BIOORGANIC CHEMISTRY **25,** 163–168 (1997)
ARTICLE NO. BH971063

Improved Method for Detecting Fluorescent-Labeled Glycoprotein on Sodium Dodecyl Sulfate–Polyacrylamide Gel

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Received January 13, 1997

An improved method for detecting glycoproteins labeled with fluorescent probe on SDS–polyacrylamide gel has been developed based on the previous method we reported previously. In the current method, instead of dansyl glycyl hydrazide (DNS-GLY-$NHNH_2$), fluorescein 5-thiosemicarbazide with a high extinction coefficient and fluorescence quantum yield was employed to label glycoprotein covalently, and the fluorescent staining of glycoproteins on the gel after SDS–PAGE used in the previous method was replaced by the fluorescent labeling of protein samples before electrophoresis to save the fluorescent probe and to shorten the experiment period. Labeled glycoproteins can be detected clearly under ultraviolet illumination after electrophoresis. The examination of several known glycoproteins indicated that the limiting detective sensitivity in the current method was about 1–2 ng of glycoproteins tested, 10–20 times higher than that in the previous method.

Liu and Jiang in 1994 reported a method for detecting glycoprotein on SDS–PAGE by using fluorescent staining with dansyl glycyl hydrazide (DNS-GLY-$NHNH_2$). The fluorescent probe was covalently conjugated to glycoprotein through the reaction between hydrazide and aldehyde generated by the oxidation of carbohydrade groups in glycoprotein. Since all of the procedures, including the staining and removal of the spare fluorescent probe, were carried out on the gel after electrophoresis, the experiment would take a long time and require a large amount of fluorescent probe. In addition, only a lower limiting detective sensitivity could be obtained in the previous method due to the lower extinction coefficient and fluorescence quantum yield of DNS-GLY-$NHNH_2$. All of these shortcomings have restricted the wide utilization of this method in practice. Recently, two procedures in the previous method have been modified. Fluorescein 5-thiosemicarbazide (FTSC), but not DNS-GLY-$NHNH_2$, was used to label glycoprotein. FTSC has a very high extinction coefficient and fluorescence quantum yield in a polar environment (*2*). The second modification was that the glycoproteins were labeled with fluorescent probe in solution before SDS–PAGE. Based on these two modifications, the limiting detective sensitivity was about 10–20 times higher than that in the previous method.

MATERIALS AND METHODS

Materials

FTSC was purchased from Molecular Probe. DNS-GLY-$NHNH_2$ was synthesized in our lab (*3*, *4*). Horseradish peroxidase (HRP) and Con A were products of the

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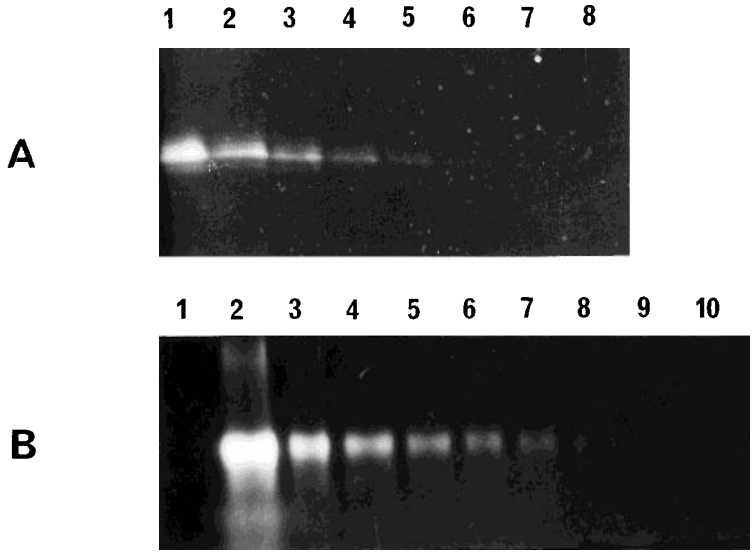

FIG. 1. The detection of HRP in the previous method and the current method. (A) Stained with DNS-GLY-$NHNH_2$. Lanes 1–8 were loaded with HRP: 0.25, 0.1, 0.05, 0.02, 0.01, 0.005, 0.002, and 0.001 μg, respectively. (B) Labeled with FTSC. Lane 1 was loaded with 0.25 μg HRP treated with the FTSC labeling procedure except with no oxidation. Then, 0.25, 0.05, 0.02, 0.01, 0.005, 0.002, 0.001, 0.0005, and 0.0002 μg labeled HRP with FTSC was loaded onto Lanes 2–10, respectively.

