

## Efficacy of Colloidal Gold-Labeled Antibody as Measured in a Barley Stripe Mosaic Virus-Lectin-Antilectin System

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### ABSTRACT

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By using colloidal gold-labeled rabbit antibody to a barley stripe mosaic virus-precipitating barley lectin isolated from barley seed, the lectin was shown to be distributed along the entire surface of the virus particle and not at the ends only. Upon standing, the specific activity of rabbit antilectin

antibody labeled with colloidal gold was gradually lost by flocculation; it should be used within 1 wk after being labeled. The function of the carbohydrate portion of barley stripe mosaic virus protein remains to be elucidated.

*Additional key words:* electron-dense marker.

Lectins are widely recognized in cell biology; however, little is known about their function, distribution in plants, or reaction with plant viruses. Several possibilities for an *in vivo* function for lectins have been suggested, including: a defense mechanism in inhibiting hyphal growth or spore germination; involvement in recognition processes; enzymatic function; and protection against seed predators (6).

Barley stripe mosaic virus (BSMV) has been shown to contain as much as 3.7% carbohydrate (11). A BSMV-precipitating lectin has subsequently been isolated from barley seed (10).

Although BSMV protein subunits are identical, they are capable of two aggregation states, one helical and the other disklike (9). We, and others (eg, M. K. Brakke, *personal communication*), have frequently noticed both aggregation forms in routine electron microscopy of leaf-dip preparations in dilute specific antiserum. Even though the protein subunits of BSMV are identical, it does not necessarily follow that the lectin in a virus-lectin complex must be evenly distributed over the entire surface of the virus particle. The carbohydrate portion may be folded in and thus inaccessible to the lectin along the length of the virus particle, but not at the ends. This could cause the ends of virus particles to bind on membranes. A special relationship between BSMV and plastid membranes has already been shown (4).

A wide variety of proteins and polysaccharides can be labeled with colloidal gold (1,2,5,7,8,12,13) and the use of this technique in research is increasing. The method of preparation determines the size of the colloidal gold particles, which can be varied from 5 to 64 nm (7).

We have investigated the distribution of lectin binding sites on BSMV rods with gold-labeled rabbit antilectin IgG. Our experience with gold-labeled antibody may be applicable to research requiring an electron-dense marker.

### MATERIALS AND METHODS

**Lectin and BSMV purification.** Barley lectin was extracted from 100-g barley seed lots with 0.1 M sodium acetate, pH 5.5, as

described by Partridge et al (10). BSMV (North Dakota 18 strain) was purified from infected cultivar Larker barley. Tissue was ground in 0.1 M  $K_2HPO_4$  (1 g/3 ml). Expressed sap was clarified by a low-speed centrifugation (10,000 rpm, 10 min in a Sorvall SS34 rotor) and by absorption of the supernatant with  $K_2HPO_4$ -CaCl<sub>2</sub> (3) followed by low-speed centrifugation. The supernatant of the second low-speed centrifugation was made to 1% Triton X-100 and layered over a 3-ml cushion of 20% sucrose in 0.05 M potassium phosphate buffer, pH 7.3, or 0.05 M tris-HCl, pH 7.0, in Spinco #30 rotor tubes. A 1 hr, 28,000 rpm, high-speed centrifugation was sufficient to pellet the virus (M. K. Brakke, *unpublished*). Virus pellets were dissolved in a small amount of either buffer and subjected to a second and third cycle of low- and high-speed centrifugation.

**Preparation of gold-labeled globulin.** Rabbit antibarley lectin IgG was purified from heat-treated (0.5 hr at 56 C) whole serum (obtained from J. E. Partridge) by precipitation with ammonium sulphate (33.3% w/v). The precipitate was pelleted by low-speed centrifugation and dissolved in physiological saline. The precipitation was done a second and third time. After the third precipitation, the IgG was dissolved in distilled water and dialyzed for a total of 2 days, first against 4 L of distilled water and then three times against 1 L of double glass-distilled water. Microaggregates were removed by low-speed centrifugation and then a high-speed centrifugation (1 hr, 38,000 rpm, in a Spinco #40 rotor) immediately before labeling with gold colloid. Only the top half of the supernatant fluid was used for labeling as done by Geoghegan and Ackerman (5). Antibarley lectin IgG was labeled at pH 7.6 (pH adjusted with 0.2 M  $K_2CO_3$ ) with gold colloid exactly as described by Geoghegan and Ackerman (5). Gold-labeled antibody was used either immediately or within 3-4 days.

