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Research Interests  

Pectin Biosynthesis  
Anti-cancer effects of Pectin  
Function of pectin in plants  

What is Pectin?  

The cell wall of plants is a polysaccharide and protein rich macromolecular structure that is essential for plant form and function and is the first entity encountered by plant symbionts and pathogens. Pectin is a major polysaccharide component of all plant primary walls. Anabolic and catabolic changes in pectin metabolism are associated with fruit ripening, organ abscission, plant defense responses, growth, and development. Oligosaccharides released from pectin induce plant defense responses and regulate plant development. Pectin is also a food fiber in fruits and vegetables and an economically important nutritional and gelling agent in foods. Pectin has beneficial effects on human health including the lowering of blood cholesterol and serum glucose levels, and the potential inhibition of cancer growth and metastasis. The goals of research in the Mohnen laboratory are to understand the biosynthesis and the biological functions of pectin. The main strategy is to study pectin biosynthetic enzymes and their genes to elucidate how pectin is synthesized. The long term goal is to use that knowledge, and transgenic plants that produce modified pectin, to determine the function of pectin in planta, to study how pectin is synthesized, and to alter pectin structure so as to produce pectins with novel health and nutritional properties and produce plants with improved agricultural value.

Biosynthesis of Pectin.  

*Galacturonosyltransferases*  

The main research project in the Mohnen laboratory is based on the premise that the most direct way to elucidate the biological functions of pectin is to understand how pectin is biosynthesized. Current efforts center on how the pectic polysaccharide homogalacturonan (HGA) is synthesized. HGA is a linear polymer of α-1,4-linked galactosyluronic acid that makes up ~60% of the pectic polysaccharides (Fig. 1). We previously identified a 4-α-galacturonosyltransferase (GalAT) that transfers UDP-GalA (and UDP-[14C]GalA) onto HGA or HGA oligosaccharides (oligogalacturonides, OGAs) using membrane preparations from tobacco, radish, pea and *Arabidopsis thaliana*. The product  

![Figure 1. Trimeric region of homogalacturonan (HGA).](image-url)
synthesized onto endogenous acceptors by membrane bound tobacco GalAT is ~105 kDa and contains up to 89% HGA, of which at least 50% is esterified\textsuperscript{13}. Detergent-solubilized tobacco GalAT \{\textsuperscript{15}\} transfers GalA from UDP-GalA onto the non-reducing end\textsuperscript{16} of exogenous OGAs with degrees of polymerization of $>9$ \textsuperscript{15}.

\[
\alpha_{1,4}\text{GalAT} \\
\text{HGA}_n + \text{UDP-GalA} \rightarrow \text{HGA}_{n+1} + \text{UDP}
\]

We showed that in pea GalAT is localized to the Golgi with its catalytic site in the Golgi lumen\textsuperscript{14}. These results are consistent with a type II membrane protein topology\textsuperscript{17} for GalAT. Under low UDP-GalA concentrations, solubilized GalAT adds predominantly one galacturonic acid onto the non-reducing end of exogenous OGA acceptors (e.g OGA of DP 15 to DP 16), while at higher concentrations of UDP-GalA ($\sim$ mM), OGAs can be extended by more than one GalA residues, suggesting that at least \textit{in vitro} the enzyme act non-processively.

We recently partially purified GalAT from \textit{Arabidopsis}, trypsinized the partially purified protein, and determined the amino acid sequence of candidate GalATs by tandem mass spectrometry sequencing (Sterling et al, in preparation). Expression of one of the candidate GalAT genes (JS36) in human embryonic kidney cells gave low levels of GalAT activity the recombinant cells, leading us to name this gene \textit{GALAT1}. Blast analysis comparison of \textit{GALAT1} with the \textit{Arabidopsis} genome identified 14 genes with $\geq 34\%$ sequence identity and $\geq 52$ sequence similarity to \textit{GALAT1}. We propose that these 15 genes comprise a GalAT gene family\textsuperscript{28}. We have also identified an additional 10 \textit{Arabidopsis} genes with slightly lower sequence similarity, but similar conserved domains, and call this the putative GalAT-like family (collaboration with Michael Hahn, unpublished). Our current efforts center on proving the function of these genes by identifying the specific enzyme activity, substrate specificity, and \textit{in vivo} function of these 25 proposed pectin biosynthetic genes. Our strategy includes heterologous expression of the genes and analysis of gene mutants. We propose that these genes encode multiple GalATs involved in the synthesis of the pectins HGA, rhamhogalacturonan I (RG-I) and rhamnogalacturonan-II (RG-II).

\begin{center}
\textbf{GalAT Superfamily}
\end{center}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{galatSuperfamily.png}
\caption{Arabidopsis GalAT Superfamily}
\end{figure}

\begin{center}
\textit{methyltransferases}
\end{center}

The enzyme that methylates HGA at the C6 carboxyl, HGA-methyltransferase
(HGA-MT), contributes to HGA function by modifying the charge on HGA and thus, the ionic and structural properties of pectin, including its gelling properties. We previously localized tobacco HGA-MT activity\(^{18,19}\) to the Golgi and showed that its catalytic site faces the Golgi lumen\(^{20}\), suggesting that HGA-MT and HGA-GalAT are both localized in the same subcellular compartment.

