# Alcian Blue Staining of Polysaccharide

A. H. Wardi and G. A. Michos. 1972. Alcian Blue Staining of Glycoproteins in Acrylamide Disc Electrophoresis. *Anal. Biochem.* 40: 607-609.

1. Grow cells under capsule-producing conditions. Prepare SDS-PAGE samples for electrophoresis.
2. Pour a 5% acrylamide gel (no resolving gel) according to Maniatis. Load samples and run at 120V.
3. Remove gel (carefully) and fix in 12.5% TCA for 30 minutes.
4. Rinse with distilled water, then incubate in 1% periodic acid (in 3% acetic acid) for 50 minutes.
5. Rinse 3 times with distilled water for 10 minutes each.
6. Reduce excess periodate and iodate by incubating gel in 0.5% potassium metabisulfite (aka potassium disulfite, $K_2S_2O_5$) for 30 minutes.
7. Wash 3 times with distilled water for 10 minutes each.
8. Stain with 0.5% Alcian blue (in 3% acetic acid) for 4 hours to overnight.
9. Destain with 7% acetic acid. Be careful, as gel may destain rapidly.

ANALYTICAL BIOCHEMISTRY 48, 621-623 (1972)

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The principal methods used for the staining of glycoproteins in paper electrophoresis include the Schiff-periodic acid (1,2), the diphenylamine (3), and the ninhydrin reaction. Among these, only the Schiff-periodic acid method has been adapted successfully for the staining of glycoprotein in cellulose acetate electrophoresis. The Schiff-periodic method, however, presents certain difficulties which limit its usefulness as a staining technique in the routine investigation of glycoproteins. These difficulties include:

(*a*) The preparation procedure of the fuchsin-$SO_2$ dye is tedious and the reagent requires continuous refrigeration.

(*b*) The glycoprotein fractions acquire only weak coloration, which fades with time.

(*c*) Glycoproteins with high carbohydrate content, especially those isolated from protease-digested tissue, fail to stain with this reagent (4).

In this report an alternative method for the staining of glycoprotein is presented. It is based on the oxidation of glycoprotein with periodate, reducing excess periodate and iodate with potassium metabisulfite, and staining of the oxidized glycoprotein with the Alcian blue reagent. The oxidation of glycoprotein with periodate results in the formation of aldehyde groups, which are transformed to bisulfite addition products upon reaction with potassium metabisulfite. The formation of these bisulfite addition groups increases the acidity of glycoprotein and enhances its reaction with the Alcian blue stain.

*Procedure.* Samples (10 $\mu$l) of serum or 3% glycoprotein solution was applied at the middle of 5 × 14 cm cellulose acetate strips previously impregnated with buffer. The electrophoresis was carried out in a barbiturate buffer of pH 8.6 (0.05 ionic strength) at 130 V for 90 min (5). The wet strips were removed from the instrument, placed between blotters, and heated at 100° for 10 min. The strips were soaked in 95% ethanol for 10 min, placed in 1% periodic acid solution (in 40% ethanol) for another 10 min, and rinsed with 40% ethanol. They were then placed in 1% potassium metabisulfite (in 40% ethanol) for 5 min, rinsed with 40% ethanol, and stained in 1% Alcian blue in 2.5% acetic acid for 15 min (6).

The strips were destained in 3 changes of 2.5% acetic acid, placed in

95% ethanol for 1 min, and cleared with a cyclohexanone-ethanol mixture (30:70, v/v). The cleared strips were heated at 100°, placed in plastic envelops, and scanned in the Beckman microzone densitometer using interference filter No. 600.

Figure 1 shows the electrophoretic pattern of the glycoprotein fractions of serum, bovine submaxillary mucin (Sigma, type 1), and bovine glycoprotein fraction VI (Mann Research Laboratories) stained by the Alcian blue method.

Figure 2 shows the densitometric curve of serum glycoprotein fractions, which gave the following values for normal serum in per cent of total: $\alpha_1$, 18.40; $\alpha_2$, 35.43; $\beta$, 18.68; and $\gamma$ 27.47.

Acid mucopolysaccharide, which may be present in glycoprotein preparations, could be visualized with Alcian blue without pretreatment with periodate and potassium metabisulfite. Under these conditions glycoprotein fractions are not stained by this reagent. It is, therefore, possible to differentiate and determine both glycoprotein and acid mucopolysaccharide when present in the same preparation.

