Detection of N-glycans on small amounts of glycoproteins in tissue samples and sodium dodecyl sulfate–polyacrylamide gels

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N-linked glycans harbored on glycoproteins profoundly affect the character of proteins by altering their structure or capacity to bind to other molecules. Specific knowledge of the role of N-glycans in these changes is limited due to difficulties in identifying precise carbohydrate structures on a given glycoprotein, which arises from the large amounts of glycoprotein required for N-glycan structural determination. Here, we refined a simple method to purify and detect trace amounts of N-glycans. During the N-glycan purification step, most contaminants were removed by two kinds of columns: a graphite carbon column and a cellulose column. N-Glycans were identified with a three-dimensional high-performance liquid chromatography (HPLC) system. Using our method, a global analysis of N-glycans from human muscle biopsy samples and mouse brain sections was possible. By combining sodium dodecyl sulfate–polyacryl- amide gel electrophoresis (SDS–PAGE) with our method, we refined analytical procedures for N-glycans from SDS–PAGE gels using hydrazinolysis to achieve a high N-glycan recovery rate. N-Glycans on as little as 1 mg of the target protein transferrin or immunoglobulin G (IgG) were easily detected. These methods allowed us to efficiently determine glycoprotein N-glycans at picomole (pmol) levels.