(1→3)-β-D-GLUCAN SYNTHASES OF PLANTS

by

Andrew Keith Jacobs, B. Sc. (Hons.)

A thesis submitted in fulfillment of the requirements
for the degree of Doctor of Philosophy

School of Agriculture and Wine
Faculty of Sciences
University of Adelaide, Waite Campus
Glen Osmond, SA 5064
Australia

June 2003

Table of Contents

Abstract

Callose is a (1→3)-β-D-glucan that is widely distributed in higher plants. During normal plant growth and development, callose is found as a transitory component of the cell plate in dividing cells, it is a major component of pollen mother cell walls and pollen tubes, and is found as a structural component of plasmodesmatal canals. Callose is also observed in abscission zones and in the phloem of dormant tissues. In addition to its role in normal growth and development, callose is deposited between the plasma membrane and the cell wall following exposure of plants to a range of abiotic and biotic stresses, including wounding, desiccation, metal toxicity and microbial attack. The involvement of the plant glucan synthase-like or *GSL* genes in the formation of callose at these various locations is investigated in this thesis. Techniques including database screening, bioinformatics, various cloning protocols, heterologous expression, analysis of protein-protein interactions, gain-of-function systems and loss-of-function systems have been used in this project. Gene isolation was conducted in ryegrass where good biochemical data existed for GSLs but subsequent functional analyses were conducted in barley, where better genetic information was available. Ultimately proof of function of a single *GSL* gene was achieved in the plant model *Arabidopsis*.

A full length cDNA of a (1→3)-β-D-glucan synthase termed *LmGSL1*, encoding 1907 amino acid residues, was isolated from *Lolium multiflorum* using molecular techniques. The deduced LmGSL1 protein is predicted to contain 14 transmembrane spanning domains with the NH_2-terminus and a large central loop, thought to contain the catalytic site, located in the cytoplasm. A homologue of this gene was isolated from barley, *HvGSL1*, for the purpose of functional analyses. cDNA fragments of the barley *GSL* gene were expressed in *E. coli* and mammalian cells, and (1→3)-β-D-glucan synthase activity assays were undertaken on purified fractions. No (1→3)-β-D-glucan synthase activity could be attributed to either of the expressed polypeptides, alone or when combined.

The involvement of the barley *GSL* gene in the formation of papillae that result from fungal challenge was investigated in single cells of barley leaf blades using a transient

gene-silencing assay. Double-stranded RNA interference (dsRNAi) constructs with homology to *HvGSL1* were produced and introduced into the epidermal cells of barley leaves using a biolistic approach. The callose deposits found in transformed leaves at papillae were indistinguishable from those found in control leaves, suggesting that HvGSL1 is unlikely to be involved in papillae formation. Regions of the barley GSL protein were further assessed for interactions with other proteins using the yeast two-hybrid system and interactions between the NH_2-terminal region of HvGSL1 and proteins expressed from several plant cDNA libraries were detected.

Deposition of callosic plugs, or papillae, at sites of fungal penetration is a widely recognised early response of host plants to microbial attack and is thought to physically impede entry of the fungus. *Arabidopis* was transformed with dsRNAi constructs designed to silence three putative callose synthase genes (*AtGSL5, 6* and *11*). Both papillary callose and wound callose were absent in lines transformed with *AtGSL5* dsRNAi constructs, and in a corresponding sequence-indexed *AtGSL5* T-DNA insertion line, but were unaffected in *AtGSL6* and *AtGSL11* dsRNAi lines. Depletion of callose from papillae in *gsl5* mutants only slightly enhanced penetration of the grass powdery mildew fungus *Blumeria graminis*. Upon infection of wild type *GSL5* plants with biotrophic powdery mildew fungi or the oomycete *Peronospora parasitica*, callose also encased haustorial complexes, which are intracellular fungal feeding structures that are vital for nutrient uptake. Most importantly, the absence of callose in papillae or at haustorial complexes correlated with effective growth cessation of several normally virulent powdery mildew species and of *P. parasitica*. The enhanced disease resistance phenotype of the previously described *Arabidopsis* mutant *pmr4* to powdery mildew is probably the result of a mutation in *AtGSL5*. It is proposed that biotrophic fungal pathogens exploit wound-inducible GSL5 callose for the maintenance of a biotrophic lifestyle.