

Abstract

MS/MS is the technology of choice for analyzing complex protein mixtures. However, due to the intrinsic complexity and dynamic range present in higher eukaryotic proteomes, prefractionation is an important step to maximize the number of proteins identified. Off-gel IEF (OG-IEF) and high pH RP (Hp-RP) column chromatography have both been successfully utilized as a first-dimension peptide separation technique in shotgun proteomic experiments. Here, a direct comparison of the two methodologies was performed on ex vivo peripheral blood mononuclear cell lysate. In 12-fraction replicate analysis, Hp-RP resulted in more peptides and proteins identified than OG-IEF fractionation. Distributions of peptide pIs and hydrophobicity did not reveal any appreciable bias in either technique. Resolution, defined here as the ability to limit a specific peptide to one particular fraction, was significantly better for Hp-RP. This leads to a more uniform distribution of total and unique peptides for Hp-RP across all fractions collected. These results suggest that fractionation by Hp-RP over OG-IEF is the better choice for typical complex proteome analysis.