Improved sensitivity for detection and quantitation of glycoproteins on polyacrylamide gels

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Summary. A simple and sensitive technique for staining glycoproteins on polyacrylamide gels utilizing periodic acid-Schiff (PAS) reagent is described. The novelty of this technique is an additional incubation of the PAS stained gels in an ethanolic solution of metabisulfite. The treatment results in an about 20-fold increase in the intensitiy of bands and the color is stable for at least 1 week at room temperature. Furthermore, the integrated optical density of the bands is proportional to the amount of glycoproteins. Amounts as small as 0.2 µg of fetuin can be conveniently determined. As compared to other PAS staining methods, this procedure offers a remarkably improved sensitivity for detection and quantitation of glycoproteins following their electrophoretic separation on polyacrylamide gels.

Most of the procedures for detection of glycoproteins on polyacrylamide gels are based on the periodic acid-Schiff reagent (PAS) reaction. The intensity of glycoprotein bands stained with PAS method depends on the number and nature of sugar moieties of individual proteins. However, for a particular glycoprotein species the stoichiometry of the chromophore binding permits quantitative determination of the protein[2]. The present report describes a modification of this commonly employed technique which brings about a many-fold increase in the sensitivity of glycoprotein detection.

Methods. Disc electrophoresis was carried out as previously described[3] in glass tubes with internal diameter 5 mm and 100 mm long containing 10% polyacrylamide gel and 0.1% sodium dodecyl sulfate (SDS). 2 standard glycoproteins, namely fetuin and ovalbumin (Sigma, Chemical Company, St. Louis, Missouri) were solubilized in a sample buffer consisting of 0.78% (w/v) SDS, 0.75% (w/v) dithiothretiol and 7% (w/v) sucrose in 12.5 mM sodium phospate buffer, pH 7.0, and electrophoresed. Thereafter the gels were removed from the tubes and processed according to Matthieu and Quarles[2]. The last step of the procedure was, however, modified. Thus, following the 18-h incubation in Schiff reagent (Merck, Darmstadt, West Germany) the gels were transferred to glass tubes containing 35 ml of 0.2% potassium metabisulfite in 40% ethanol-5% acetic acid and incubated for 90 min at 55 °C with occasional shaking. The treatment resulted in an appreciable increase in the band intensity, although the red color also developed in both the void parts of the gels and in the bath fluid. Incubation exceeding 90 min did not influence the intensity of the color. The gels were subsequently destained with 40% ethanol-5% acetic acid. Usually a 24-h wash with occasional agitation and several changes of the fluid removed all the stain from the background. Gels were scanned at 520 nm in a Vitratron densitometer. Glycoproteins were quantitated by determination of areas under the recorded peaks.

Results and discussion. As shown in the table the incubation of the Schiff reagent-treated gels in the ethanol-containing solution of metabisulphite increases the intensity of fetuin and ovalbumin bands by about 18 and 22 times respectively as compared to the intensity developed after the wash with an aqueous solution of metabisulphite[2]. Virtually identical results were obtained with a methanol-containing solution, although the development of the color was slightly delayed. The concentration of ethanol in the incubation medium is far above the critical one, although the ratio of the medium to the Schiff reagent-containing gel should be preserved. A deviation from this ratio by more than 20% may decrease the intensity of staining. The color of bands is stable for at least 1 week at room temperature in a dark. No staining of glycoproteins was detectable in gels when the treatment with periodic acid was omitted.

Figure 1 depicts the correlation between the amount of standard proteins and the peak areas of the bands. At the same protein concentration the intensity of fetuin staining was greater by about 3-fold than the intensity of ovalbumin bands, reflecting the quantity and type of carbohydrate content of these proteins[4,5]. The linearity was preserved up to a concentration of approximately 50 and 120 µg of

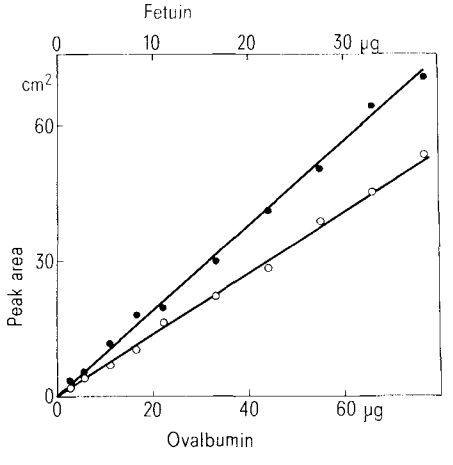

Figure 1. Correlation between the amounts of fetuin (closed circles) and ovalbumin (open circles) and the peak areas of the bands. Points represent average values from 2–4 independent determinations.

