NOTE

Immunocytochemistry of Wheat Storage Proteins: Effect of Particle Size of Protein A-Gold on Labeling Intensity

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ABSTRACT

Prolamins in wheat endosperm were localized by immunocytochemistry with colloidal gold particles of different sizes as markers. These proteins were localized in the protein bodies and in vesicles associated with the Golgi apparatus. The intensity of labeling was significantly different, depending on the size of the gold particles. Intense labeling of protein bodies and Golgi-complex vesicles was obtained with 5- and 10-nm gold markers. Labeling was much less efficient with 20-nm particles. Our observations suggest that the size of protein A-gold markers determines, in large part, their binding efficiency. Consequently, careful attention should be given to size when choosing gold markers for immunocytochemical studies, especially when quantification is a consideration.

Localization of macromolecules by immunocytochemistry has progressed rapidly since Nakane and Pierce (1966) first successfully employed enzyme-labeled antibodies to visualize tissue antigens. Immunolocalization techniques have provided valuable information on the spatial distribution of antigens within cells. The precise localization of antigens provides clues to their biosynthetic pathways and sheds light on their functional role (Horisberger 1984).

In recent years, a great variety of immunocytochemical methods have been used to localize macromolecules in plants (Herman 1988, Mishkind et al 1987). In most cases, the antigens are tagged by the antibodies, which then can be detected by markers that are directly or indirectly attached to them (Romano and Romano 1984). Various markers are currently in use for light and transmission electron microscopy. During the last 10 years, however, colloidal gold particles have been most extensively used as markers, both for transmission and scanning electron microscopy.

We are interested in understanding the cellular routes of protein body formation in cereals. By using the immunogold localization technique, we have provided evidence for the condensing role of the Golgi apparatus in prolamin trafficking in cereal caryopses (Krishnan et al 1988, 1990). Initially we used 10-nm gold particles as markers. Recently we began using 20-nm gold particles and have noticed that the number of gold particles specifically bound to the protein bodies and Golgi apparatus-derived vesicles decreased significantly. Since no studies have been done on the influence of the size of the gold particles on localization of cereal proteins, we decided to systematically examine this phenomenon. Here we report that the size of the gold particle greatly influences its efficiency as a marker.

MATERIALS AND METHODS

Wheat (*Triticum aestivum* L. cv. Chinese Spring) grains were obtained from E. R. Sears, University of Missouri, Columbia. Grains were germinated on moist filter papers in the dark for three days and were transplanted to small clay pots containing garden soil. The plants were grown in a glasshouse as described previously (Krishnan et al 1986). Caryopses at the soft-dough stage (about 15 days after anthesis) were removed from the center of the panicle and processed for histological studies. Small pieces of endosperm tissue were dissected with a razor blade and fixed in 2.5% glutaraldehyde in 50m*M* sodium phosphate buffer, pH 7.2 (Krishnan et al 1990). The tissue was postfixed with 2% aqueous osmium tetroxide, dehydrated in a graded acetone series, infiltrated, and embedded in Spurr's resin (Spurr 1969), essentially as described previously (Krishnan et al 1986).

Prolamin Localization

Immunogold localization of prolamins used thin sections mounted on uncoated nickel grids. These were floated on drops of 50m*M* Tris-HCl, pH 7.5, containing 0.15*M* NaCl, 0.1% Tween-20, and 1% (w/v) bovine serum albumin (TTBS) for 30 min. The sections were then incubated for 60 min with wheat prolamin antibodies (Krishnan et al 1988) that had been diluted 1:100 with TTBS, and were then incubated with protein A-gold particles (Sigma Chemical Co., St. Louis, MO) for 45 min. Three different sizes of protein A-gold particles were used: 5 nm (2×10^{14} particles per milliliter), 10 nm (2.7×10^{13} particles per milliliter) and 20 nm (2.8×10^{12} particles per milliliter). In one set of experiments, the gold-particle concentrations were adjusted to similar absorbance levels, while in the second set, the gold particles were adjusted to an equal protein-A concentration (1 mg/ml). After several rinses with TTBS and distilled water, the sections were stained sequentially with 0.5% aqueous uranyl acetate for 30 min and with 0.4% aqueous lead citrate for 30 min. Sections were examined with a JEOL JEM 100B electron microscope (JEOL Ltd., Tokyo) at 100 kV.

