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Abstract
Lipid rafts are specialized microdomain of plasma membrane characterized by its enrichment of cholesterol, sphingolipids, and GPI-anchored proteins. Previous studies have indicated that lipid rafts play an important role in vecicular trafficking and receptor-mediated signal transduction. To date, the studies on the lipid rafts proteome mostly utilized nongel-based methods, such as a combination of liquid chromatrography and mass spectrometry. In contrast, lipid rafts proteins are poorly resolved by two-dimensional gel electrophoresis (2-DE), despite its ability to separate thousands of proteins in one gel. The aim of this study is to develop a high resolution separation strategy based on 2-DE and to couple with tandem mass spectrometry to profile the lipid rafts. First, the lipid rafts in THP-1 cells were separated by density gradient centrifugation and further confirmed by immunoblotting analysis using markers of lipid rafts and ornagelle's membrane. For separation of raft components by 2-DE, a variety of detergents were tested. The results showed that the raft components are better separated by a combination of ASB-14 and CHAPS with respect to the pattern and the number of protein spots resolved. The identities of 17 spots have also been determined by mass spectrometry, including signaling molecules, cytoskeleton proteins, vesicular trafficking, and endoplasmic reticulum components. As lipid rafts are involved in transmembrane signaling, the established 2-DE approach was applied to explore the change of raft components in control and fractalkine-treated THP-1 cells. Image analysis of the 2D profiles indicated that there are 12 differentially expressed protein spots in the lipid rafts fraction of fractalkine-treated cells. Among them, 6 spots are up-regulated and 6 down-regulated. In summary, we have successfully established a strategy using high resolution 2-DE to separate the lipid rafts components. Coupling with protein identification by mass spectrometry, the 2DE-based strategy will not only be useful to establish the lipid rafts proteome but also lead to a better understanding of raft-associated signaling network.
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