**Immunoelectron microscopy.** For immunoelectron microscopy carbon-backed collodion-covered grids were used. Grids were washed between each reagent application with 0.05 M potassium phosphate or tris buffer containing 0.1% bovine serum albumin as an aid in spreading the negative stain. A drop of rabbit BSMV antiserum diluted 1/200 in 0.01 M ammonium acetate, pH 6.0, was placed on a grid for 2 min and excess fluid was removed by touching with a filter paper strip. Barley stripe mosaic virus (0.08 mg/ml) in potassium phosphate or tris buffer was placed on the grid for 15-30 sec, drained by touching the grid to a filter paper strip, and followed with barley lectin (1 mg/ml) in 0.1 M ammonium acetate for 2-3

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min. After three 10-min washes, gold-labeled anti-barley IgG was applied and left for 1 hr at room temperature. Grids were washed three times for 10 min and negatively stained with 1% PTA, pH 7.0. In control tests, antiserum-coated grids with BSMV were incubated as above without barley lectin, or gold-labeled horseradish peroxidase was used instead of anti-barley lectin globulin.

**Colloidal gold protein A labeling.** Protein A from *Staphylococcus aureus*, Cowan strain, was isolated by the method of Sjöquist et al (14) and also purchased from Sigma and Pharmacia. The gold-labeling procedure for protein A was as described for rabbit anti-barley lectin IgG (5). The procedure for the gold-labeled protein A was the same as rabbit IgG except after the lectin treatment, unlabeled rabbit anti-lectin IgG was used and then gold-labeled protein A. Grids were examined in a Zeiss 10A electron microscope.

## RESULTS AND DISCUSSION

**Distribution of lectin on virus particles.** Colloidal gold can be seen distributed along the length of the virus particles and also at both ends (Fig. 1A). Control tests showed only scattered gold particles or clusters not associated with virus (Fig. 1B). The attachment of BSMV to the virus antibodies on the collodion membrane is visible in the control (Fig. 1B). The large clusters of gold-labeled anti-barley lectin IgG (Fig. 1A) is partially caused by the tendency of gold-labeled antibody to form clusters. Colloidal gold-labeled antibody was not mainly present as monodispersed

