

4. Discussion

An assay method for RG-GalT, which transfers a Gal residue to a Rha residue in the RG-I polysaccharide backbone, was constructed in the present study (Fig. 1). RG-GalT is crucial for the biosynthesis of RG-I galactan because the enzyme produces the acceptor for further galactosylation events. Although the exact linkage position between the transferred Gal and Rha residues in the backbone of RG-I was not determined in this study, galactosidase digestion and MS/MS analysis of the product showed that RG-GalT transferred the Gal residue to an RG-I oligosaccharide through a β-linkage (Fig. 1B and C). A unique characteristic of the enzyme is its substrate specificity. This enzyme transfers a Gal residue onto RG-I oligosaccharides ranging from DP 9 to 14 (Fig. 2A), and the Gal residue was transferred to the fourth Rha residue from the non-reducing end of GR_{10}-PA (Fig. 1C). These results suggest that the enzyme recognizes several residues at the non-reducing end of acceptor substrates (Fig. 1A). Such characteristics of RG-GalT are distinct from those of galactan-elongating galactosyltransferases such as Arabidopsis GALS1, GALS2, and GALS3 (Liwanag et al., 2012). A *GALS*-deficient Arabidopsis mutant exhibits the addition of a single residue of Gal to the RG-I backbone (Ebert et al., 2018), suggesting that RG-GalT and GALSs are necessary and sufficient to synthesize RG-I galactan. However, it is not clear yet whether the GALSs transfer Gal residues to the single residue of Gal added to the RG-I polysaccharide backbone, the enzymatic product of RG-GalT.

Two pectin biosynthesis models—consecutive GT and domain synthesis models—have been proposed (Atmodjo et al., 2013). The former model proposes that glycosyltransferases consecutively transfer glycosyl residues from nucleotide sugars to synthesize complex pectin structures. The latter model proposes that an enzyme transfers oligo- or polysaccharides *en bloc* onto a growing polysaccharide. Suggestive data in support of the hypothesis that the galactan domain is transferred onto an acidic pectin domain have been reported (Stoddart and Northcote, 1967); however, it is not clear which model would be applied for RG-I galactan synthesis based on the current biochemical characteristics of RG-GalT (this study) and GALS (Ebert et al., 2018). To solve this problem, identification of the RG-GalT gene and further biochemical characterization of RG-GalT and GALSs is needed.

Enzyme hyperactivation was observed for RG-GalT in the presence of amphiphilic compounds such as quaternary ammonium surfactants or cationic polyelectrolytes (Fig. 3A). Such hyperactivation has been observed for several enzymes (Sintra et al., 2014; Kurinomaru et al., 2014). Cationic polyelectrolyte was reported to be effective for increasing both the anionic substrate-binding affinity to the enzyme and the turnover number of its catalysis (Kurinomaru et al., 2014). Similar effects were observed for RG-GalT in the presence of BAC (Table 2). Although the precise mechanism underlying the hyperactivation of this enzyme was not elucidated, electrostatic interactions among the enzyme, substrates, and an amphiphilic compound would be responsible for this hyperactivation because a larger decrease in the activity of the hyperactivated enzyme upon NaCl addition was observed. Amphiphilic compounds that were effective for hyperactivation in this study have structural characteristics with moderate hydrophobicity and positive electrical charges. These characteristics might also contribute to their non-covalent binding to the enzyme and/or substrates to induce activation. Such hyperactivation was not observed for other pectin glycosyltransferase, such as rhamnosyltransferase (Uehara et al., 2017) or galacturonosyltransferase (Akita et al., 2002), suggesting that structural characteristics of RG-GalT are mainly involved in its hyperactivation. This activation could be practically used for the identification of the gene encoding RG-GalT based on its gene-deficient mutant or a recombinant protein because this enzyme activity from Arabidopsis is quite low or because the expression level of a recombinant Golgi-localized membrane protein in heterologous host cells is frequently low.

Author contribution statement

N.M. and T. I designed the experiments. N.M., Y.T., B.W., and H.K. performed all the experiments. Tomoya Imai carried out the MS analysis of oligosaccharides. T.I. analyzed the data and wrote the whole manuscript. All authors read and approved the manuscript.

Conflicts of interest

The authors declare that they have no conflict of interest.

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References

Akita, K., Ishimizu, T., Tsukamoto, T., Ando, T., Hase, S., 2002. Successive glycosyltransfer activity and enzymatic characterization of pectic polygalacturonate 4-α-galacturonosyltransferase solubilized from pollen tubes of *Petunia axillaris* using pyridylaminated oligogalacturonates as substrates. Plant Physiol. 130, 374–379.
Atmodjo, M.A., Hao, Z., Mohnen, D., 2013. Evolving views of pectin biosynthesis. Annu. Rev. Plant Biol. 64, 747–749.
Caffall, K.H., Mohnen, D., 2009. The structure, function, and biosynthesiss of plant cell wall pectic polysaccharides. Carbohydr. Res. 344, 1879–1900.
Carpita, N.C., 1996. Structure and biogenesis of the cell walls of grasses. Annu. Rev. Plant Physiol. Plant Mol. Biol. 47, 445–476.
Ebert, B., Birdseye, D., Liwanag, A.J.M., Laursen, T., Rennie, E.A., Guo, X., Catena, M., Rautengarten, C., Stonebloom, S.H., Gluza, P., Pidatala, V.R., Andersen, M.C.F., Cheetamun, R., Mortimer, J.C., Heazlewood, J.L., Bacic, A., Clausen, M.H., Willats, W.G.T., Scheller, H.V., 2018. The three members of the Arabidopsis glycosyltransferase family 92 are functional β-1,4-galactan synthases. Plant Cell Physiol. 59, 2624–2636.
Gorshkova, T., Mokshina, N., Chernova, T., Ibragimova, N., Salnikov, V., Mikshina, P., Tryfona, T., Banasiak, A., Immerzeel, P., Dupree, P., Mellerowicz, E.J., 2015. Aspen tention wood fibers contain β-(1→4)-galactans and acidic arabinogalactans retained by cellulose microfibrils in gelatinous walls. Plant Physiol. 169, 2048–2063.
Harholt, J., Jensen, J.K., Sørensen, S.O., Orfila, C., Pauly, M., Scheller, H.V., 2006. ARABINAN DEFICIENT 1 is a putative arabinosyltransferase involved in biosynthesis of pectic arabinan in *Arabidopsis*. Plant Physiol. 140, 49–58.
Khodaei, N., Fernandez, B., Fliss, I., Karboune, S., 2016. Digestibility and prebiotic properties of potato rhamnogalacturonan I polysaccharide and its galactose-rich oligosaccharides/oligomers. Carbohydr. Polym. 136, 1074–1084.
Kong, Y., Zhou, G., Abdeen, A.A., Schafhauser, J., Richardson, B., Atmodjo, M.A., Jung, J., Wicker, L., Mohnen, D., Western, T., Hahn, M.G., 2013. Galacturonosyltransferase-