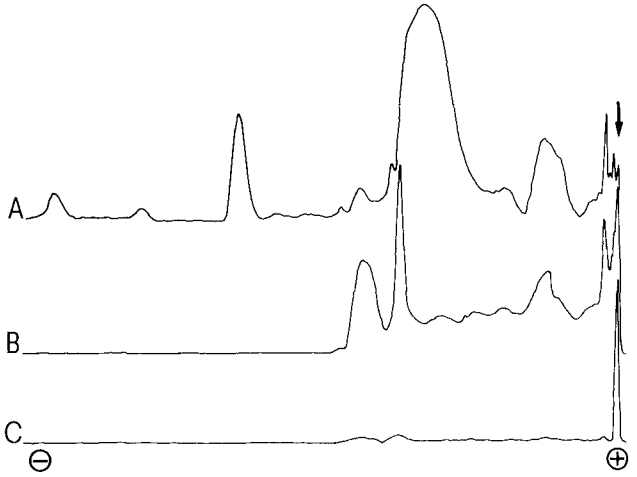

Figure 2. Densitometric profiles of human serum proteins separated by disc gel electrophoresis and stained with Coomassie Brilliant Blue[3] (A), the modified PAS technique (B) and the original PAS technique of Matthieu and Quarles[2] (C). Gels were loaded with 52 µg of serum protein. The arrow indicates the top of the gels.

protein per gel for fetuin and ovalbumin, respectively. Amounts as low as 0.2 µg of fetuin and 0.7 µg ovalbumin can be conveniently quantitated using the high-sensitivity setting of the densitometer Thus, within these limits of linear relationship, standard curves covering various ranges of protein concentration can be plotted.

When whole human serum was electrophoresed and stained, a linear relationship between the amount of protein applied on the gel and the peak area was observed for all the glycoprotein bands (within certain limits for individual bands). On the other hand, a number of serum proteins (stained by Coomassie blue) did not stain with this technique (fig. 2). Also in a sample of CNS myelin proteins only the bands representing glycoproteins were stained. Other proteins, without glycosyl residues, were not stained although they were present in much higher concentrations in the sample. Thus, although this study did not determine the chemistry of the alcohol-dependent reaction, it may be tentatively concluded that the chromophore binds specifically and stoichiometrically to glycoproteins.

The simple and rapid procedure described here offers remarkably improved sensitivity for detection and quantitation of glycoproteins following their electrophoretic separation on polyacrylamide gels.

The comparison of 2 methods for glycoprotein staining in polyacrylamide gels. The gels were loaded with either 32.8 µg of fetuin or 65.5 µg of ovalbumin. The figures represent average values and SD from 4 experiments

Method	Peak area (cm^2)	
	Fetuin	Ovalbumin
Original technique of Matthieu and Quarles	3.6 ± 0.3	2.1 ± 0.2
Modified technique	64.3 ± 3.1	45.3 ± 4.5

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0014-4754/84/030303-02$1.50 + 0.20/0

Harvesting large amounts of Drosophila embryos at precisely defined stages

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Summary. A simple method is described which makes it possible to eliminate the retained eggs of *Drosophila melanogaster* and to harvest a large amount of embryos at a defined stage of development. It is based upon carbon dioxide anesthesia, which brings about the expulsion of eggs remaining in the uterus.

One of the problems when working with *Drosophila* embryos is the difficulty of harvesting large amounts of embryos at precisely defined early stages of development. This is due to the fact that some females do not lay recently fertilized eggs, but retain them for a variable time[1,2], so that the age-span of collected embryos does not correspond exactly to the laying period because there are 10–15% of older embryos. Since mitotic cycles at early stages are very rapid (10–60 min^{1-3}), even a modest contamination by older embryos creates a significant proportion of cells or nuclei from stages more advanced than that of the desired sample[4].

Several methods have been proposed to avoid the effects of egg retention, such as collecting the eggs at a desired stage one at a time[3,5] or obtaining enough virgin females and allowing them to be fertilized just after the start of laying[4,6]. These arduous methods do not permit the collection of the large amounts of embryos needed for certain purposes, especially molecular studies. A light-dark cycle causes a burst of egg laying during the light to dark transition[2], producing a certain degree of homogeneity, but only once a day, so that this method necessitates the rearing of very large populations. In this paper we describe a method of harvesting large amounts of *D. melanogaster* embryos with

Table 1. Percentage of embryos at each early stage when a defined stage reaches the maximum

Stages	Time at which the stages reach their maximum (min)															
	A				B				C				D			
	90	145	195	255	90	145	195	255	90	145	195	255	80	135	180	245
Syncytium	**84.3**	9.9	3.1	3.1	**91.1**	6.7	4.9	4.9	**99.6**	11.8	8.4	8.4	**100**	9.0	9.0	9.0
Syncytial blastoderm	4.2	**75.5**	5.2	2.1	6.2	**83.5**	0.9	0.5	0.4	**87.7**	4.8	1.1	0.0	**91.0**	3.4	2.1
Cellular blastoderm	4.2	6.8	**71.0**	1.0	0.9	7.1	**80.4**	2.2	0.0	0.4	**83.9**	1.0	0.0	0.0	**87.6**	0.0
Gastrula	7.3	7.8	17.7	**93.9**	1.8	2.7	13.8	**92.4**	0.0	0.0	2.9	**89.4**	0.0	0.0	0.0	**88.9**

Embryos harvested after a laying period reach successive stages, each at a reproducible time after the start of laying. This time is expressed in minutes. A, B, C and D are the conditions shown in the figure, and the homogeneity of the sample in a defined time is expressed by the percentage of embryos at each stage. CO_2 treatment before laying (C and D) clearly shows the elimination of the retention effect especially at a short laying interval (30 min) since contaminating embryos are unfertilized eggs or dead embryos from earlier stages.