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Abstract

A new method for staining sialoglycoproteins in polyacrylamide gel after disc electrophoresis is described. The method utilizes the reaction of sialic acids with an acidic ninhydrin reagent which yields a stable color with an absorption maximum at 470 nm. After electrophoresis, the polyacrylamide gel is placed in a test tube and heated with 5 ml of the acidic ninhydrin reagent for 10 min in a boiling water bath. Sialoglycoproteins are detected as brown bands. No additional procedure such as destaining is necessary. When 20 micrograms fetuin, a sialoglycoprotein, per gel is applied, the band remains visible for at least 2 h. Stained gel can be scanned with a gel scanner at 470 nm. When the stained gel was dried on a sheet of polypropylene filter, the color was stable for at least one month. The present method is superior to the method using Stains-all (3,3'-diethyl-9-methyl-4,5,4',5'-dibenzothiacarbocyanine) in specificity and simplicity for the detection of sialoglycoproteins.

KEYWORDS: sialoglycoprotein, polyacrylamide gel electrophoresis, staining, acidic ninhydrin reaction

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Visualization of Sialoglycoproteins in Polyacrylamide Gels by Acidic Ninhydrin Reaction

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A new method for staining sialoglycoproteins in polyacrylamide gel after disc electrophoresis is described. The method utilizes the reaction of sialic acids with an acidic ninhydrin reagent which yields a stable color with an absorption maximum at 470 nm. After electrophoresis, the polyacrylamide gel is placed in a test tube and heated with 5 ml of the acidic ninhydrin reagent for 10 min in a boiling water bath. Sialoglycoproteins are detected as brown bands. No additional procedure such as destaining is necessary. When 20 μ g fetuin, a sialoglycoprotein, per gel is applied, the band remains visible for at least 2 h. Stained gel can be scanned with a gel scanner at 470 nm. When the stained gel was dried on a sheet of polypropylene filter, the color was stable for at least one month. The present method is superior to the method using Stains-all (3, 3'-diethyl-9-methyl-4, 5, 4', 5'-dibenzothiacarbocyanine) in specificity and simplicity for the detection of sialoglycoproteins.

Key words : sialoglycoprotein, polyacrylamide gel electrophoresis, staining, acidic ninhydrin reaction

Sialoglycoproteins have been detected in polyacrylamide gel after disc electrophoresis by staining with a cationic carbocyanine dye, Stains-all (3, 3'-diethyl-9-methyl-4, 5, 4', 5'-dibenzothiacarbocyanine) (1, 2). We have reported a new specific method for determining free and bound sialic acids (3-5) using acid ninhydrin reagent 2 of Gaitonde (6). In the present paper, we report a new and simple method for the detection of sialoglycoproteins in polyacrylamide gel using the same acid ninhydrin reagent.

Materials and Methods

Chemicals. Chemicals for electrophoresis and acid ninhydrin reagent were purchased from Wako Pure Chemical Ind., Ltd., Osaka, Japan. Coomassie brilliant blue R-250 was obtained from Nacalai Tesque Inc., Kyoto, Japan. Fetuin and asialofetuin (Type I) were obtained from Sigma Chemical Co., St. Louis, MO, USA. Polypropylene prefilter (P12-WP-090-25), a membrane filter, was obtained from Micro Separations Inc., Westford, MA, USA.

Disc polyacrylamide gel electrophoresis. Disc electrophoresis was performed as described (7) using separating (7% polyacrylamide, pH 8.9) and stacking (2% polyacrylamide, pH 6.7) gels and Tris-glycine buffer, pH 8.3 as the electrode buffer. One-hundred μ l of fetuin

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solution (1 mg/ml) or 10 μ l of peritoneal fluid containing about 14 mg of protein per ml was applied, and electrophoresis was performed for 70 min. Bromophenol blue was used as a marker.

Procedure for staining with the acidic ninhydrin reagent. After the electrophoresis, the position of bromophenol blue was marked by inserting a small piece of stainless wire into the polyacrylamide gel. The gel and 5 ml of acidic ninhydrin reagent were placed in a test tube (10 mm i. d. \times 12 cm), heated in a boiling water bath for 10 min, and then cooled in a water bath at 25 $^{\circ}$ C. No additional procedure such as destaining was necessary. As the gel in the acidic ninhydrin reagent shrank to about 88 % of the original size during the heating, the gel length was corrected for the original length when electrophoretic profiles of gels stained by different staining methods were compared. The stained gel was scanned at 470 nm using a gel scanner (CS-900, Shimadzu Seisakusho, Kyoto, Japan).