RESULTS

Wheat caryopses at the soft-dough stage contain numerous starch grains and protein bodies of various shapes. At this developmental stage, an extensive network of rough endoplasmic reticulum is evident. The protein bodies often are surrounded by a loose network of membranes (Figs. 1–3). Numerous Golgi bodies with several small vesicles also are common (Figs. 4–6).

As expected, prolamins were primarily localized within the protein bodies (Figs. 1–3). Protein bodies were intensely labeled in thin sections of wheat endosperm that had been incubated with prolamin-specific antisera and 5-nm protein A-gold particles (Fig. 1). When 10-nm protein A-gold particles were used (Fig. 2), the binding of the markers, although satisfactory, was less intense than with the 5-nm particles. However, when 20-nm markers were used (Fig. 3), the binding of protein A-gold particles was drastically reduced. The number of 5-, 10-, and 20-nm protein A-gold particles were calculated to be $1,370 \pm 98$, 120 ± 7, and $31 \pm 2/\mu m^2$, respectively. Since the particle preparations were of different concentrations (i.e., the numbers of gold particles per unit of volume varied), we incubated sections with protein

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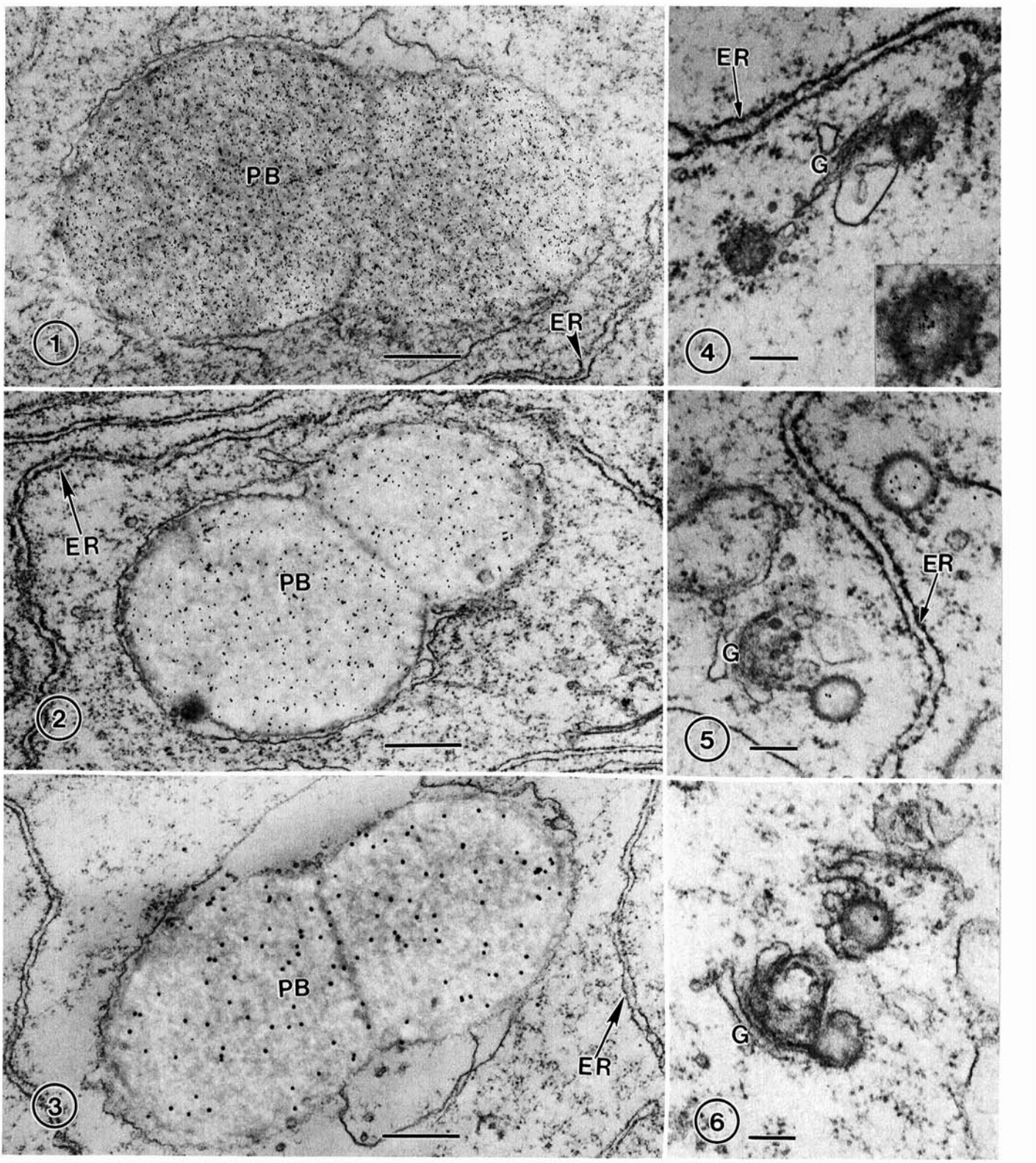
A-gold particles that were adjusted to a constant $A_{520} = 0.50$. This treatment, however, did not alter the labeling efficiency of the different-sized gold particles (data not shown).

