Simple Method for Detecting Glycoproteins Dot-blotted or Electro-blotted on to a Polyvinylidene Difluoride Membrane

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A simple method is described for detecting glycoproteins which had been dot-blotted or electro-blotted on to a polyvinylidene difluoride (PVDF) membrane after sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. This method is based on the periodate oxidation of glycoproteins on a PVDF membrane with subsequent staining by the chromophoric hydrazide, 4-N,N-dimethylamino-4'-azobenzene sulfonyl hydrazide. The glycoproteins could be visualized as red-colored spots or bands in the range of 0.16–0.31 μg.

Key words: glycoprotein; PVDF membrane; blotted; sugar; hydrazide

A powerful tool for the purification of microquantities of proteins is SDS-polyacrylamide gel electrophoresis and subsequent electroblotting of the proteins on to an immobilizing membrane. By using a chemically stable membrane such as PVDF, the immobilized protein is then directly available for chemical analysis to obtain information on the amino acid sequence(1) or amino acid composition.(2) This technique is also applicable to glycoproteins by adopting a staining method specific for the relevant glycoprotein. There are several reports on the detection technique for glycoproteins on a PVDF membrane,(3,4) these being based on the periodic acid-Schiff (PAS) method.(3)

We describe here a simple procedure for detecting glycoproteins dot-blotted or electro-blotted on to a PVDF membrane after SDS-polyacrylamide gel electrophoresis by using the chromophoric hydrazide, 4-N,N-dimethylamino-4'-azobenzene sulfonyl (DABS) hydrazide.

Glycoproteins dot-blotted or electro-blotted on to a PVDF membrane (protein-sequence membrane, 0.2 μm pore size, Bio-Rad Lab., Richmond, CA, U.S.A.) were stained with DABS-hydrazide under optimized conditions. DABS-hydrazide was prepared from DABS-chloride by reaction with hydrazine as described previously.(6) DABS-hydrazide (5 mg) was dissolved in 5 ml of ethanol and mixed with 10% aqueous trichloroacetic acid (TCA) at 39:1 (v/v) just before use. Glycoproteins each blotted on to a PVDF membrane (6.5 × 5.5 cm) were oxidized with 10 ml of 1.65% periodic acid in 5% aqueous acetic acid for 20 min at room temperature. The PVDF membranes were washed three times with 50 ml of distilled water for 10 sec each to remove the excess periodic acid and then treated with 10 ml of 0.5% sodium metabisulfite in 5% aqueous acetic acid for 3 min. After washing again three times with distilled water, the PVDF membranes were incubated in 5 ml of the DABS-hydrazide solution for 20 min at 60°C. The membranes were then washed with ethanol to remove the excess reagent and treated with 0.02% borane-dimethylamine in ethanol for 3 min at room temperature. This manipulation significantly reduced the background, although any longer incubation time did not increase the sensitivity. The glycoproteins on the PVDF membranes were visualized as red-colored spots or bands after successively washing with distilled water and 1N HCl.

This staining procedure required less than 50 min to complete. The detection limit was examined for several glycoproteins each dot-blotted onto a PVDF membrane by using Bio-Dot microfiltration apparatus (Bio-Rad Lab.). Ovalbumin (lot M3M9365) and bovine serum albumin (lot M3K9967) were from Nacalai Tesque (Kyoto, Japan), and fetal calf serum fetuin (lot I29F9585) and bovine pancreatic trypsin (lot 17F0188) were from Sigma (St. Louis, Mo., U.S.A.). An acorn barnacle lectin (BRA-2)(7) and soybean agglutinin(9) were prepared as described, BRA-2 containing 0.6% mannose, 0.2% fucose and 2.3% N-acetylgalactosamine.(9) Ovalbumin, soybean agglutinin and BRA-2, whose carbohydrate contents were 3.3%, 6.5% and 3.1%, respectively, could be detected at 0.31 μg, while fetuin containing 22% carbohydrate could be detected even at 0.16 μg (Fig. 1). This detection limit is lower than that of the conventional PAS method, whereas non-glycoproteins like trypsin and bovine serum albumin were not stained as expected.

SDS-polyacrylamide gel electrophoresis was carried out by the method of Laemmli(10) with mini slab gels (8 × 9 cm, 1.0 mm thick) of 12.5% of polyacrylamide gel. Ten μg of each sample was subjected to gel electrophoreses, after which the gel was electroblotted on to a PVDF membrane by using semi-dry electro-blotting apparatus (Trans-Blot SD, Bio-Rad Lab.) according to the method of Lauciene.11) The electro-blotted PVDF membranes were then stained by the DABS-hydrazide method. Glycoproteins (ovalbumin, fetuin, soybean agglutinin and BRA-2) were specifically stained with DABS-hydrazide as shown in Fig. 2. The detection limit was not determined, because the efficiency of the glycoprotein transfer was not optimized in this study.

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Fig. 1. Staining of Dot-blotted Samples on a PVDF Membrane by the DABS-Hydrazide Method.

A, Ovalbumin; B, fetal calf serum fetuin; C, soybean agglutinin; D, BRA-2; E, bovine pancreas trypsin; F, bovine serum albumin.
The amount of each sample loaded on the PVDF membrane was 1, 2.5 μg; 2, 1.25 μg; 3, 0.62 μg; 4, 0.31 μg; and 5, 0.16 μg.

Fig. 2. Detection of Glycoproteins Electro-blotted on to a PVDF Membrane by the DABS-Hydrazide Method.

Ten μg of each sample was subjected to SDS-polyacrylamide gel electrophoresis. (A) Staining patterns with Coomassie brilliant blue. (B) Staining patterns by the DABS-hydrazide method. A, Ovalbumin; B, fetal calf serum fetuin; C, soybean agglutinin; D, BRA-2; E, bovine pancreas trypsin; F, bovine serum albumin.

The application of this method is shown in Fig. 3, which illustrates stained bands of the water-soluble fraction of defatted soybean (Miyagishirome) powder on the gel and on the electro-blotted PVDF membrane after SDS-gel electrophoresis. Although a number of protein bands were stained by Coomassie brilliant blue, 82 kDa, 76 kDa, 56 kDa and 37 kDa bands were detectable as glycoproteins. These bands on the PVDF membrane could be subjected to an amino acid analysis\(^\text{12}\) or amino-terminal sequencing\(^\text{13}\) without further manipulation.

The main use of this method is to determine whether a preparation contains glycoprotein or not after blotting on to a PVDF membrane. It can be applied to localization of the glycosylation sites within a glycoprotein by the combined use of peptide mapping and dot-blotting. Furthermore, DABS-hydrazide itself can be used for a sugar composition analysis of the blotted glycoproteins.\(^\text{14}\)

Fig. 3. Staining Patterns of the Water-soluble Fraction of Soybean after SDS-Polyacrylamide Gel Electrophoresis and Subsequent Electro-blotting on to a PVDF Membrane.

(A) Stained with Coomassie brilliant blue. (B) Stained by the DABS-hydrazide method.

References