DOCTORAL THESIS

Development of redox proteomics methods and the identification of redox-sensitive proteins in arabidopsis

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Date of Award:
2015

Abstract

Cellular redox homeostasis mediates a wide range of physiological and developmental processes. Various stresses trigger over-production of reactive oxygen/nitrogen species which leads to oxidative modifications of redox-sensitive proteins. Identification and characterization of redox-sensitive proteins are important steps toward understanding molecular mechanisms of stress responses.

In the study, a high-throughput quantitative proteomic approach termed OxiTRAQ was developed for identifying proteins whose thiols undergo reversible oxidative modifications in Arabidopsis cells subjected to oxidative stress. In this approach, a biotinylated thiol-reactive reagent is used for differential labeling of reduced and oxidized thiols, and the biotin-tagged peptides are affinity-purified and labeled with iTRAQ reagents for quantitation. This approach allows identification of the specific redox-regulated cysteine residues in proteins and offers an effective tool for elucidation of redox proteomes.

With this approach, we identified 195 cysteine-containing peptides from 179 proteins whose thiols underwent oxidative modifications in Arabidopsis cells following the treatment with hydrogen peroxide. A majority of those redox-sensitive proteins, including several transcription factors, were not identified by previous redox proteomics studies. Besides, this method was also used to identify proteins that underwent oxidative modifications in Arabidopsis cells subjected to 15 minute treatment of salicylate (a key signaling molecule in the plant defense pathway) or flg22 (a *peptide* from bacterial flagellin that induces pathogen associated *molecular patterns-triggered* immunity). In total, 127 peptides from 111 distinct proteins were identified as salicylate- and/or flg22-responsive redox-sensitive proteins. Among the identified redox sensitive proteins are many regulatory proteins including those involved in chromatin remodeling, transcription, nucleocytoplasmic shutting, and posttranslational regulation.

Furthermore, *in vivo* 15N metabolic labeling method combined with a cysteine-containing peptide enrichment technique was applied to identify proteins that undergo oxidative modifications in plants in response to pathogen attack. The identification of redox-sensitive proteins provides a foundation from which further study can be conducted toward understanding the biological significance of redox signaling in plant stress response.

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