Protein A Gold-Silver Staining Method for Light Microscopic Immunohistochemistry

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Summary. A new sensitive method has been established for light microscopic immunohistochemistry. It involves a protein A gold technique instead of immunoglobulin-gold method, followed by an improved procedure of physical development. In this procedure, two selected reagents were employed: bromohydroquinone, a more potent developing agent than hydroquinone, and specifically purified gum arabic solution. The results obtained by this method in the pancreatic islet tissues of the rat under a variety of histochemical control conditions have substantiated both the high specificity and fidelity of this method.

The immunohistochemical methods employing colloidal gold-labeled immunoglobulins are subject to the serious limitation that immunoglobulins of all animal species will not necessarily bind firmly to colloidal gold. To circumvent such a limitation, an indirect immunohistochemical method named the protein A gold technique has been developed, in which a protein A-colloidal gold complex was used as the second stepreagent (ROMANO and ROMANO, 1977; ROTH and Binder, 1978; BENDAYAN et al., 1980; ROTH et al., 1981a, b; ROTH, 1982). The protein A gold technique for immunohistology was originally developed for electron microscopy, and ROTH (1982) was the first to successfully employ this technique for light microscopy, demonstrating reddish reaction products in the rat endocrine pancreas.

In the present study, attempts have been made to establish an improved method for the protein A gold technique for light microscopy, in which the contrast of the immunoreactive sites obtained by the protein A gold technique are intensified by means of our improved procedure of physical development. The method presented here has been tentatively named the protein A gold-silver staining.

MATERIALS AND METHODS

Pieces of tissues from the splenic portion of the adult rat pancreas were fixed in Bouin's fluid for 18 hr, dehydrated in a graded ethanol series, cleared in xylene and embedded in paraffin. Sections were cut at a thickness of 4 μm, deparaffinized, hydrated and subjected to the following staining procedures.

The sections were: 1) immersed for 15 min in three changes of 0.01 M phosphate buffered saline (PBS) (pH 7.4); 2) treated for 60 min with 1% ovalbumin in PBS;
3) incubated for 60 min with guinea pig anti-porcine insulin serum (DAKO corporation, USA) diluted 1:400 with 1% bovine serum albumin in 0.01 M phosphate buffered saline (BSA-PBS) at room temperature in a moist chamber; 4) rinsed in four changes of PBS for 30 min; 5) treated for 15 min with 1% ovalbumin in PBS; 6) incubated for 30 min with protein A gold complex preparation (E-Y Lab. Inc., USA, particle size, approximately 10 nm) diluted 40 times with BSA-PBS; 7) rinsed in four changes of 0.01 M phosphate buffer (pH 7.4) for 30 min; 8) physically developed with a solution of the formula noted below for 45-50 min at 20°C in a dark room; 9) washed in running tap water for 5 min and then immersed for 1 min in a photographic fixer diluted 5 times with water; 10) rinsed in running tap water for 10 min; 11) briefly counterstained with 0.1% nuclear fast red in 5% alumimum sulfate aqueous solution; 12) dehydrated, cleared and mounted in a Bioleit.

The formula of the developing solution used at step (8) is as follows: Solution A: 20% gum arabic aqueous solution, 45 ml; 10% silver nitrate aqueous solution, 1 ml. The gum arabic solution was prepared by centrifugation at 18,000 rpm for 30 min at 0°C and separation of the supernatant for use. Solution B comprised: distilled water, 15 ml; bromohydroquinone, 200 mg; citric acid, 300 mg. The working developing solution was prepared by mixing solutions A and B in a dark room under a photographic safe lamp illumination.

To test the specificity of the above reaction, five types of control staining procedures were performed: 1) incubation with the antiserum previously adsorbed with porcine insulin, followed by treatment with the protein A gold solution and the physical development: for 100 μl of undiluted antiserum, 1 mg insulin (Sigma Chem. Co., USA); 2) incubation with the specific antiserum, followed by 60 min incubation with unlabeled protein A (0.2 mg/ml, Sigma Chem. Co., USA), then with the protein A gold solution and finally with the developing solution; 3) incubation with normal guinea pig serum diluted with BSA-PBS (1:400) followed by treatment with the protein A gold solution and the physical development; 4) omission of the antiserum and protein A gold steps followed by the physical development; 5) omission of the physical development only.

RESULTS AND DISCUSSION

In sections of the pancreas immunostained for insulin by means of the present protein A gold-silver staining procedure, reaction products were visualized as particles of black color in the cytoplasm of centrally located islet cells, presumably B cells (Fig. 1). In these sections, non-specific stainings of other endocrine and exocrine elements were not recognizable. At higher magnifications, the reaction products were found to be localized in secretory granules (Fig. 2). None of the black-colored particles was seen in any portions of the pancreas, whenever sections were subjected to the control staining procedures including steps (1), (2), (3), (4) and (5) described above in Materials and Methods.

The present results obtained by the experimental and control procedures have shown that the precipitation of the black-colored particles by this protein A gold-silver method is specific for the immunoreaction and that the reaction products of the protein A gold are greatly intensified by physical development. In the physical developers employed in the present study, improvements have been made so that hydroquinone used in previous reports (HOLGATE et al., 1983; SPRINGALL et al., 1984) was replaced by a more potent derivative, bromohydroquinone, and the gum arabic solution was purified by high speed-centrifugation. On the basis of the data of photographic chemistry (Lee
and Brown, 1977), it is evident that bromohydroquinone exhibits a significantly higher developing capacity than hydroquinone. Such improvements are thought to result in

Fig. 1. Rat pancreatic islet stained for insulin with protein A gold-silver technique and briefly counterstained with nuclear fast red. Reaction products are visualized as black particles in the cytoplasm of B cells. Non-specific stainings of other endocrine and exocrine cells are not recognized. ×260

Fig. 2. At a higher magnification, the reaction products are found to be localized in the secretory granules of B cells. ×1,300
a well-contrasted image which is free from any notable non-specific stainings. Since protein A shows an affinity to the Fc fragment of immunoglobulin among different animal species (Forsgren and Sjöquist, 1966; Goding, 1978), the use of the protein A gold as the second step-immunoreagent instead of the immunoglobulin-gold is believed to make the present method more widely available than the immunogold silver techniques previously reported (Holgate et al., 1983; Springall et al., 1984).

This paper has reported on a new immunostaining method by the use of protein A gold instead of immunoglobulin-gold combined with silver staining agents, offering sensitivity higher than in previous methods. It can be expected that this method will be widely applicable to immunohistochemistry at the light microscopic level.

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