GOLD-LABELED ENZYME-HISTOCHEMICAL DETECTION OF GLUCOSE-6-PHOSPHATE AND PHOSPHOENOLPYRUVATE

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It is well known that carbohydrate components present in the structure of glycoproteins play an essential role in the functioning of these proteins. They determine their conformational structure, responsible for intercellular and intermolecular interaction and protection against proteolytic enzymes, they participate in immunological reactions, and so on [3, 8]. The possibility of detecting these compounds in histological tissue sections under normal and pathological conditions is very interesting. Histochemical techniques for the detection of carbohydrate residues of glycoproteins that are available are not, however, distinguished by their simplicity or reliability [6]. Accordingly, in recent years the histochemical labeling approach has been actively developed, in research involving the use of labeled components of a biochemical reaction in tissue sections. At the ultrastructural level, lectins labeled with colloidal gold [11] and also enzymes utilizing the corresponding carbohydrates as substrate [7, 8] have been used. Parallel studies using different methods have demonstrated the high specificity of the use of enzymes labeled with colloidal gold [7]. The aim of this investigation was to develop reagents for detecting carbohydrate components of glycoproteins, represented by glucose-6-phosphate (G6P) and phosphoenolpyruvate, on the basis of labeling specific enzymes with colloidal gold, and also to develop a modified technique for obtaining reagents which will allow dyes to be obtained in sufficient amounts for investigations at the light optical level on sections prepared in the usual way.

EXPERIMENTAL METHOD

Human placental tissue was obtained from three women with a normal course of pregnancy immediately after full-term delivery, and transferred into 4% paraformaldehyde or into 1% glutaraldehyde ("Serva," West Germany) in phosphate buffer. Liver tissue was taken form three adult male Wistar rats after decapitation of the animals, and kept in the same fixing solutions. The tissues were fixed for 5 h at 5°C. After washing, the tissues were taken through increasing concentrations of alcohols to xylol and embedded in paraffin wax. Sections 4 μm thick were mounted on albuminized slides, and before investigation they were dewaxed and rehydrated in the usual way.

Colloidal gold was obtained as in [4]. After carrying out Zsigmondy's reaction [11] the optimal amount of protein required to stabilize the solution of the colloid was selected. Stabilization was done in two ways. In the first, only glucose-6-phosphate dehydrogenase (G6PD), from "Serva," in a concentration of about 0.5 mg/ml was added to the solution of colloidal gold (calculated for the final solution containing gold); in the second way, a mixture of 0.1 mg/ml of G6PD and about 1 mg/ml of human serum albumin ("Reanal," Hungary, calculated for final solution containing gold) was added. The samples were than centrifuged at 10,000*g* for 1 h, the residue was resuspended in the smallest possible volume of supernatant, and the product was stored at −4°C.

Pyruvate kinase ("Reanal") in a concentration of 0.1 mg/ml and human serum albumin ("Reanal") in a concentration of 0.3 mg/ml (calculated for final concentration with gold) were added to a solution of colloidal gold, after which the mixture was centrifuged, resuspended, and the products restored as described above.

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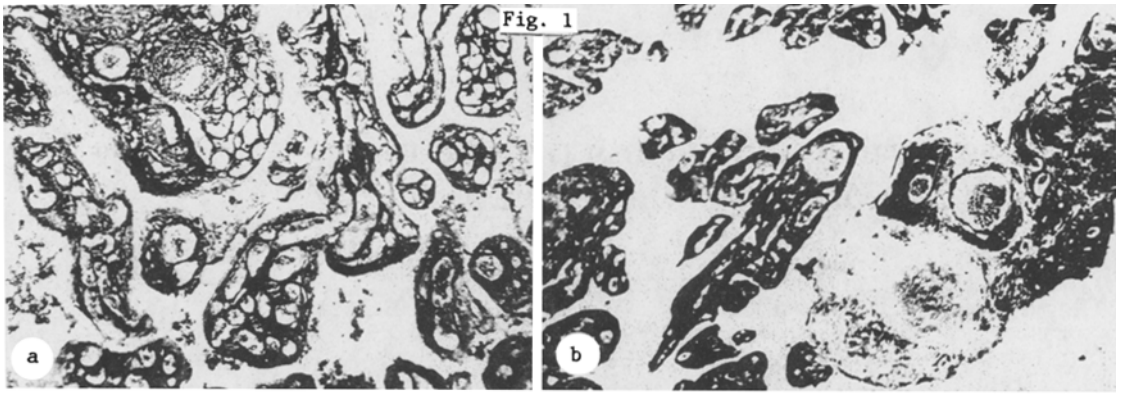

