Biochemical aspects of the cell wall of Fusarium oxysporum
Schoffelmeer, E.A.M.

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SUMMARY

In this thesis research is described that focuses on several biochemical aspects of the cell wall of the fungal plant pathogen *Fusarium oxysporum*. We have not only identified components of this cell wall but also determined its structure. Furthermore, the components that are associated with covalently bound cell wall glycoproteins (CWPs) were identified and finally we found compelling evidence for the involvement of a glycosylphosphatidylinositol anchor (GPI-anchor) in linking CWP to β-glucan. Since little was known about the *F. oxysporum* cell wall composition we started with a biochemical and structural analysis (chapter 2). Biochemical analysis revealed several macro-molecular structures, notable glycoproteins and polysaccharides composed of β-1,3-, α-1,3- linked glucose (glucans) and α-1,4- linked N-acetylglucosamine (chitin). Using specific antibodies, β-1,6-glucan was also detected in this cell wall (chapter 3). Both glucan and chitin are major components of this cell wall as glucose and N-acetylglucosamine represent 25% and 10% of the cell wall dry weight, respectively. Also mannose is an abundant monosaccharide that represents ~29% of the cell wall dry weight. Minor monosaccharides in this cell wall appeared to be galactose and uronic acids; ~9 and ~10% of the cell wall dry weight, respectively. Proteins make up only 7-8% of cell wall dry weight and appear to be extensively decorated with glycan chains (chapter 3). These glycan chains not only consist of mannose (chapter 3) but also contain galactose, uronic acid and glucose. As such, glycoproteins are a considerable component of the cell wall. Structural analysis of the wall by electron and fluorescence microscopy revealed a bilayered structure. The layer close to the plasma membrane consists predominantly of carbohydrates, notably chitin and glucan. CWPs are found in the outer layer of the cell wall exposed to the environment.

As demonstrated in chapter 2 the cell wall contains CWPs that resist SDS/β-mercaptoethanol extraction suggesting that they are covalently bound to the cell wall. To investigate the nature of this linkage glycoproteins were subjected to a more detailed analysis (chapter 3). About 69% of the covalently bound CWPs were β-1,3-glucanase extractable, indicating a link with β-1,3-glucan. Antibodies that specifically recognized β-1,3-glucan confirmed this notion. Besides β-1,3-glucan, high molecular weight CWPs (± 200 kDa) also contain β-1,6-glucan that was not detected on low molecular weight CWPs (< 80 kDa). The linkage between CWPs and β-glucan was further investigated by using aqueous hydrofluoric acid (HF), an agent that is known to break phosphodiester bonds. Aqueous HF treatment of CWPs resulted in loss of β-1,3-glucan from the high molecular weight CWPs, indicating that the β-1,3/β-1,6-glucan is linked to the CWPs via a phosphodiester bond. These observations suggest that cell walls of the mycelial fungus *Fusarium oxysporum* may contain
a similar structural unit as found in the budding yeast *Saccharomyces cerevisiae*, namely CWP- '{P-}→β1,6-glucan→β1,3-glucan. Only the CWPs of low molecular weight (< 80 kDa) contain β-1,3-glucan but lack β-1,6-glucan, suggesting that these CWPs are directly linked to β-1,3-glucan.

Release of CWPs from the *Fusarium* cell wall by aqueous HF and more specifically the release of the β-1,3-/β-1,6- heteroglucan from CWPs by this agent, suggested that a phosphodiester bond is involved in linking CWPs to β-glucans. This phosphodiester bond might be part of a GPI-anchor. GPI-anchors are attached to proteins that possess a C-terminal hydrophobic domain. Attachment of a GPI-anchor results in membrane localization of this protein. To obtain evidence for GPI-anchoring, and determine additional characteristics of *Fusarium* CWPs we set out to clone a gene encoding such a protein. To this end we obtained the partial amino acid sequence of an N-terminal peptide from a 60 kDa CWP that was released by aqueous HF (chapter 4). Based on this information a gene designated *FEM1* (*Fusarium* Extra-cellular Matrix protein) was cloned. The primary translation product of *FEM1* revealed an N- and C-terminal signal peptide. The former directs the protein into the secretory pathway, whereas the latter has the hallmark of a GPI-anchor attachment/cleavage signal. The presence of this signal together with the high abundance of serine and threonine residues that potentially serve as attachment sites for O-glycan chains are features that are shared with CWPs of *S. cerevisiae*. Addition of glycan chains probably accounts for the difference between the size of the mature protein of 17.5 kDa and the estimated molecular weight of 60 kDa. To demonstrate that we obtained the gene encoding the 60 kDa CWP, a gene construct was generated that would result in a histidine tagged FEM1. Cell walls isolated from a *Fusarium* isolate transgenic for a tagged-*FEM1* gene were treated with aqueous HF and released a His-tagged CWPs of the expected size, clearly showing that we cloned the right gene. Whether the putative GPI-attachment/cleavage site functions as such was studied by creating a *FEM1* gene that would result in a C-terminal truncation of FEM1. A *Fusarium* transformant expressing this gene was not able to incorporate FEM1 into its cell wall, indicating that the GPI-anchor attachment/cleavage site plays an essential role in linking this CWP into the cell wall. This shows for the first time that GPI-anchoring of CWPs as a means to link CWPs covalently into the cell wall is not only restricted to yeast CWPs but also occurs in filamentous fungi like *Fusarium*.