Procedure for drying polyacrylamide gel after staining with acidic ninhydrin reagent. Gel stained by the acidic ninhydrin reaction as described above was put in

the slit (8 \times 80 mm) of a polyethylene frame (23 \times 100 \times 0.8 mm) and placed between two sheets of polypropylene prefilter, which were sandwiched between several sheets of Whatman 3MM filter paper (Fig. 5B). The sandwiched gel was dried using a gel dryer at 65 $^{\circ}$ C under reduced pressure. The dried gel was sealed in a polyethylene envelope in order to avoid exposure to ammonia vapor. The color in the dried gel was stable at least for one month.

Staining with Stains-all. The staining with Stains-all was performed according to the methods of Green and Pastewka (1) and of King and Morrison (2). The stained gel was scanned at 655 nm.

Other staining method. Total proteins were stained with Coomassie brilliant blue R-250. The stained gel was scanned at 600 nm.

Determination of sialic acids in solution. Determination of sialic acids and sialoglycoproteins was performed by acidic ninhydrin reaction (3-5). In some experiments, polyacrylamide gels after electrophoresis were sectioned into 3.7-mm lengths. Each piece of the gel was crushed with a glass rod in a test tube, and the proteins were

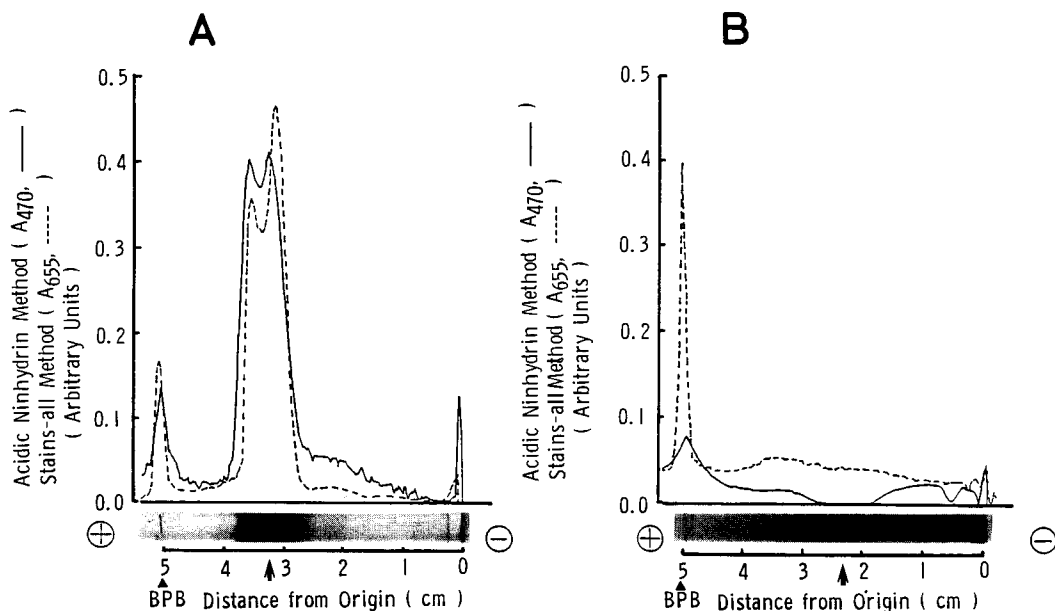


Fig. 1 Detection of fetuin by acidic ninhydrin reaction after disc polyacrylamide gel electrophoresis. Electrophoresis was performed using 7 % polyacrylamide gel (pH 8.9) as separating gel, 2 % polyacrylamide gel (pH 6.7) as stacking gel and Tris-glycine buffer (pH 8.3) as the electrode buffer. In A, 100 μ g of fetuin was applied to each of 3 separate gels. After electrophoresis, two gels were stained with the acidic ninhydrin reagent or Stains-all (0.04 %) and scanned at 470 and 655 nm, respectively. The third gel was stained with Coomassie brilliant blue R-250, and a photograph is shown at the bottom. Details of the electrophoresis and staining are described under Materials and Methods. In B, asialofetuin was applied and stained in the same manner. Arrows indicate the position of fetuin in A and asialofetuin in B.

extracted with 1ml of 5M acetic acid while heating in a boiling water bath for 60min. Aliquots of the extracts were subjected to the acidic ninhydrin reaction. In order to confirm the presence of sialic acids, extracts of sections exhibiting high acidic ninhydrin reaction were pooled. Sialic acids in the pooled extracts were isolated according to a previously reported method (5), and absorption profiles were recorded.

