Abstract

Characterization of plant protein complexes by multiple chromatography and quantitative mass spectrometry techniques

Uma K Aryal1, 2, Eileen Mallery3, Mark C. Hall2, Jun Xie3, Yi Xiong4, Daisuke Kihara4, Daniel B. Szymanski3

Department of Agronomy 1, Biochemistry 2, Statistics 3 and Computer Science 4, Purdue University, West Lafayette, IN 47907

Introduction

Cytosol is an intracellular fluid containing non-organellar molecules such as ions, metabolites, nucleic acids, and proteins. It allows interactions between compartmentalized metabolic processes. While cellular organelles undertake specialized functions, many fundamental processes such as glycolysis, part of the PP pathway, protein biosynthesis, and degradation can take place in the cytosol. Virtually all proteins are present in, and function as, oligomeric complexes. Determination of the composition and modification of protein complexes is therefore an important research goal. This project seeks to develop new mass spectrometry-based methods to determine the composition of endogenous protein complexes in the cytosol of Arabidopsis.

Methods

Plant Growth Conditions
A. thaliana (Col-0) seeds were cold treated at 4°C for 4 days and cultivated at 22°C under sterile condition using 0.5 X Murashige and Skoog (MS) mineral salts with BactoAgar™ under constant light. Ten days old seedlings were transferred to a new plates containing the same MS medium and grown for 3 weeks before harvesting for protein extraction.

Cytosolic Fraction Separation
Proteins were extracted under non-denaturing condition and cell-fractionated by differential centrifugation to obtain cytosolic fraction (Figure 2). Protein complexes in the cytosolic fraction were resolved by SEC. SEC fractions probed with a polyclonal anti-PEPC antibody. (B) Western blot showing the enrichment of PEPC in the cytosolic fraction. PEPC1 and PEPC2 were detected in the cytosolic fraction.

Protein Identification
Proteins in each SEC fraction were analyzed by MALDI-TOF mass spectrometry system (Figure 3) to establish the presence of protein complexes. Protein complexes in SEC fractions were digested with trypsin and analyzed by LTQ-Orbitrap. MS data were processed using Xcaliber (Thermo) and searched against the NCBInr protein database using MASCOT v2.2.07 (Matrix Science) for protein identification. Protein quantification was performed using Progenesis LC-MS software.

Results

Proteins in different cell fractions are shown in Figure 3 showing differences in proteins, particularly in 200k pellet and supernatant fractions. Table 1 shows the number of proteins identified in each fraction. SEC effectively separated cytosolic protein complexes with majority of proteins showing peak intensity in fraction 9 and 11. Only one peptide for chloroplast-encoded large subunit of RUBISCO, and 26 peptides for nuclear-encoded small subunit of RUBISCO which has a cytosolic pool, were detected. Western blot analysis revealed that ENOLASE partitions between cytosol and endomembrane compartment. Our method provides a robust and high throughput platform for studying plant protein complexes.

Conclusions

• The combination of cell fractionation, SEC and LC-MS is an effective strategy for determining the apparent mass of endogenous cytosolic protein complexes.

Proteins localize in multiple compartments. For example, PEPC resides almost entirely in cytosol whereas others such as ENOLASE can localize in multiple organelles. Using LC-MS, we observed 18 peptides of the cytosolic ENOLASE2/ENO2. Sucrose gradient and western blot also showed that ENO2 was also associated with membrane, and co-purified in ER-enriched membrane. This was also confirmed by LC-MS. This result suggests that we can use cell-fractionation method with LC-MS profiling to localize plant protein complexes.

Future Plan

1. Prediction of 100 protein complex composition in cultured cells, and their subunits partitions between cytosol and endomembrane.
2. Prediction and localization of 100 cytosolic protein complexes from Arabidopsis and soybean leaf, and verification using mutants.

References


Contact: uaryal@purdue.edu