Title Phosphoproteomic analysis of ethylene signaling pathway in Arabidopsis thaliana

Author Hao Li and Ning Li

Citation Thesis, Doctor of Philosophy (Biology), Hong Kong University of Science and Technology. 210 pages. 2008.

Keywords Phosphoproteomic; Ethylene; Gene expression; Arabidopsis mutant

Abstract

The plant hormone ethylene regulates a variety of stress responses and developmental adaptation in plants. Ethylene signal transduction is one of the most intensively studied cell signaling subjects in plant biology over the past decade. In the former forward genetic screen research in our lab, based on the stimulation effect of long-term treatment (12 hours) of ethylene on hypocotyl gravitropism of light-grown *Arabidopsis* seedlings, a mutant, *egy1-1,* was isolated and characterized to have a duel-phenotype, reduced chlorophyll accumulation and abnormal ethylene-dependent hypocotyl gravicurvature. In the present study, we have adopted the phosphoproteomics approach to investigate the differential protein phosphorylation in response to a 12-hour ethylene treatment in an ethylene-insensitive mutant *ein2.* A total of 318 phosphopeptides were identified from both the ethylene-treated and -untreated etiolated *ein2* seedlings, of which 72 phosphopeptides were detected at least three times by Q-TOF nanoLC-MS/MS analysis. The ratio of the number of phosphopeptides identified from ethylene-treated *ein2* seedlings to that of the untreated is 0.82, suggesting a general reduction of phosphorylated proteins in *ein2* mutant following an ethylene exposure. Overall, we have identified 3 ethylene-inducible and 3 ethylene-repressible unique phosphopeptides, respectively. Genes encoding proteins related to osmolarity and aluminum-induction are representatives of ethylene-induced phosphoproteins, whereas genes encoding calnexin precursor and RNA recognition motif (RRM)-containing protein are representatives of ethylene-suppressed ones. Most of phosphorylation events occur on Ser (66.9%) and Thr (28.4%) residues and only a few on Tyr (4.7%) residues. More than 55% of phosphoproteins are related to those signaling components and factors responsible for regulation of gene expression. Bioinformatic analysis of 72 repetitively identified unique protein phosphopeptides revealed two groups of most frequently occurring phosphopeptides, which contain the highly conserved phosphorylation motifs, PRVD/G *S* x and *S* PDYxx,, respectively. Analysis of the ethylene-altered phosphopeptides also reveals 5 unique protein phosphorylation motifs, 2 of which are ethylene-inducible, whereas the rest 3 are ethylene-repressible. *EIL1, ERF* transcription factors and Hua Enhancer 4 were all found to contain one of the putative protein phosphorylation motifs that may be ethylene responsive in terms of phosphorylation status. The *in vitro*

kinase assays validated the phosphorylation of both groups of proteins identified either from phosphoproteomics and bioinformatics prediction. The ethylene-dependent differential phosphopeptides were substantiated by the kinase assays and dot-blot experiments. These results suggest that ethylene signals may be transduced by phosphor-relay to nuclear transcriptional events *via* both *EIN2* -dependent and -independent pathways.