Evaluation of Macrophage Cell Membrane Preparation for Proteomics Analysis

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INTRODUCTION

In the proteomics science, powerful technologies are applied for the comprehensive identification and characterization of membrane proteins. However, these molecules impose technical difficulties. While 2DE has a high capacity for separation and visualization allowing, for instance, quantification of volume and post-translational modifications, the highly hydrophobic, low-abundant and basic proteins are not amenable to this approach. Non-gel methods have therefore the potential range for ionized basics, hydrophobic and high molecular weight proteins. Nevertheless, the limited post-processing capacity of transmembrane protein operates, is difficult, assessing the conditional level of knowledge and knowledge-based enrichment. Regardless the tactic chosen, enrichments in conjunction with efficient extraction and solubilization are crucial.

In this study, we used J774 macrophage cell lysates to determine optimal extraction and solubilization conditions for the application of gel- and non-gel-based methods.

MATERIALS & METHODS

Crude membranes were obtained by ultracentrifugation of cell homogenates prepared by Triton X-100 lysis buffer. A whole membrane extraction buffer were assessed for extraction capacity at 20 °C, pH 8.0. CM, crude membranes. CM/ASB14, proteins collected by the inner membrane (B) using a Micromass A@ldiTM spectrometer (Manchester, UK).

1D-MALDIIMS approach for enriched plasma membrane sample preparations

Bottom-up Shotgun 2D-LC MS/MS of crude macrophage membrane preparations

Scheme of protocols for preparation of crude membranes and plasma membrane enriched proteins

RESULTS & DISCUSSION

Two plasma membrane samples were used, crude and plasma enriched samples. For this purpose, we optimized our sample extraction, we used standard conditions for crude membrane preparations. Also, a non-gel based approach was assessed in crude membrane preparations. The plasma membrane enriched samples were explored by 2DE-PAGE and 1D-PAGE and tryptic peptides identified by MALDI-TOF.

Vector control was evaluated for its ability to induce high number of clear and well-focused protein signals on 2DE gels. The simplest and most straightforward one was to condition protein extraction procedure at the concentration of 10 and 15 mM DHPC (1, 2-diheptanoyl-10 mM Tris HCl, pH 8; 7 M urea, 2 M thiourea, 5 mM TBP) and 10% ASB14. For both cases, DHPC induces a two-phase separation (membrane clumping). In this case, it would be required to block the plasma membrane, to visualize and subcellular localization with more. The subcellular localization (15) of these steps is required for the experimental reproducibility. Thus the 2D-PAGE can also be used as a tool to assess the level of peptide fractionation. The plasma-membrane enriched samples were explored by 1D-MALDIIMS and tryptic peptides identified by MALDI-TOF.

Membrane fractions were removed for protein content determination. Peptides eluted consecutively steps. Proteins in the second dimension gels were visualized using Sypro Ruby stain (Sigma). Isoelectric focusing (IEF) and second-dimensional gel.

MALDI/MS analysis

MULTICOMPOUND ANALYSIS

MATERIALS & METHODS

Material and methods: 1D-MALDIIMS approach for enriched plasma membrane sample preparations

Crude membrane samples were loaded onto a RP trapping column. The samples are then eluted onto the analytical column. Pump 2. Flow: 5,000 µl/min. The column was connected to a Nanoflow device. Chromatogram showing UV traces from the SCX runs of the crude membrane aliquots onto a RP trapping column. The samples are then eluted onto the analytical column. Pump 2. Flow: 5,000 µl/min.

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