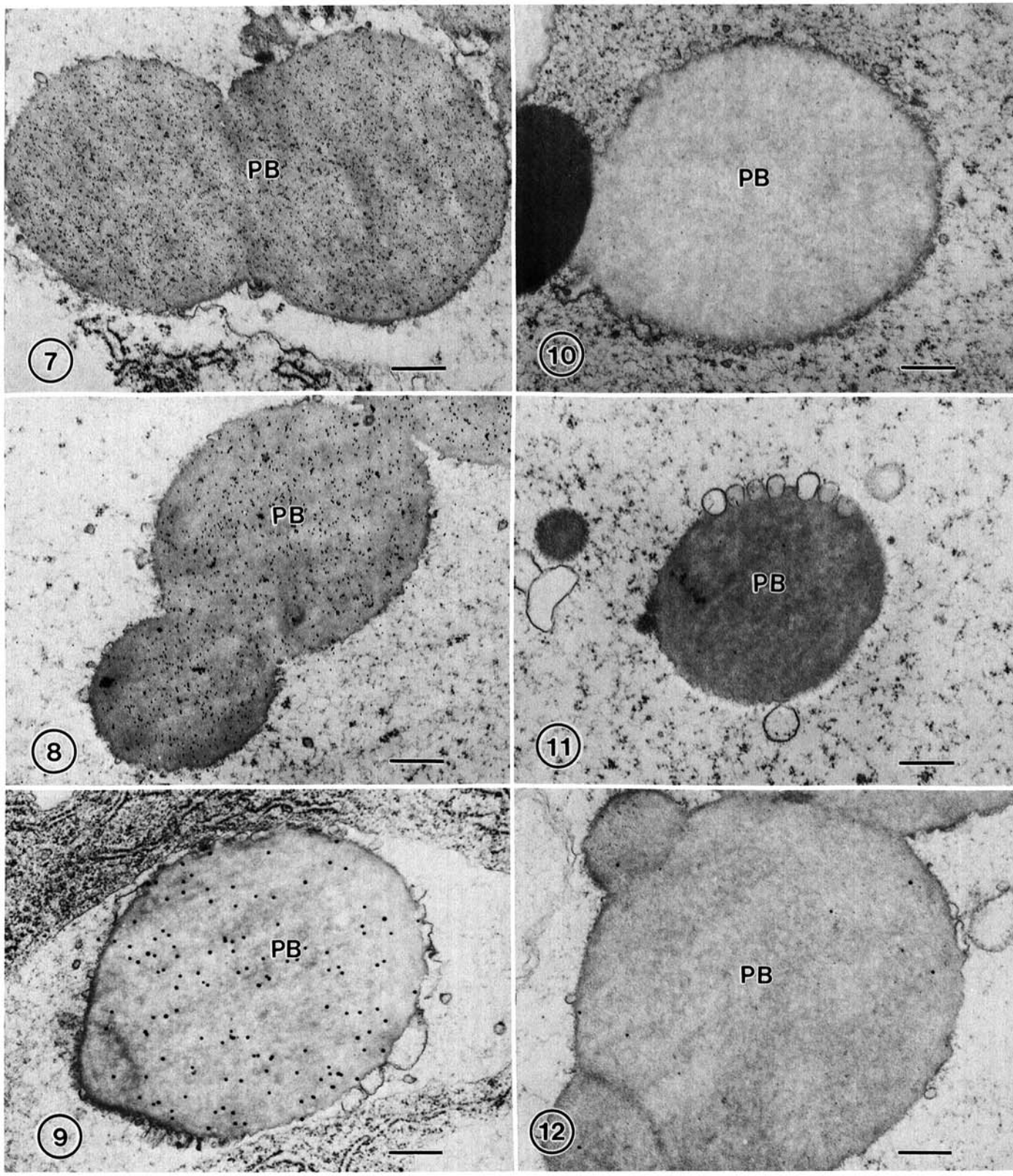
Since the specificity of the gold labeling is dependent on the amount of protein A (the concentration of which is different in the different-sized gold particles), we also examined the effect of using a similar concentration of protein A on labeling efficiency (Figs. 7–9). Interestingly, the labeling efficiency obtained was very similar to that obtained when protein A-gold particles were adjusted to a constant absorbance level. Quantification of 5-,