Determination of protein concentration. Determination of protein concentration was performed according to the method of Lowry *et al.* (8).

Results and Discussion

Fetuin was applied to 3 separate disc gels and electrophoresed in parallel. Each gel was stained with acidic ninhydrin reagent, Stains-all or Coomassie brilliant blue. The 2 gels stained with the former 2 reagents were scanned at 470 and 655 nm, respectively. The densitometric and visual patterns of these 3 gels are shown in Fig.

1. In Fig. 1A, the peak of the absorption curve at 470 and 655nm in the middle of the gels corresponded with the band stained with Coomassie brilliant blue R-250. As shown in Fig. 1B, this peak was not observed when asialofetuin was electrophoresed and stained under the same conditions. These results indicate that sialic acids in fetuin were stained by the acidic ninhydrin reaction as shown in the previous study, which was performed with sialic acid and sialoglycoprotein solutions (3-5). A calibration curve constructed after electrophoresis, acidic ninhydrin reaction, and densitometry using 50-200 μ g of fetuin per gel was linear. However, the curve constructed after Stains-all reaction was not linear.

Fig. 2 shows the disc electrophoresis and staining of the peritoneal fluid of mice bearing Ehrlich ascites tumor cells. Densitometric peaks 1, 2 and 3 observed after the acidic ninhydrin reaction coincided with visual bands observed after Coomassie brilliant blue staining as shown in

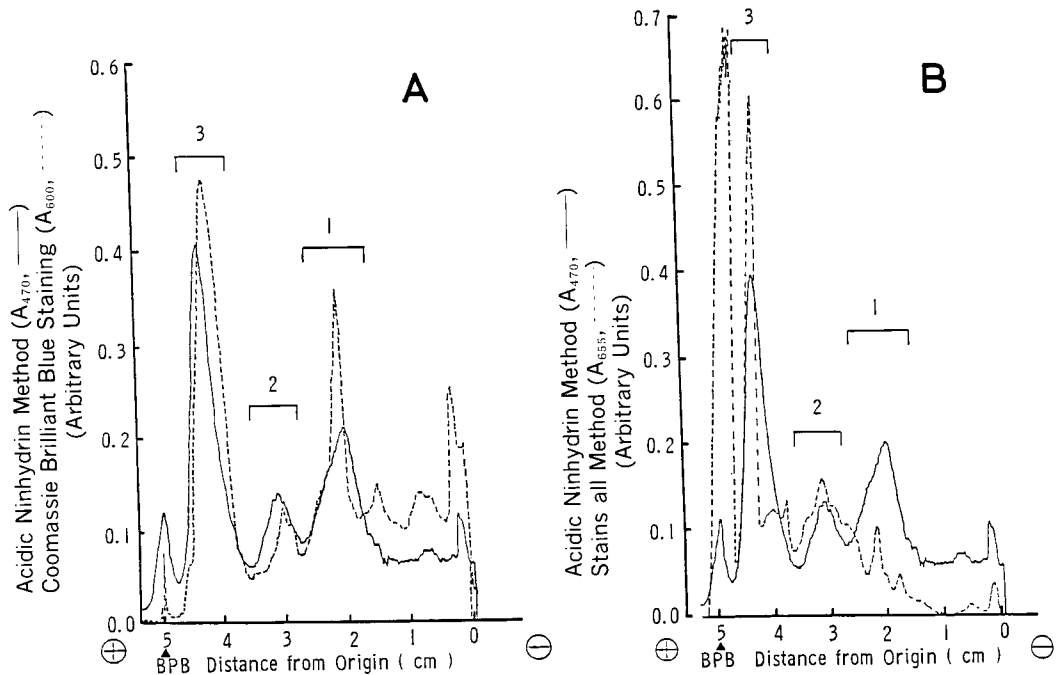


Fig. 2 Detection of sialoglycoproteins in peritoneal fluid of mice bearing Ehrlich ascites tumor cells after disc polyacrylamide gel electrophoresis. Electrophoresis and staining were performed in the same manner as in Fig. 1. Gel staining was performed with the acidic ninhydrin reagent and Coomassie brilliant blue R-250 in A and with the acidic ninhydrin reagent and Stains-all in B.