Shanghai Dong Feng Biochemical Technology Company. Cinnamomin was isolated from the seeds of the camphor tree according to the method of Jiang and Liu (*5*). Human transferrin (hTf) was kindly given by Professor Y. M. Fong. All other reagents were of A.R. grade.

Methods

Oxidation of the carbohydrate groups in glycoprotein was carried out in a manner similar to the method of Zamecnik *et al.* (*6*). Labeling buffer (0.2 M NaAc, pH 5.6) was added to the sample solution containing 10–20 μg glycoproteins to a final concentration of 0.01 M NaAc (termed the labeling sample). The 500-fold molar excess of $NaIO_4$ over protein was added to the labeling sample and the mixture was incubated at room temperature for 90 min in dark. After removal of the remaining oxidants by adding a twofold molar excess of Na_2SO_3 over $NaIO_4$ under the same conditions, an approximately 10-fold molar excess of FTSC over protein was added to label glycoprotein and the labeling mixture was kept at 37°C in the dark. After 5 h of incubation, the labeling mixture containing labeled-glycoprotein was directly loaded to SDS gel to run electrophoresis. The staining of glycoproteins with DNS-GLY-NH-NH_2 was exactly the same with that in the previous paper (*1*). The fluorescent bands of the labeled glycoproteins were examined and analyzed in wet or dry gels under an ultraviolet lamp.

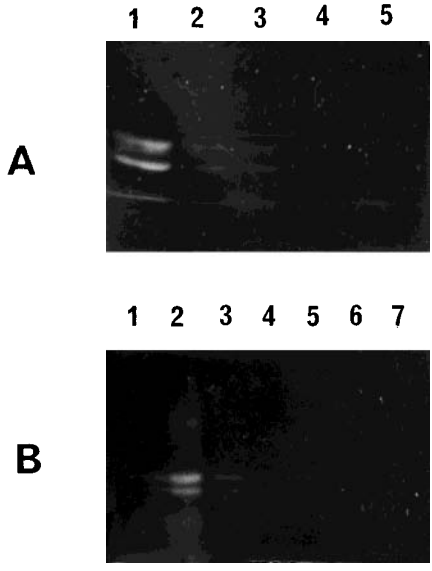

FIG. 2. The detection of cinnamomin with the previous method and the current method. (A) stained with DNS-GLY-$NHNH_2$. Lanes 1–5 were loaded with 0.1, 0.05, 0.02, 0.02, and 0.005 μg cinnamomin, respectively. (B) Labeled with FTSC. Lane 1 was loaded with 0.05 μg cinnamomin treated with the same FTSC labeling procedure except with no oxidation. Then, 0.05, 0.01, 0.005, 0.002, 0.001, and 0.0005 μg labeled cinnamomin with FTSC was loaded onto Lanes 2–7, respectively.

RESULTS

Three glycoproteins were used to compare the sensitivity of the previous and the current methods. In Fig. 1, different amounts of HRP were loaded onto lanes 1–8 of the SDS gel. Figure 1A shows the result obtained in the previous method. After staining with DNS-GLY-$NHNH_2$, the bands containing above 10 ng HRP (Fig. 1A, Lane 5) could be seen on the gel, while in the current method (shown in Fig. 1B), the minimum amount of HRP detected was only 1 ng (Fig. 1B, Lane 8), demonstrating about 10 times more sensitivity than the previous method. Figure 2 shows the results of cinnamomin, a newly isolated ribosome-inactivating protein from the seeds of the camphor tree. Cinnamomin is known to consist of two polypeptide chains with a carbohydrate (*7*). From Fig. 2A, it can be seen that the minimum amount of cinnamomin detected in the staining with DNS-GLY-$NHNH_2$ was 20 ng, while in the labeling with FTSC, the minimum detected amount was 2 ng, demonstrating 10 times more sensitivity than the previous method. In fact, the limiting detection sensitivity for the current method might be higher (see Fig. 3). The minimum hTf amounts to detect in the previous method and the improved method were 20 ng (Fig. 3A, Lane 3) and 1 ng (Fig. 3B, Lane 6), respectively. The same result can be also obtained from Fig. 1. Careful examination of Fig. 1 indicated that 0.5 ng HRP could be also detected (Fig. 3B, Lane 9).