Freeze-fracture evidence of gel-phase lipid in membranes of senescing cowpea cotyledons

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Abstract. The structural details of membrane organization in germinating and senescing cotyledons of cowpea (Vigna unguiculata (L.) Walp.) were studied by thin section and freeze-fracture electron microscopy. Germination- and senescence-related changes in the ultrastructure of parenchymal cells of cowpea cotyledons, as detected in thin sections, closely resemble those described for other leguminous seeds. Additionally, electron-dense deposits associated with the membranes, particularly the plasmalemma and endoplasmic reticulum, were seen to increase with advancing senescence. Freeze-fracture electron microscopy demonstrated that the membranes of cotyledons of 2-d-old seedlings appear to be normal, with evenly dispersed intramembranous particles. However by 4 d, small areas or domains of the plasmalemma were free of intramembranous particles. These particle-free areas increased in both size and number as senescence progressed. We interpret these particle-free areas to be structural evidence for lateral phase separations of the membrane lipids into microdomains of gel-phase lipid from which intrinsic membrane proteins are excluded. Our results support wide-angle X-ray diffraction studies which have demonstrated the presence of gel-phase lipid in senescing bean cotyledons.

Key words: Lipid (gel phase) – Membrane structure – Microdomain – Senescence (structural changes) – Vigna.

Introduction

During germination and growth of the seedling, parenchyma cells of the cotyledons of most legumes, such as cowpea, undergo depletion of storage protein followed by total cellular senescence in 8–10 d (Öpik 1966; Ashton 1976). Studies with physical techniques such as wide-angle X-ray diffraction (McKersie et al. 1976, 1978; McKersie and Thompson 1977; Lees and Thompson 1980) and electron-spin resonance (McKersie et al. 1978), have detected increased amounts of membrane gel-phase lipid, decreased membrane fluidity, and an increase in the phase-transition temperature to be associated with advancing senescence. The occurrence of gel-phase lipid in both natural and model membrane systems has been correlated with loss of selective permeability (Haest et al. 1972; Papahadjopoulos et al. 1973; Barber and Thompson 1980), and a decrease in membrane-bound enzyme activity (McKersie and Thompson 1977; Thompson et al. 1978; Lees and Thompson 1980). Thus, gel-phase lipid may play an important role either in the cause or at least the expression of senescence in cotyledons.

Most biochemical and biophysical studies of membrane fluidity provide information about the physical-chemical status of the average or the predominant membrane fraction of the sample. However, if small portions of a membrane, or only certain membrane types, undergo fluidity changes, these may not be detected. For example, electron-spin-resonance (ESR) studies of membranes of both senescing Phaseolus cotyledon and rose petal membranes labeled with various spin probes do not indicate the presence of a phase transition with senescence, although an increase in the general viscosity of the membranes has been detected (McKersie et al. 1978; Legge et al. 1982). Wide-angle X-ray diffraction studies, on the other hand, implicated the presence of a phase transition in membranes of senescing rose petals (Legge et al. 1982) as well as Phaseolus cotyledons (McKersie and Thompson 1977; McKersie et al. 1978; Lees...
and Thompson 1980) by demonstrating the presence of gel-phase lipids in membranes from both tissues. This apparent inconsistency has been explained by the probability that the spin probe preferentially inserts into more fluid regions of the membrane (Shimshick and McConnell 1973; McKersie et al. 1978).

Freeze-fracture electron microscopy can be complementary to the above-mentioned physical techniques by allowing a more direct visualization of the numerous membrane types in situ. Thus, if small but important changes in membrane fluidity occur, or if only certain membrane types change, these perturbations may be detected by the freeze-fracture technique. We have studied the membranes of senescing cotyledons using freeze-fracture electron microscopy. We have demonstrated, in the plasmalemma and protein-body membrane, areas which are free of intramembranous particles (IMPs). These IMP-free areas increase in both frequency and size with advancing senescence. We interpret these areas to be regions of gel-phase lipids.

Material and methods

Seeds of cowpea (Vigna unguiculata (L.) Walp. cv. California Blackeye No. 5; a gift from Al Eckard, University of California, Riverside) were planted in vermiculite without presoaking and kept in the dark during germination. Cotyledons were removed 2, 4, 6, and 8 d after planting, and samples from the midportion of several cotyledons at each stage were taken for freeze-fracture electron microscopy. We have demonstrated, in the plasmalemma and protein-body membrane, areas which are free of intramembranous particles (IMPs). These IMP-free areas increase in both frequency and size with advancing senescence. We interpret these areas to be regions of gel-phase lipids.

Results

After planting and imbibition of the seeds, cowpea cotyledons undergo the normal sequence of changes during germination and senescence described for other legumes (Öpik 1966; Ashton 1976). The radicle emerged shortly after 1 d. The cotyledons showed incipient external signs of senescence by day 4 when some shrinkage was apparent. Cotyledons of 6- and 8-d-old seedlings showed considerable shrinkage, and some discoloration was apparent by 8 d.

Thin sections of cotyledons 2 d after planting exhibited an ultrastructural organization similar to that described for other protein-storing cotyledons (Fig. 1) (Öpik 1966; Harris and Chrispeels 1975). The plasmalemma was continuous and stained with the normal characteristics (Fig. 2). The protein bodies were surrounded by a single membrane and the endoplasmic reticulum (ER) was abundant between and around the protein bodies (Fig. 3).

The three-dimensional organization of the ER, as apparent in freeze-fracture replicas (Figs. 4, 5), indicated that both cisternal and tubular forms of ER were present. Additionally, the density of IMPs in each of the faces of the various membrane types (plasmalemma, protein bodies, ER) was observed. In all cases, the exoplasmic fracture (EF) face had a lower IMP density than the protoplasmic fracture (PF) face for each membrane type (Figs. 5–7). The IMP density of both ER membrane faces appeared to be lower than the density of corresponding faces of the protein bodies (Fig. 5) and the plasmalemma (Figs. 6, 7). All membranes of 2-d-old cotyledons had apparently random distributions of IMPs in both the EF and PF faces (Figs. 5–7).

Four d after the start of germination, thin sections showed that the protein bodies in the parenchyma cells farthest from the vascular tissue had begun to deteriorate. This degradation appeared to take two forms, and was apparent either as a general loosening of the matrix (Fig. 8), or as a localized loss of protein, resulting in electron-transparent pockets (Fig. 8). The ER in these cells again appeared to be present in both tubular and cisternal forms (Fig. 8).

Freeze-fracture confirmed the organization of the ER and proved that the IMP density and distribution in both faces of the ER (Fig. 9) and protein body (Fig. 10) was similar to that seen in 2-d-old cotyledons. Replicas of the EF face of the plasmalemma of 4-d-old cotyledons were also indistinguishable from those of 2-d-old cotyledons (com-