Inclusions within Lipid Droplets in Some Plant Seeds

Brief Report

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With 9 Figures

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Summary

Inclusions have been found within lipid droplets in cells of a number of air-dry seeds when investigated by freeze-etching. The inclusions invariably appear as depressions in the replicas. It is deduced that the inclusions are gaseous, and evidence from artificial preparations supports this.

1. Introduction

Many plant seeds have a high content of lipid which occurs in the form of droplets, and these appear homogeneous when viewed in thin section in the electron microscope (e.g., in Ricinus communis, Mollenhauer and Totten 1970; Oryza sativa, Buttrose and Soffky 1973). However, Schwarzenbach (1971) presented some micrographs showing inclusions in spherosomes of Ricinus communis and he interpreted these as remnants of prospherosomal vesicles.

When investigating Oryza sativa embryos by freeze-etching, it was found (Buttrose and Soffky 1973) that lipid droplets frequently had inclusions represented by circular depressions in their fracture faces. This communication reports on the nature of these inclusions and on their presence in a range of seeds.

2. Material and Methods

Tissues examined were coleoptiles of rice (Oryza sativa L. cv. Kulu) and wheat (Triticum aestivum L. cv. Gabo); cotyledons of sunflower (Helianthus annuus L. cv. Polestar), pistachio (Pistacia vera L.), almond (Prunus amygdalus Batsch.), and cucumber (Cucumis sativus L. cv. Short Green); and storage tissue of hazel nuts (Corylus avellana L.) and Quandong nuts [Santalum acuminata (R. Br.) D. C.]. Rice coleoptiles were taken from developing grains, mature grains, and from grains at the onset of germination. Sunflower
cotyledons were taken from mature seeds and from seeds at the onset of germination. Other tissues were from mature non-germinating seeds only. Blocks of tissue were mounted in either 100% glycerol or water on gold specimen discs, and within 10 seconds were frozen in liquid Freon 22, cooled to approximately $-150^\circ C$. Freeze-etch studies were also made of the following preparations: 1. a lipid fraction from sunflower seeds obtained by gently grinding cotyledons in a mortar and pestle with water and collecting the supernatant following centrifugation; 2. emulsions of commercial domestic sunflower oil and water (1:2, v:v and 6:1, v:v); c) a sunflower oil/air foam prepared by whipping sunflower oil cooled in a dry ice bath. Rice coleoptiles and sunflower cotyledons were prepared for thin-sectioning as described previously (Buttrose and Soffky 1973).

3. Results

In thin section lipid droplets had a homogeneous content (Fig. 1). In replicas fracture faces of lipid droplets often contained circular depressions, and there were no corresponding circular elevations (Figs. 2, 3, and 4). These depressions ranged in size from 0.1 $\mu$m in rice coleoptile (lipid droplets 0.5 $\mu$m diameter) to up to 2.0 $\mu$m in sunflower cotyledon (lipid droplets 3 $\mu$m diameter). They were found in all the tissues listed in the previous section. In rice coleoptile and sunflower cotyledon, frequency of occurrence of depressions varied between zero and a density as illustrated in Fig. 2 (170 per 216 $\mu$m$^2$). Frequency of depressions in those tissues other than rice coleoptile and sunflower cotyledon which were examined was low. Depressions were not observed in freeze-etch replicas of lipid droplets from immature rice coleoptiles taken from developing grains, nor coleoptiles of germinating grains. The concave face of the circular depressions was smooth relative to surrounding cross-fractured lipid (Figs. 3 and 4) which in turn was smoother than surrounding cytoplasm in preparations that had been exposed to water before freezing (Fig. 3). The concave face had a finely granular surface which was dotted with 25 nm particles (Figs. 4 and 5). Approximately-circular depressions with similar characteristics were also seen in fracture faces of 1. the lipid fraction isolated from sunflower

$L = $ lipid droplet, CW = cell wall, $PB =$ protein body, $SO =$ sunflower oil, $A =$ surface of air/sunflower oil interface, $\bigcirc =$ direction of platinum shadowing, $W =$ water, $D =$ depression, $C =$ cytoplasm

Fig. 1. Thin-section appearance of lipid droplets in rice coleoptile. $\times 10,000$
Fig. 2. Replica of rice coleoptile cells in which numerous lipid droplets contain a depression. Air-dry tissue frozen in water within 10 seconds. $\times 10,000$
Fig. 3. Depressions in lipid droplets of sunflower cotyledon. Tissue imbibed for 10 minutes before freezing. $\times 10,000$
Fig. 4. A depression in sunflower lipid droplet. Air-dry tissue frozen in water within 10 seconds. $\times 20,000$