Note

Ultrastructure of Protein Bodies in Embryonic Axes of Soybean Seeds (Glycine max cv. Enrei)

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Received January 11, 1990

In mature seeds, storage proteins are kept mainly in specialized organelles called protein bodies, which are classified into several types according to their origin and structural complexity.1,2) Cotyledonary protein bodies in some legumes including soybean have a homogeneous matrix and rare globoids, while in others large and frequent globoids were observed in the protein bodies.3) Embryonic axis and cotyledon develop from one zygote during seed maturation. It has been reported4,5) that the composition and accumulation profile of storage protein in the embryonic axis are different from that in cotyledon. Little information, however, had been obtained on the structure of protein bodies in embryonic axis.

Soybean seeds (Glycine max cv. Enrei) were harvested in 1986 in Nagano Prefecture, Japan and stored at 4°C. Isolated embryonic axes were cut into pieces of about 2mm³, fixed overnight in 2.5% glutaraldehyde in 0.1M Na-cacodylate buffer (pH 6.7) containing 2mM MgCl₂, and postfixed for 2 hr in 1% OsO₄ in the same buffer. The fixed materials were dehydrated using an ethanol series and embedded in Spurr's 6) epoxy resin. After staining with uranyl acetate and lead citrate, ultrathin sections were examined with a JEM-1200EX microscope.

Elements in electron-dense globoids in protein bodies were analyzed by energy dispersive X-ray (EDX) microanalysis.7) Sections were carbon-coated to strengthen the collodion support film, and examined with JEM-2000FX transmission electron microscope and AN10000 X-ray microanalysis system (Link Systems Ltd.).

Figure 1 shows the ultrastructure of cortex cells and stele cells in embryonic axes. Protein bodies in cortex cells had inclusional structure composed of an inner envelope, an electron transparent region, referred to as a soft globoid in this paper, and electron-dense globoids. Outside of the inclusional structures, the protein bodies were filled with dispersed form of proteinous matrix. Protein bodies found in the ultrathin sections were usually smaller than 1μm in diameter.

Protein bodies in the section of stele cells were larger and more predominant than those in cortex cells (Fig. 1B). The protein bodies also contained evident globoids and dispersed proteinous matrix, but the inner envelope and the soft globoid were sometimes not observed around the globoids. The electron transparent region had been occupied with globoids in the course of biogenesis of protein bodies (data not shown). Protein bodies in both tissues were surrounded by a clearly visible single membrane (white arrows in Figs. 1A and 1C), and lipid bodies lined

Abbreviation: EDX analysis, energy dispersive X-ray microanalysis.
The presence of phosphorus and several metal elements, Zn, Mg, K, Ca, and Fe is indicated by peaks located at 2.01, 1.01, 8.63, 1.25, 3.31, 3.69, and 6.40 corresponding to the respective locations of the P-Kα, Zn-La, Zn-Kα, Mg-Kα, K-Kα, Ca-Kα, and Fe-Kα emission lines.

An EDX spectrum showed the presence of phosphorus and other minerals in the globoids from embryonic axes (Fig. 2). According to previous papers, P, K, and Mg are generally present in phytate globoids, and Ca values vary greatly from absent to large amounts. Lott and Buttrose (1977) identified P, K, Mg, and a trace of Ca in the globoid crystals of soybean cotyledons. In the globoids of soybean embryonic axes, Mg and Ca were relatively abundant, and K was found only in traces, although it must be considered that K might be removed during sample preparation. Besides these major components of phytate, small peaks of Fe and Zn were also detected in the globoids. These two elements had been found in oats and rice. The copper peak is probably an artifact from the use of copper grids. In the globoids, a slight amount of osmium was found forming a weak shoulder of the peak of phosphorus, implying the presence of protein in the phytin globoids.

In summary, protein bodies in embryonic axes of soybean seeds had inclusional structures composed of an inner membrane, soft globoids, and evident phytin globoids. Mineral composition of phytin globoid in the protein body was different from that in cotyledonary protein bodies. Although further examination is needed to characterize the inner membrane and soft globoid surrounding the phytin globoid, it is of interest how the inclusional structure of protein bodies in embryonic axes is formed during seed development.

Acknowledgment. We thank Mr. T. Watabe and the staff at JEOL Japan for their help in the EDX analysis.

References