

Structural and Biochemical Insight into *Populus trichocarpa* β -1,4-Galactan Synthase

Background

- GALACTAN SYNTHASE (GalS) enzymes are categorized as inverting glycosyltransferases (GT) from family 92 (GT92) in the CAZY database. GalS enzymes are involved in synthesizing β -1,4-linked galactan side chains of rhamnogalacturonan-I (RGI) that accumulate during tension wood formation.

Approach

- The 3D structure of GalS1 was solved using X-ray crystallography (Fig. 1). Key catalytic residues were identified using deep-evolutionary analysis and confirmed by biochemical analysis. Docking and molecular dynamic simulations were used to gain further insight into the mechanism of galactan chain biosynthesis.

Results

- We found that GalS1 structure contains an ancillary carbohydrate-binding module at its N-terminus that binds specifically to the backbone of RGI. This represents the founding member of a new family (CBM95).
- The stem-domain plays a structural role in homodimer formation, interacting across GalS1 monomers in a ‘handshake’ pose, and is essential for GT activity and protein stability.
- By mining over half a million GT-A fold sequences and comparing them to those in the new GT92 family, key donor binding residues were identified and confirmed by biochemical analyses.
- Combined with Docking and MD simulations, data shows that GalS1 utilizes an S_N2 single-displacement reaction mechanism.

Significance

- We solved the first experimentally determined 3D structure of a GT92 enzyme, leading us to discover a new family of carbohydrate-binding module, CBM95. Analysis of the CBM substrate specificity, together with UDP-Gal (donor) and 1,4- β -D-galactotetrose (acceptor) substrate binding led to the proposal of a new model for complex pectin biosynthesis (Fig. 2) leveraging a CBM to potentiate interaction with the backbone during chain elongation.

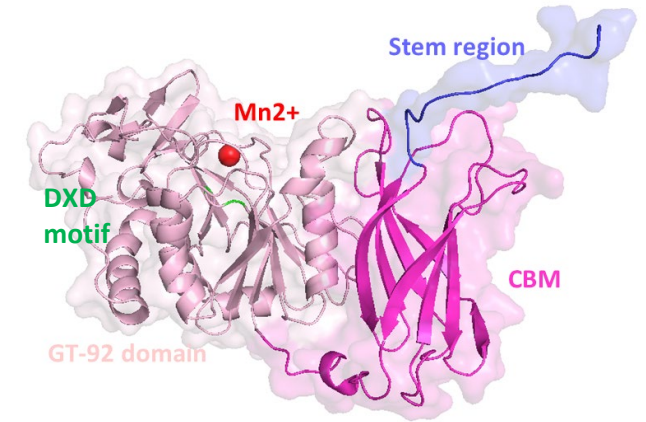


Figure 1. Monomer of GalS1 highlighting the stem domain (blue), CBM95 (magenta), and core GT-A domain (light pink).

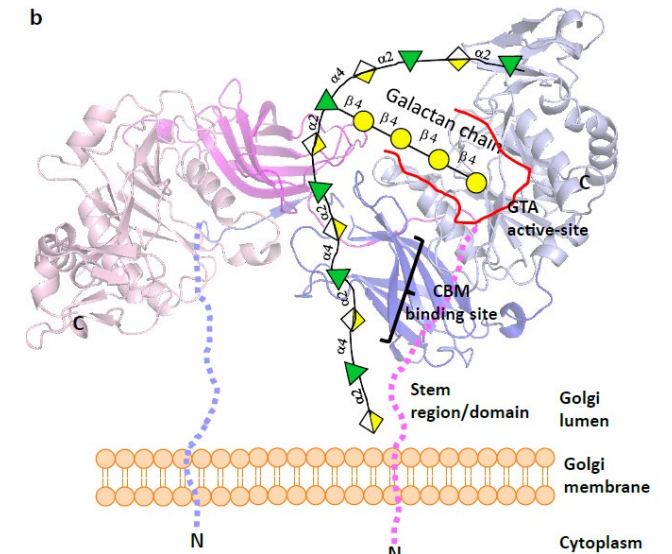


Figure 2. Proposed schematic representation of RGI binding and galactan chain elongation by homodimeric GalS1.