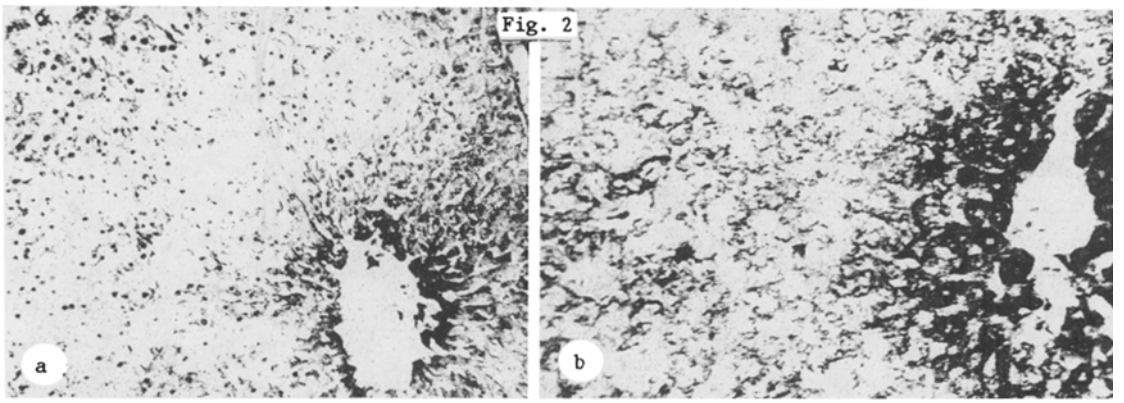

Fig. 1. Human placenta at full term. a) Sections treated with G6PD, labeled with colloidal gold. G6P residues distributed uniformly in tissue of a villus. 150×. Without counterstaining or intensification. Original colors red with white; b) sections treated with pyruvate kinase, labeled with colloidal gold. Phosphoenolpyruvate residues uniformly distributed in tissue of a villus. A villus with a low content of carbohydrate components, represented by phosphoenolpyruvate, is present. 150×. Without counterstaining or intensification.

Fig. 2. Rat liver. a) Sections treated with G6PD, labeled with colloidal gold. Intensely stained cytoplasm of centrilobular hepatocytes and cell nuclei. 120×. Without counterstaining or intensification; b) sections treated with pyruvate kinase, labeled with colloidal gold. Cytoplasm of centrilobular hepatocytes intensely stained. Cell nuclei unstained. 150×. Without counterstaining or intensification.

Sections prepared as described above were diluted with distilled water (to 1:4) superposed on the sections, and allowed to stand overnight in a humid chamber at room temperature. The sections were then taken through alcohols to xylol and mounted in Canada balsam.

The following controls were set up for labeled G6PD: 1) G6PD (unlabeled) was first applied to the section, followed by labeled G6PD; 2) labeled G6PD was mixed with G6P (about 10 mg/ml, final concentration), and after 1 h the whole of this mixture was superposed on the tissue section and allowed to stand overnight; 3) human serum albumin, labeled with colloidal gold, was superposed on the tissue section. No reaction whatever took place in any of the controls.

The following controls for labeled pyruvate kinase were set up: 1) unlabeled pyruvate kinase was applied to the section first, followed by the labeled form; 2) labeled pyruvate kinase was mixed with phosphoenolpyruvate (about 10 mg/ml, final concentration), and 1 h later the whole of this combination was applied to the tissue section as described above; 3) human serum albumin, labeled with colloidal gold, was superposed on the section. No staining was observed in any of the control sections.