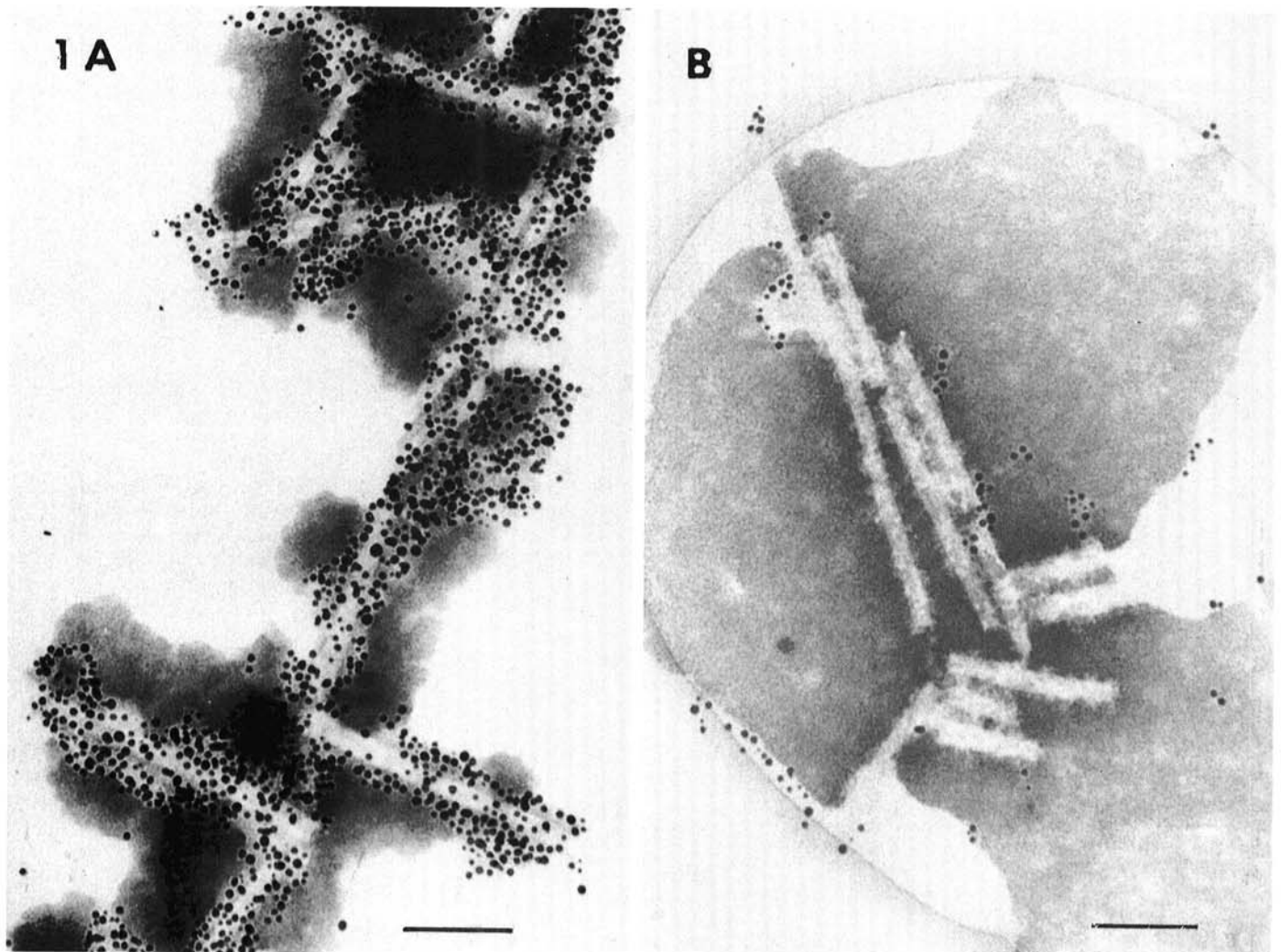
particles. Clustering of several or more particles was common and could not be prevented. Tris buffer could not be used in lectin-antilectin experiments because it dissociated BSMV-lectin precipitates or prevented BSMV-lectin binding.

The gold-labeled anti-lectin antibody clustered along the entire length and both ends of the BSMV particles and this indicates that the lectin is distributed along the entire particle and is available for antibody reaction. The end-on binding of BSMV to membranes (4) is therefore probably not mediated by lectin.

Most of the labeled antibody formed large aggregates upon storage in 0.01% sodium azide at 4 C and gradually flocculated out of solution during a 1-wk period. We attempted to overcome the aggregation problem and achieve higher resolution by using gold-labeled protein A and unlabeled rabbit anti-barley lectin IgG. Although the colloidal gold was stabilized by protein A and did not precipitate after the addition of NaCl, it did not bind specifically to rabbit antibody. Commercially obtained protein A was used in subsequent experiments and it also failed to react with rabbit antibody after gold labeling.

Other workers have used gold-labeled protein A as electron-dense markers (2,12,13). However, to our knowledge there have been no reports of its successful use in the USA.

Colloidal gold-labeled specific antibody can be prepared relatively easily and rapidly, is visible at lower magnifications than the considerably less electron-dense ferritin, and exhibits little nonspecific attachment to plastid membranes as does ferritin. These properties may make it more useful than ferritin in immunological electron microscopy.



**Fig. 1.** Electron micrographs of barley stripe mosaic virus (BSMV). **A**, Negatively stained purified BSMV reacted with barley lectin and then colloidal gold-labeled rabbit anti-barley lectin antibody. **B**, Control test without barley lectin. Bars represent 100 nm.

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