

107d Use of Two-Dimensional Electrophoresis for Studying Protein Phosphorylation

Nancy Kendrick, Jon J Johansen, and Matthew Hoelter

Many eukaryotic cellular processes involve protein phosphorylation as evidenced by the 518 kinases revealed in the human genome sequence. Unraveling function of phosphorylated proteins will be key in understanding many disease processes. Two-dimensional electrophoresis (2D) allows separation of phosphorylated protein charge isoforms from the unphosphorylated form, and facilitates elucidation of phosphorylation sites. However, sample solubilization for 2D is often a problem. In this study we examine the effects of SDS on resolution of phosphorylated proteins by comparing 2D patterns of mouse liver phosphorylated proteins detected with an anti-phosphotyrosine antibody and Western blotting. Phosphatase treatment is used to confirm protein identity. Samples prepared in urea and SDS buffer are compared using the carrier ampholine 2D system and Progenesis Discovery software. Theoretical and practical limits to resolving phosphoprotein isoforms are discussed. Examples of 2D patterns of known phosphorylated proteins are shown.