**Synthesis of UDP-GalA**

The location of pectin synthesis in the Golgi leads to the question of where the nucleotide-sugar substrates are synthesized and of how the substrates gain access to the enzyme. We have proposed that the UDP-GalA synthesizing enzyme, UDP-GlcA 4-epimerase, is located on the cytosolic side of the Golgi and that the UDP-GalA is transported into the Golgi lumen by a UDP-GalA:UMP antiporter\(^{14}\) (model A below). While this model is consistent with the topology of some nucleotide-sugar biosynthetic enzymes in animals and plants, there are also indications that some nucleotide biosynthesis enzymes, such as UDP-glucuronic acid decarboxylase, may actually reside in the Golgi (model B below)\(^{10}\). Thus, until the definitive subcellular location of UDP-GlcA 4-epimerase is confirmed experimentally, two models for the location of UDP-GlcA 4-epimerase, must be considered. Our preliminary data confirm that UDP-GlcA 4-epimerase co-fractionates with Golgi membranes (Adams and Mohnen), suggesting that the epimerase is membrane bound and not free in the cytosol. If UDP-GalA is synthesized on the cytosolic side of the Golgi, it is likely transported into the Golgi via a UDP-GalA:UMP antiporter in the Golgi membrane. Regardless of whether the UDP-GalA is synthesized on the cytosolic or lumenal side of the Golgi, the UDP released upon transfer of the GalA from UDP-GalA onto HGA would be hydrolyzed by a Golgi-localized nucleotide-5'-diphosphatase (NDPase) into UMP and inorganic phosphate. The nucleoside monophosphate would then presumably be transported out of the Golgi by the nucleotide-sugar:nucleoside monophosphate antiporter.

![Diagram A: UDP-GlcA 4-epimerase located on the cytosolic face of the Golgi](image1)

![Diagram B: UDP-GlcA 4-epimerase in the Golgi lumen](image2)

**Biological activity of Pectin in Humans and Animals**

A developing research area in the Mohnen lab is the investigation of the beneficial effects of pectin on human health. Pectin has multiple beneficial effects on human health\(^\text{7}\) including the lowering of blood cholesterol and serum glucose levels\(^\text{3}\), and the potential inhibition of cancer growth and metastasis\(^\text{4}\), and the inhibition of fibroblast growth factor-receptor interactions\(^\text{21}\).
Some of these effects appear to occur via the induction of apoptosis and/or the interfering with ligand:receptor interactions. However, neither the specific pectin structure with these activities nor the precise molecular mechanisms of pectin's activities are known. In a collaborative project with Vijay Kumar\(^2\) (Section of Urology, Medical College of Georgia), we are studying the effects of different pectins on cell apoptosis and on cancer metastasis\(^2\). Prostate cancer is the most common malignancy and the second leading cause of death in American men. Specifically we are studying the effects of different pectins on apoptosis in several different human prostate cancer cell lines and identifying the specific structure(s) in pectin that inhibit human prostate cancer cell growth. The goal of these studies is to determine the molecular mechanism(s) by which pectin inhibits prostate cancer. A longer-term goal is to develop recommended diet changes and/or pectin-based nutraceutical or pharmaceutical strategies to combat the incidence and lethality of prostate cancer, and other types of cancer, and to promote human health.

**Biological Activity of Pectic Oligosaccharides in Plants.**

A long term research interest of the Mohnen lab is how the biologically active oligosaccharide fragments released from pectin - oligogalacturonides (OGAs) - regulate plant development\(^2\)\(^\text{b}\)\(^\text{c}\). OGAs with a degree of polymerization (DP) >9 regulate *in vitro* morphogenesis and *de novo* meristem formation in tobacco thin cell-layer explants (TCLs)\(^2\)\(^\text{d}\)\(^\text{e}\)\(^\text{f}\). OGAs inhibit root formation and/or induce *de novo* flower shoot formation in TCLs, with a half-maximum morphogenesis response at ~400 nM. TCLs cultured in the presence of OGAs also show enhanced polarity of tissue enlargement and organ formation at the basal end of the explant and correspondingly less at the apical end. Thus, OGAs regulate both the polarity of TCL morphogenesis and the type of meristems formed on the tissue explants.

**Reference List**

14. Sterling J, Quigley HF, Orellana A, Mohnen D: The catalytic site of the pectin biosynthetic enzyme α-1,4-galacturonosyltransferase (GalAT) is located in the lumen of the Golgi. Plant Physiology 2001; 127: 360-71
15. Doong RL, Mohnen D: Solubilization and characterization of a galacturonosyltransferase that synthesizes the pectic polysaccharide homogalacturonan. Plant Journal 1999; 207: 512-7
29. Jackson, C.L., Dreaden, T.M., Beal, T.L. I., Eid, M., Debra, M. Kumar, V. Mohnen, D. “Pectin Induces Apoptosis in Prostate Cancer Cells” (in preparation)

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