like5 is involved in the production of Arabidopsis seed coat mucilage. Plant Physiol. 163, 1203–1217.
Kurinomaru, T., Tomita, S., Hagihara, Y., Shiraki, K., 2014. Enzyme hyperactivation system based on a complementary charged pair of polyelectrolytes and substrates. Langmuir 30, 3825–3831.
Lau, J.M., McNeil, M., Darvill, A.G., Albersheim, P., 1985. Structure of the backbone of rhamnogalacturonan I, a pectin polysaccharide in the primary cell walls of plants. Carbohydr. Res. 137, 111–125.
Laursen, T., Stonebloom, S.H., Pidatala, V.R., Birdseye, D.S., Clausen, M.H., Mortimer, J.C., Scheller, H.V., 2018. Bifunctional glycosylransferases catalyze both extension and termination of pectic galactan oligosaccharides. Plant J. 94, 340–351.
Liwanag, A.J.M., Ebert, B., Verhertbruggen, Y., Rennie, E.A., Rautengarten, C., Oikawa, A., Andersen, M.C.F., Clausen, M.H., Scheller, H.V., 2012. Pectin biosynthesis: GALS1 in *Arabidopsis thaliana* is a β-1,4-galactan β-1,4-galactosyltransferase. Plant Cell 24, 5024–5036.
McCartney, L., Ormerod, A.P., Gidley, M.J., Knox, J.P., 2000. Temporal and spatial regulation of pectic (1-4)-β-D-galactan in cell walls of developing pea cotyledons: implications for mechanical properties. Plant J. 22, 105–113.
Mikshina, P.V., Petrova, A.A., Gorshkova, T.A., 2015. Functional diversity of rhamnogalacturonans I. Russ. Chem. Bull. Int. Ed. 64, 1014–1022.
Ohashi, T., Jinno, J., Inoue, Y., Ito, S., Fujiyama, K., Ishimizu, T., 2017. A polygalacturonase localized in the Golgi apparatus in *Pisum sativum*. J. Biochem. 162, 193–201.
Ridley, B.L., O'Neill, M.A., Mohnen, D., 2001. Pectins: structures, biosynthesis, and oligogalacturonide-related signaling. Phytochemistry 57, 929–967.
Schols, H.A., Vierhuis, E., Bakx, E.J., Voragen, A.G.J., 1995. Different populations of pectic hairy regions occur in apple cell walls. Carbohydr. Res. 275, 343–360.
Sintra, T.E., Ventura, S.P.M., Coutinho, J.A.P., 2014. Superactivity induced by micellar systems as the key for boosting the yield of enzymatic reactions. J. Mol. Catal. B Enzym. 107, 140–151.
Stoddart, R.W., Northcote, D.H., 1967. Metabolic relationships of the isolated fractions of the pectic substances of actively growing sycamore cells. Biochem. J. 105, 45–59.
Takenaka, Y., Kato, K., Ogawa-Ohnishi, M., Tsuruhama, K., Kajiura, H., Yagyu, K., Takeda, A., Takeda, Y., Kunieda, T., Hara-Nishimura, I., Kuroha, T., Nishitani, K., Matsubayashi, Y., Ishimizu, T., 2018. Pectin RG-I rhamnosyltransferases represent a novel plant-specific glycosyltransferase family. Nature Plants 4, 669–676.
Uehara, Y., Tamura, S., Maki, Y., Yagyu, K., Mizoguchi, T., Tamiaki, H., Imai, T., Ishii, T., Ohashi, T., Fujiyama, K., Ishimizu, T., 2017. Biochemical characterization of rhamnosyltransferase involved in biosynthesis of pectic rhamnogalacturonan I in plant cell wall. Biochem. Biophys. Res. Commun. 48, 130–136.
Voiniciuc, C., Engle, K.A., Günl, M., Dieluweit, S., Schmidt, M.H., Yang, J., Moremen, K.W., Mohnen, D., Usadel, B., 2018. Identification of key enzymes for pectin synthesis in seed mucilage. Plant Physiol. 178, 1045–1064.
Western, T.L., Skinner, D.J., Haughn, G.W., 2000. Differentiation of mucilage secretory cells of the Arabidopsis seed coat. Plant Physiol. 122, 345–355.
Zykwinska, A.W., Ralet, M.-C.J., Carnie, C.D., Thibault, J.-F.J., 2005. Evidence for in vitro binding of pectin side chains to cellulose. Plant Physiol. 139, 397–407.