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Glycoprotein Staining following Electrophoresis on Acrylamide Gels

Polyacrylamide gel electrophoresis has proved to be an excellent tool for the separation of proteins and glycoproteins from both plant and animal sources. A number of effective staining procedures are available for detecting protein bands, employing amido black, nigrosine, Coomassie Brilliant Blue and Procion Blue R.S. Generally, some modification of the periodic acid-Schiff (PAS) technique has been applied to the detection of glycoproteins following electrophoresis on paper, cellulose acetate, and acrylamide gels.

The PAS staining procedure of Kōiw and Grönwall (1) that had been applied to Whatman No. 1 filter paper was modified by Keyser (2) and Clarke (3) for acrylamide gel electrophoresis. Berenson *et al.* (4) also described a staining procedure which was attributed to Bodman (5) but is essentially similar to that of Keyser (2). In these laboratories we have had little success with the Keyser staining procedure on our acrylamide gel electrophoresis cylinders containing glycoprotein of both plant and animal sources. The glycoprotein bands either failed to develop or were weakly represented. Earlier, McGuckin and McKenzie (6) had pointed out that, although the procedure of Kōiw and Grönwall produced excellent results on Whatman No. 1 paper, it failed to give satisfactory results on heavier papers and the reproducibility of the staining is dependent on the concentration of HCl in the dye bath and subsequent wash. It therefore seemed obligatory to examine some of the steps in the published PAS procedure to account for the inconstancy of the method

applied to acrylamide gels and to devise an improvement that is essentially based on that of Clarke (3).

At the USDA, aliquots of sample preparation representing 50-98 μg each of κ -casein and β -lactoglobulin, and potato tuber preparation containing 500-900 μg proteins were photopolymerized in 2.5% sample gels. The latter incorporated either 10% sucrose or 2% Ficoll¹ or were made without the inclusion of a carbohydrate. At Kent State University, 2 μl rat serum was photopolymerized in 2.5% sample gel containing 20% sucrose and the separation was in 5% chemically polymerized acrylamide gel. The USDA study employed a similar 7.5% resolving gel. Most were

TABLE 1
Staining of Glycoproteins in Acrylamide Gel

Step	Gel treatment at 25°	Time interval, min
1	Immerse in 12.5% trichloroacetic acid (25-50 ml/gel)	30
2	Rinse lightly with distilled water	0.25
3	Immerse in 1% periodic acid (made in 3% acetic acid)	50
4	Wash 6 \times for 10 min each in 200 ml distilled water/gel with stirring or shaking or wash overnight with a few changes	60 or ON ^b
	If 60 min washing was used, check last wash with 0.1 N AgNO ₃ and when test is negative for IO ₃ ⁻ , continue washing with 2 more changes	20
5	Immerse in fuchsin-sulfite stain in dark ^a	50
6	Wash with freshly prepared 0.5% metabisulfite 3 \times for 10 min each (25-50 ml/gel)	30
7	Wash in distilled water with frequent changes and motion until excess stain is removed	ON
8	Store in 3-7.5% acetic acid	

^a Prepared according to McGuckin and McKenzie (5).

^b Overnight.

cylindrical gels 4 or 5 mm in diameter, although in some cases vertical rectangular gels (2.5 \times 10 mm) were prepared in rectangular glass tubing. The electrophoresis was run at ambient temperatures or at 10° with 3-4 mA per gel depending on the cross-sectional area. At both laboratories the buffer system was that of Davis (7) and the comparative protein stain was 1% amido black in 7.5% acetic acid or Coomassie Blue in trichloroacetic acid (8). β -Lactoglobulin was included as a negative glycoprotein control for the PAS stain.

Consideration was given to the possibility that ethanol fixation of glycoproteins may be necessary for the PAS stain. ¹Mention of company or trade names does not imply endorsement by the Department of Agriculture over others not named.

the protein within the gel may have been incomplete, causing diffusion of the protein on further treatment of the gel. However, gels which failed to produce a positive glycoprotein pattern with the Keyser procedure did provide good protein patterns when amido black or Coomassie Blue stains were substituted for the fuchsin-sulfite reagent in the Keyser staining procedure. Nevertheless, the gels suffer from a not fully reversible dehydration when employing ethanol as the protein fixative and gel dehydration may also act to retard penetration of subsequent reagents. Trichloroacetic acid fixation permits all further steps in the staining procedure to be accomplished in aqueous media. Concentrations of 5, 10, and 12.5% produced bands of equal sharpness.

Table 1 describes our expansion of Clarke's periodic acid-Schiff procedure applied to acrylamide gels as derived from this study.

