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Note

Silver stain devised to indicate glycoproteins in polyacrylamide gels revealing hemoproteins and hemin

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The identification of glycoproteins, electrophoretically separated in polyacrylamide gels, has been a focus of increasing attention, with various staining procedures employed[1–3]. However, the relatively low sensitivities of those methods have limited their usefulness.

The periodic acid–silver (PA–Ag) staining method of Dubray and Bezard[4] exhibited superior sensitivity for the staining of human serum glycoproteins, as observed in this laboratory. Glycoproteins such as haptoglobin[5], α_2 macroglobulin[6] and transferrin[7] stained while serum albumin did not. However, when serum was hemolyzed (containing hemoglobin), the hemoglobin (Hb) subunit bands separated in sodium dodecylsulfate (SDS) polyacrylamide gels also stained intensely for glycosylation. Since in some cases of diabetes a portion of human Hb has been seen to be glycosylated[8,9], this result was thought to indicate a diabetic condition in the human donor. However, when commercially available human met-Hb and other nonglycosylated hemoproteins were resolved in SDS gels, all stained intensely. The staining reaction with hemoproteins and hemin was observed to be independent of periodic acid oxidation.

EXPERIMENTAL

Materials

Human serum was supplied by the Red Cross of Portland, OR, U.S.A. The low-molecular-weight marker kit was purchased from Pharmacia, while human met-hemoglobin, sperm whale myoglobin and cytochrome *c* were obtained from Sigma. Electrophoresis was performed on a mini-slab (10 × 8 cm) apparatus made by Idea Scientific of Corvallis, OR, U.S.A.

Polyacrylamide gel electrophoresis

The electrophoresis involved the use of SDS according to Laemmli[10] with 15% acrylamide slab gels 0.8 mm thick. Samples were dissolved in 0.0625 *M* Tris–HCl, pH 6.8, 2% SDS, 5% β-mercaptoethanol, 10% glycerol and 0.001% bromophenol blue and were heated at 100°C for 5 min.

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Staining of gels

The gels were stained according to the PA–Ag procedure of Dubray and Bezard[4] and for peroxidase activity according to Francis and Becker[11].

RESULTS AND DISCUSSION

When human serum was electrophoresed in SDS gels and stained using the PA–Ag method, proteins such as α_2 macroglobulin, haptoglobin and transferrin were indicated while serum albumin was not. Since the first three proteins are glycosylated[1–3] while albumin is not[12], the staining results supported the contention that this silver-stain method was specific for glycoproteins. However, when human hemolyzed plasma samples were electrophoresed and stained, the Hb subunit bands also gave a positive reaction. When commercially supplied human Hb was resolved in SDS gels and PA–Ag treated, the subunit bands again reacted positively. Since normal human Hb is not glycosylated[8,9], it was apparent that another component of the Hb was reacting with the ammoniacal silver under the conditions. In order to demon-

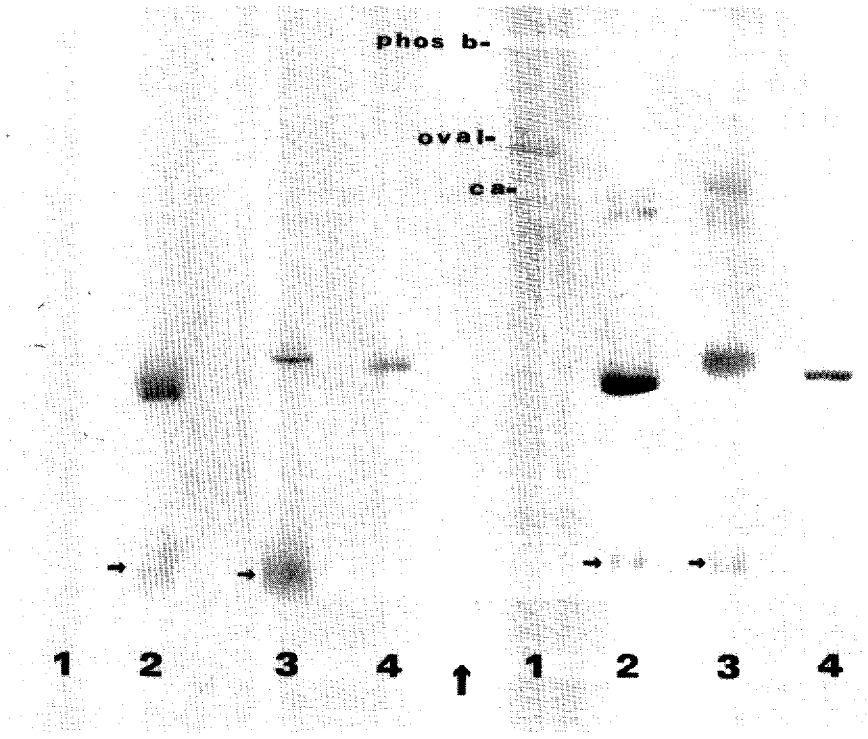

Fig. 1. A 15% polyacrylamide SDS slab gel containing: (1) Pharmacia low-molecular-weight markers; (2) human Hb; (3) sperm whale Mb; (4) cytochrome *c*. The gel half left of the arrow was stained for peroxidase activity (note that none of the molecular weight marker proteins were indicated). The gel half on the right reveals the silver staining of duplicate samples. The small arrows indicate hemin. Of the molecular weight marker proteins, ovalbumin (oval) with a molecular weight of 43,000 dalton stained intensely while phosphorylase *b* (phos *b*) at 94,000 dalton and carbonic anhydrase (ca) at 30,000 dalton stained faintly.

strate the possible role of the heme moiety in the reaction, other hemoproteins such as sperm whale myoglobin (Mb) and cytochrome *c* (neither one of which is glycosylated) were also run in SDS gels. The PA–Ag staining of these samples resulted in a positive reaction of each (Fig. 1). The evidence strongly suggested that the heme component was reacting with the silver. Myoglobin had been reported to react with the PA–Ag stain when used as a molecular weight marker in another study[13].

In order to support the contention of a heme–silver interaction, two experiments were conducted. The first involved the resolution of the hemoproteins along with the molecular weight standard in SDS gels. The samples were applied so that the gel could be bisected (after electrophoresis) resulting in duplicate samples in each half. One-half was stained for peroxidase activity[11] and the other using the PA–Ag procedure[4]. The result is shown in Fig. 1. The hemoprotein bands along with the diffuse band previously identified as hemin[11] stained for peroxidase activity. The silver-stained gel also revealed the hemoprotein and hemin bands. Of the proteins in the molecular weight standard sample, ovalbumin stained intensely with silver while phosphorylase *b* and carbonic anhydrase faintly stained. This is the same result as observed in the original PA–Ag paper[4].

The second experiment involved removing the heme group from Hb and Mb using the method of Teale[14]. The dehemed and hemed globins were electrophoresed in SDS gels and compared regarding peroxidase activity and silver staining. The hemed proteins reacted with both stains while their dehemed counterparts failed to (data not shown). The staining of hemoproteins and hemin using the PA–Ag method was observed to be independent of the periodic acid oxidative step.

The purpose of this report is to present evidence that a sensitive silver stain devised to indicate glycoproteins specifically also reveals hemoproteins and hemin in SDS gels. Since hemoproteins are present in both plant and animal tissues, the results of this study should be taken into account when assaying a sample for glycoproteins using the periodic acid–silver staining procedure of Dubray and Bezard.

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