Figs. 1–6. Immunocytochemical localization of prolamins in wheat caryopses. Thin sections of wheat endosperm were incubated with prolamin antisera and protein A-gold markers of different sizes. **1–3.** Thin sections labeled with 5-, 10-, and 20-nm gold particles, respectively. Note the drastic reduction in binding efficiency with increasing size of the markers. Bars = 0.5 μm. **4–6.** Wheat Golgi complexes that were labeled with prolamin antiserum and 5-, 10-, and 20-nm protein A-gold markers, respectively. Bars = 0.2 μm. **4.** Insert (lower right corner) is an enlarged view of one of the Golgi complex vesicles, showing the labeling with 5-nm markers. **ER** = endoplasmic reticulum, **G** = Golgi apparatus, **PB** = protein body.

10-, and 20-nm bound particles over protein bodies revealed that there were 1,210 ± 91, 198 ± 13, and 28 ± 1 gold particles per micrometer squared, respectively. These results suggest that the size of protein A-gold markers determines, in large part, their binding efficiency. Control experiments in which preimmune serum was substituted for prolamin-specific antiserum or buffer resulted in negligible background labeling over protein bodies (Figs. 10–12).

Consistent with earlier reports (Buttrose 1963, Bechtel et al 1982, Parker and Hawes 1982, Kim et al 1988), we also were able to demonstrate that the prolamins are localized in Golgi apparatus-derived vesicles (Figs. 4–6). These vesicles are intensely



Figs. 7–12. Immunocytochemical localization of prolamins in wheat caryopses. **7–9.** Thin sections labeled with 5-, 10-, and 20-nm gold particles, respectively, showing the effect of using similar protein-A concentration on the labeling efficiency of different-sized gold particles. Bars = 0.4 μm. **10–12.** Control sections labeled with preimmune serum and then with 5-, 10-, and 20-nm gold particles, respectively. Bars = 0.4 μm. **PB** = protein body.

labeled when 5-nm gold particles are used as markers (Fig. 4). On occasion, even the cisterna stacks of the Golgi apparatus are labeled. However, because of their small size, the 5-nm particles are not visible at lower magnifications. The 10-nm gold probes also gave satisfactory results (Fig. 5), but the 20-nm gold particles were inefficient as markers in detecting the prolamins within the Golgi apparatus-derived vesicles (Fig. 6). Also, in several instances, proteins present in the Golgi vesicles were unreactive with these large particles (Fig. 6).

DISCUSSION

Our study establishes that the size of the gold particle can influence its marking efficiency in immunocytochemical studies of cereal grains. Generally, the smaller particles (5 and 10 nm) are better markers than the larger ones. However, the use of 5-nm particles is restricted to studies done at high magnification, because they are otherwise not easily visible. The 10-nm gold markers, which are relatively large and exhibit good marking efficiency, may be best for general immunolocalization studies.

In immunogold studies, the marker is complexed with a carrier, such as protein A, that exhibits a high affinity for the Fc portion of the immunoglobulin G molecule (Forsgren and Sjoquist 1966). It has previously been shown that gold markers of different sizes exhibit differences in their ability to complex with immunoglobulins (De May 1986). This observation, in conjunction with the fact that the concentration of gold particles is lower in preparations of larger protein A-gold markers, could explain the lower marking efficiency of larger particles.

Colloidal gold markers of different sizes have been successfully used in double or multiple immunocytochemical labeling studies (Bendayan 1982, Tapia et al 1983). Such studies have provided valuable information on the spatial distribution of these antigens and for quantification (Horisberger 1984). However, one shortcoming is that the larger gold particles are inferior with respect to sensitivity (Geuze et al 1981) and can result in imprecise quantification of antigens. Several factors, including steric hindrance, repulsion between gold particles, and penetration characteristics of gold markers (Slot and Geuze 1981) need to be addressed in cases where quantification is a consideration.

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