PROBING MOLECULAR INTERACTIONS IN COMPLEXES OF GEL-SEPARATED PROTEINS BY MASS SPECTROMETRY

Kenneth Mackun and Kevin M. Downard

School of Molecular and Microbial Biosciences, University of Sydney, Sydney, Australia

Proteins usually work in association with other molecules to perform vital functions within the cell. The investigation of the molecular basis for these interactions is therefore an essential part of the functional characterisation of proteins, contributing to our understanding of many important biological processes. Advances in protein mass spectrometry over several decades have led to it being an established core technology for proteomics. This involves the large-scale study of proteins expression within particular cells or tissues under distinct physiological and environmental conditions. The coupling of two-dimensional polyacrylamide gel electrophoresis (PAGE) with mass spectrometry currently forms the basis of most proteome analyses, allowing for the separation and identification of thousands of proteins *en masse* with the assistance of database searching tools.

We have previously demonstrated the ability to study protein interactions by mass spectrometry through both the preservation of protein complexes on a matrix-assisted laser desorption ionization (MALDI) sample plate (1,2) and following their direct detection within the confines of a mass spectrometer (3). Despite some perceived and legitimate limitations of MALDI for these studies, the technique has the advantage that it can be mostly easily coupled to a gel-based separation strategy.

Here we demonstrate for the first time the use of native PAGE coupled with mass spectrometry to probe the molecular interactions within protein complexes [4]. Several protein systems have been studied including antigen-antibody and enzyme-peptide complexes. The recovery of both protein binding partners and intact protein complexes from native gels has been developed and optimized, and their conformational integrity confirmed by both circular dichroism (CD) spectroscopy and electrospray ionization mass spectrometry (ESI-MS). Proteins and complexes were recovered from unstained and negative stained gels to avoid the denaturing effect of typical stains. The protein complexes were treated with site-specific proteases to release the non-binding regions from each of the binding partners. The non-binding segments are separated from their bound counterparts by ultrafiltration coupled to centrifugation and the regions bound subsequently characterized through both MS and MS/MS experiments.

The approach is amenable to high sample throughput and automation, and when coupled with the MALDI-based subtractive approach (1,2) we have developed, would allow protein interactions to be studied by a proteomics-based strategy without the separation of the bound and non-bound protein domains.

References:

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