EXPERIMENTAL RESULTS

The absence of staining in the control tests indicates high specificity of staining. The enzymes used also were distinguished by high substrate specificity. For instance, we know that phosphoenolpyruvate is the only substrate to be catalyzed by pyruvate kinase [10].

Carbohydrate components represented by G6P were present in the human placenta in all villi relatively uniformly in the form of homogeneous structures (Fig. 1a). Staining with pyruvate kinase, labeled with colloidal gold, revealed villi with a low content of phosphoenolpyruvate residues, probably reflecting their altered metabolic activity (Fig. 1b). Carbohydrate components of glycoproteins, represented by G6P and phosphoenolpyruvate, located in the cytoplasm of the hepatocytes, had intralobular differentiation (distribution of the intensely stained cells was centrilobular). G6P residues were detected in large quantities in the nuclei of the hepatocytes, but their distribution throughout the lobule was not observed. Carbohydrate components, represented by phosphoenolpyruvate, were not found in the cell nuclei (Fig. 2a, b). Recently research workers have paid great attention to the role of glycoproteins in the functioning of the cell nucleus [5, 8, 9]. They are considered to play an important role in translation processes [5]. A high concentration of glycoproteins in the nucleus has been demonstrated biochemically [9], histochemically [6], and histochemically with labeled enzymes [8, 11]. The results indicate an important role of G6P residues and the relatively less importance of components represented by phosphoenolpyruvate. It is interesting to note that nuclei of the syncytium of human placenta have no specific staining (Fig. 1a, b). This may perhaps reflect some embryonic features of functioning of the cell nucleus.

By the methods presented above it is possible to obtain dyes in the amounts necessary for light-microscopic investigation. On stabilization of the solutions of colloidal gold with pure enzyme (G6PD) the final yield of the reagent was very low — about 0.25 ml from 10 ml of gold. On the addition of albumin to the stabilizing solution, the final yield of reagent was greatly increased — to 2 ml. The staining properties of the reagents under these circumstances, in the presence of the same concentration of colloidal gold (estimated by the color of the reagent applied to the tissue) were virtually indistinguishable. From our point of view this can be explained as follows; to stabilize colloidal gold particles about 60 molecules of protein are necessary, but it is not at all essential that all these molecules should be valuable enzyme molecules which, additionally, stabilize colloidal gold solutions badly. The addition of albumin sharply increased the quantity of stabilizing protein, although albumin itself with colloidal gold does not possess staining properties. Only one of the stabilizing molecules reacts with a histochemical determinant present in tissue [1]. Thus if part of the stabilizing protein consists of albumin, this ought not to affect the total reacting capacity, but on the other hand, it causes a sharp increase in the yield of reagent — to amounts sufficient for light-microscopic investigations and for clinical use. In appropriate modifications the method can be used at the ultrastructural level also. The results may find an application in scientific research and in the diagnosis of tumors and inflammatory conditions.

LITERATURE CITED

1. M. Bendayan, Techniques in Immunocytochemistry, Vol. 3, New York (1985), pp. 179-201.
2. C. De. Roe, P. J. Courtoy, and P. Baudhuin, J. Histochem. Cytochem., **35**, 1191 (1987).
3. G. Frens, Nature Phys. Sci., **241**, 20 (1973).
4. R. V. Izzo, Lab. Invest., **53**, 373 (1985).
5. F. W. K. Kan and P. Pinto da Saliva, J. Cell. Biol., **102**, 575 (1986).
6. A. L. Lathem and S. Brooks, J. Histochem., **19**, 405 (1987).
7. L. Londono and M. Bendayan, Eur. J. Cell Biol., **45**, 88 (1987).
8. L. Londono and M. Bendayan, J. Histochem. Cytochem., **36**, 1005 (1988).
9. R. K. Margolis, C. P. Crockett, W. L. King, and R. V. Margolis, Biochim. Biophys. Acta, **451**, 465 (1976).
10. A. E. F. H. Meijer et al., Histochemistry, **88**, 501 (1988).
11. J. Roth, J. Histochem. Cytochem., **31**, 987 (1983).