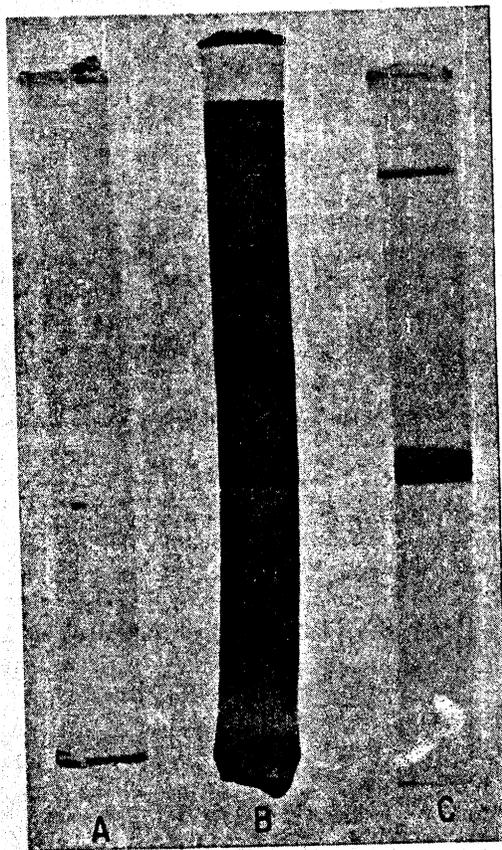


FIG. 1. Cylindrical acrylamide gels of rat serum proteins: (A) stained by Keyser's PAS method (weak bands did not register on photograph); (B) stained with amido black; (C) PAS stained.

Complete removal of the periodic acid is imperative in order to obtain a stained gel with a clean background. This is usually assured by overnight washing, provided the gel is not overexposed to periodic acid; in the latter case, removal by washing is exceedingly difficult. A laboratory-constructed continuous washer employing a centrifugal recirculating pump with a mixed bed resin of Amberlite MB-1 has been successfully used to reduce the periodic acid removal time from overnight to 2-3 hr.

The results of this study suggest that the Keyser procedure may not provide sufficient oxidation time, under the conditions of the scheme,

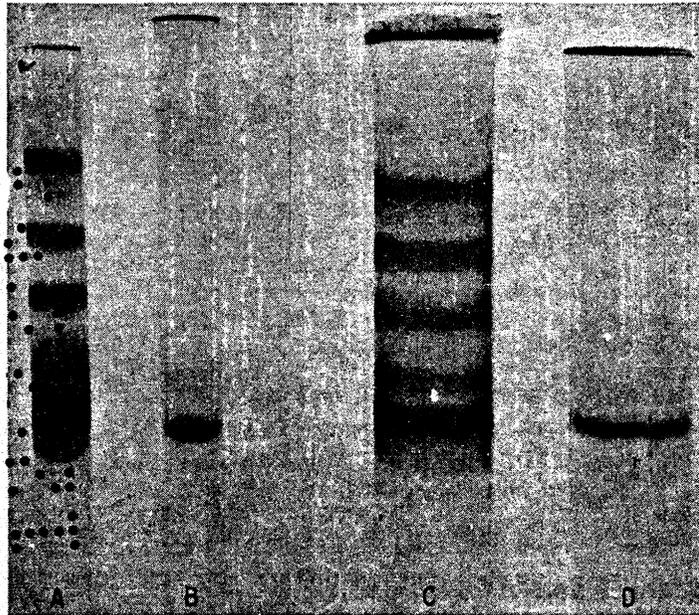


FIG. 2. Acrylamide gels of potato tuber protein: (A) cylindrical gel stained with Coomassie Blue; (B) cylindrical gel—PAS stained; (C) rectangular gel stained with Coomassie Blue; (D) rectangular gel—PAS stained.

and thus is much less sensitive. Rat serum known to contain glycoproteins high in carbohydrate content produced weak bands with the Keyser procedure (Fig. 1) while those of κ -casein (5.3% carbohydrate) and potato tuber glycoproteins generally were negative. However, with the PAS procedure described here, strong, sharp bands were observed with the same quantities of these materials.

The Keyser method was applied by its author on acrylamide gel slabs of 3 mm thickness (2). Our unsuccessful efforts with it were largely on cylindrical gels (5 mm diameter) but were also negative when applied to the rectangular gels of 2.5×10 mm. Both spherical and rectangular

gels, however, gave positive glycoprotein staining when treated by the procedure described in Table 1. These are shown in Figures 1 and 2 paired with a gel containing the same sample material stained with Coomassie Blue or amido black. The β -lactoglobulin control, as expected, did not stain for glycoprotein.

It was found preferable to prepare the sample gel without the usual incorporation of sucrose (7). In this case, the washing time was simply independent on removal of periodic acid. Following electrophoresis and staining, those which contained sucrose developed a deep colored background extending 20-25% of their length if the gels were washed for only 80 min after periodic acid oxidation (Table 1). Clear backgrounds, however, were obtained if overnight washing was used, leaching out the unbound oxidation products. Substituting Ficoll (a high polymer of sucrose) for sucrose in the gel presented no improvement.

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