Fig. 2A. Fig. 2B shows the densitometric profiles of gels stained by reaction with the acidic ninhydrin reagent and Stains-all. By the acidic ninhydrin reaction, peak 1 was defined as a fairly large peak, but the Stains-all reaction defined it as only a tiny peak, and it was difficult to identify peak 1 with the naked eye.

In order to confirm the presence of sialic acids, the gel was sectioned every 3.7 mm after the electrophoresis. Each section was extracted with 1 ml of 5M acetic acid. Sialic acid in the extract was determined by the acidic ninhydrin reaction. As shown in Fig. 3, peaks observed by scanning at 470 nm corresponded to peaks of gel sections which contained sialoglycoproteins determined by the acidic ninhydrin reaction. Section 1 exhibited a high sialic acid content. Thus, it was assumed that section 1 was contaminated with a

part of the stacking gel containing denatured proteins when gel sections were prepared.

A sialic acid fraction was prepared according to a previously reported method (5) from the pooled extracts of gel sections corresponding to peaks 1-3 in Fig. 2, and absorption spectra of these peaks after the acidic ninhydrin reaction were recorded. As shown in Fig. 4, these spectra were similar to that obtained with N-acetylneuraminic acid solution. Stains-all has been used at a concentration of 0.00125% to stain sialoglycoprotein in SDS-polyacrylamide gel (2). In the present study, various concentrations of the reagent were examined. However, results were not very reproducible. Moreover, the color produced by Stains-all faded rapidly, while the color obtained by staining with the acidic ninhydrin reaction was stable (4). As shown in Fig. 5,

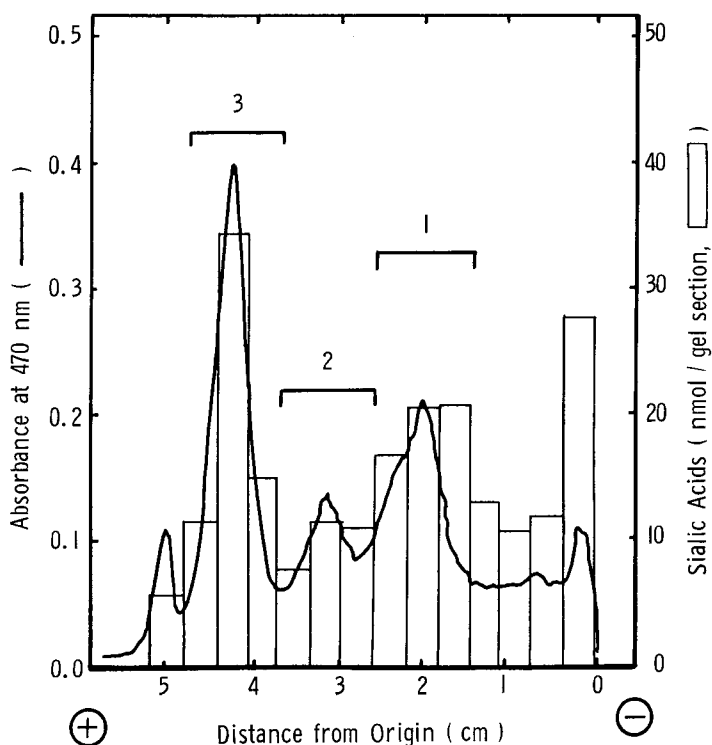


Fig. 3 Determination of sialic acid in gel slices after disc polyacrylamide gel electrophoresis of mouse peritoneal fluid. Fluid containing 1.2 mg of protein per gel was loaded. Two gels were electrophoresed in parallel. After the electrophoresis, one gel was stained with acidic ninhydrin reagent and scanned at 470 nm. The other gel was sectioned into 3.7-mm lengths. The determination of sialic acid in each gel section was performed by the acidic ninhydrin reaction. Details are described under Materials and Methods.