*Summary.* The Alcian blue method is shown to be an effective technique for the staining of glycoproteins isolated from protease-digested vitreous humor and defatted brain tissue. Application of this method to acrylamide gel electrophoresis of glycoproteins will be reported separately.

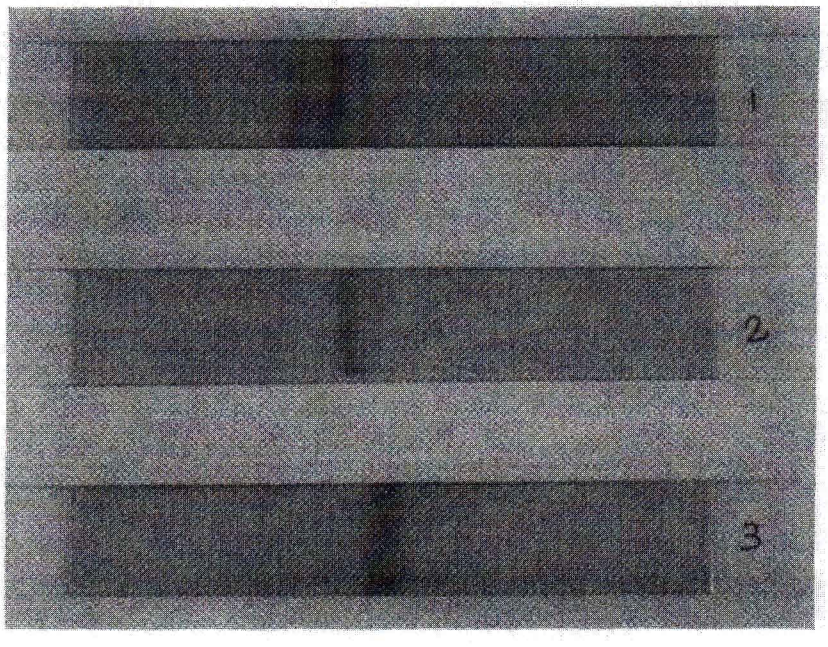


FIG. 1. Electrophoretic pattern of glycoproteins of serum (1) submaxillary mucins (2) (Electrophoresis was run for 1 hr), and bovine glycoprotein fraction VI (3) stained with Alcian blue (see text).

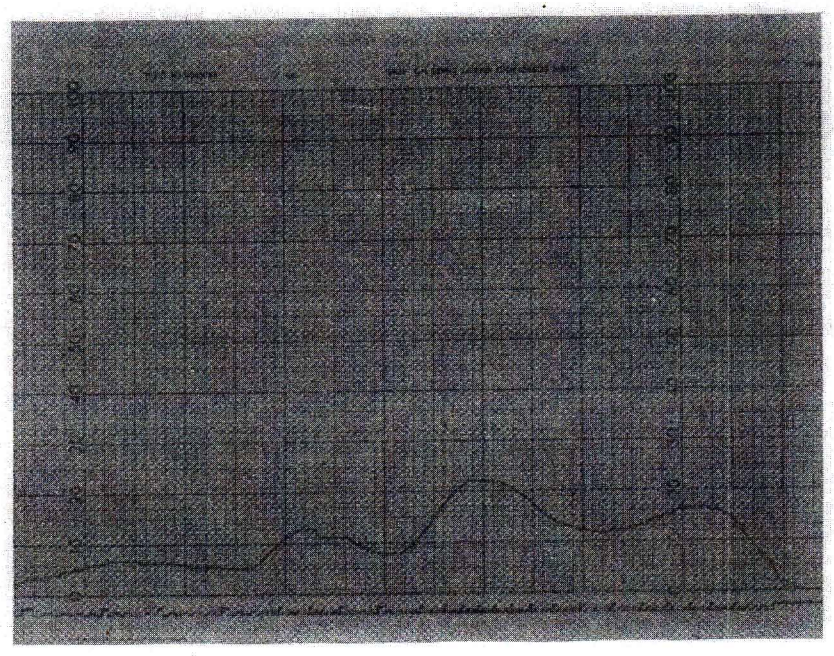

FIG. 2. Densitometic curve of serum glycoprotein fractions.

## ACKNOWLEDGMENTS

This work was supported in part by a research grant (5 RO1 NS05932-06), National Institute of Neurological Diseases and Stroke.

The authors also wish to thank Sheila Bickling, George Michos, and Ranbir Varma for their excellent technical assistance.

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*Received December 1, 1971*