

(05-1-71)

A Proteomic Approach to Apoplastic Proteins Involved in regeneration of Plant Cell Walls

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The plant cell wall is a dynamic structure that plays a critical role not only in determining cell shape and formation of the plant body, but also in interactions with environmental factors including those required for nutrition, response to abiotic stress and biological attack by other organisms. More than 2000 putative cell wall related genes are estimated to occur in the genome database of *Arabidopsis thaliana*. These genes are considered to encode proteins in categories with differing biological functions. Despite these genomic approaches, a complete picture of the concerted action of these cell wall proteins in the cell wall construction and remodeling processes is largely unknown.

To clarify the mechanisms of cell wall construction, we used a proteomic approach to investigate the proteins secreted into cell wall spaces during cell wall regeneration from the protoplasts of *Arabidopsis* suspension cultured cells. We focused on cell wall proteins loosely bound to the cell wall architecture and extractable with 1 M KCl solutions from: (1) native cells, (2) protoplasts that had been allowed to regenerate their cell walls for 1 h, and (3) protoplasts allowed to regenerate their cell walls for 3 h. We adopted a non-destructive extraction procedure without disrupting cellular integrity, thereby avoiding contamination from cytoplasmic proteins. Using two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) and matrix-assisted laser desorption ionization-time-of-flight/mass spectrometry (MALDI-TOF/MS), we separated, mapped, and identified 71 proteins derived from the native cell wall, and 175 and 212 proteins derived from the 1 h- and 3 h- regenerated protoplasts, respectively. Quite different sets of proteins with differing status of their post-translational modifications, including phosphorylation and glycosylation, were identified in the three protein fractions. This indicated dynamic *in muro* changes in the cell wall proteins during cell wall regeneration in the protoplasts. The analysis revealed a set of enzymes specifically involved in cell wall expansion and construction in suspension cultured cells. This approach has also determined a set of cell wall proteins that had not been predicted to be localized in cell wall spaces.

These results strongly indicate the usefulness of a proteomic approach for cell wall dynamics. Functional analyses for each of these newly identified cell wall proteins, together with their biochemical characterization and expression analyses, should extend our understanding of the whole picture of cell wall dynamism from a genomic point of view, and give an invaluable molecular basis for metabolomic approaches of this complex supermolecule.