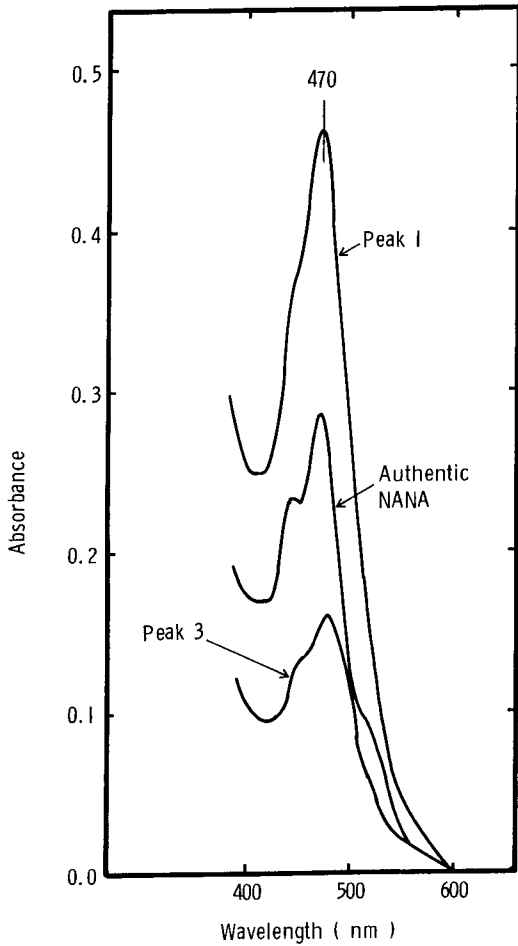


Fig. 4 Absorption profiles of gel extracts reacted with acidic ninhydrin reagent. Sialic acid fractions were prepared from the extracts corresponding to peaks 1 to 3 of Fig. 3 and reacted with acidic ninhydrin reagent. Absorption profiles obtained with the extracts corresponding to peaks 1 and 3 and with authentic N-acetylneuraminic acid (NANA) are shown. For details, see Materials and Methods.

the position of fetuin in the gel was recognizable even after about 4 weeks if the gel was dried on a polypropylene sheet and kept away from ammonia vapor.

The present study shows that sialoglycoproteins in polyacrylamide gel can be detected by a simple and specific reaction with an acidic ninhydrin reagent, and that the acidic ninhydrin method is superior to the Stains-all method in its simplicity, specificity and reproducibility.

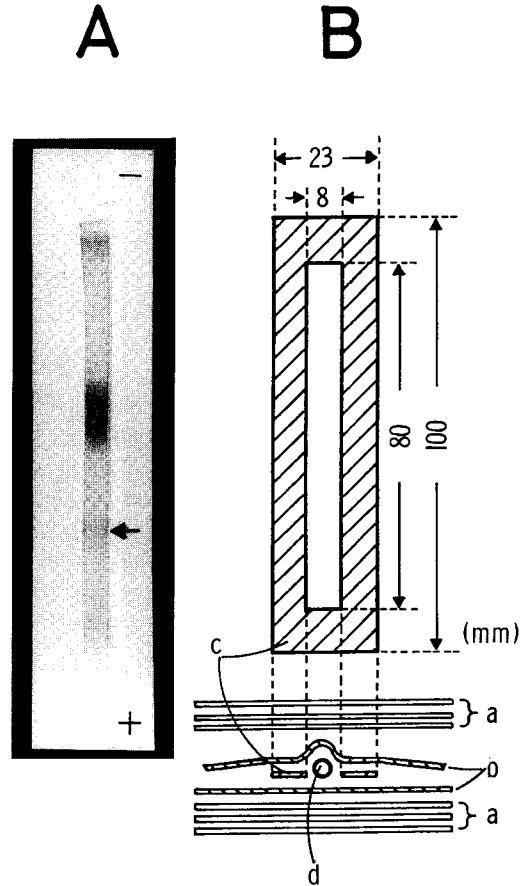


Fig. 5 Dried gel and polyethylene frame used for drying gels. Fetuin was electrophoresed in a disc polyacrylamide gel and stained by acidic ninhydrin reaction. The gel was dried on a polypropylene sheet using a polyethylene frame (Fig. 5B) as described in Materials and Methods. Arrow indicates the position of bromophenol blue. a, Whatman 3MM filter paper; b, polypropylene prefilter; c, polyethylene frame; d, gel.

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