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Microbodies in the cells of essential oil secreting glands

Artemios M. BOSABALIDIS

Department of Botany, School of Biology, Aristotle University, Thessaloniki 54124, Greece
Tel: +30 2310 998365, Fax: +30 2310 998389, E-mail: artbos@bio.auth.gr

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Abstract. The microbodies in the cells of three types of essential oil-secreting glands were studied. The secretory structures were the peltate oil hairs of *Origanum dictamnus*, the oil cavities of *Citrus deliciosa* and the oil ducts of *Apium graveolens*. Microbodies have an average diameter of 0.4 μm which is about three times smaller than that of the typical peroxisomes (1.5 μm). Their matrix is not densely fine-granular as in the peroxisomes, but it consists of a loose specky substance leaving bright irregular interspaces. Furthermore, they appear only during the stage of essential oil secretion and are relatively numerous.

Keywords: microbodies, essential oil glands.

Introduction

Plant microbodies have been ultrastructurally described (first by Mollenhauer et al. 1966) as spherical to ovoid cytoplasmic structures, 1.0-1.5 μm in diameter, surrounded by a single membrane and containing a dense fine-granular matrix. Microbodies have been observed in higher plants (Fellows & Boyer 1978), algae (Silverberg 1975), ferns (Cran & Dyer 1973), fungi (May & Barth 1977), desmids (Kiermayer 1970), etc. In plant organs, they are ordinarily met in green leaves and fatty cotyledons (Newcomb 1982), roots (Rocha & Ting 1970), petals, tubers and fruits (Huang & Beevers 1971), root nodules (Newcomb et al. 1985), etc. As concerns their intracellular localization, microbodies have been found in close association with chloroplasts and mitochondria (Lütz & Moser 1977), liposomes (Trelease et al. 1974), nucleus (Gruber & Frederick 1977), endoplasmic reticulum (Pueschel 1980), etioplasts (Schopfer et al. 1975), chromoplasts (Sitte 1974), dictyosomes (Silverberg & Sawa 1973), a system of cytoplasmic tubules (Galatis & Apostolakos 1976), etc.

Plant microbodies are ordinarily distinguished into peroxisomes, glyoxysomes and non-specialized microbodies (Gerhardt 1978, Beevers 1979). Peroxisomes and glyoxysomes have been extensively investigated and their function was considered to be associated with photorespiration and conversion of fat to sucrose, respectively. Microbodies of root nodules have been also investigated in detail and were found to be implicated in ureide synthesis (Hanks et al. 1983, Newcomb et al. 1985). Non-specialized microbodies have been observed in a wide spectrum of plant tissues, but no particular regard has been paid to them and no information has been provided about their structure and possible function.

In the present article, the microbodies in the cells of essential oil secreting glands were studied. Three types of plant oil glands, i.e. peltate oil hairs, oil cavities and oil ducts were investigated. Our study comprises only anatomical and ultrastructural data and may give a stimulation to biochemists dealing with terpene biosynthesis to extend our data to biochemical/cytochemical investigations on isolated oil gland microbodies.

Material and methods

Young leaves of *Origanum dictamnus* L. (Lamiaceae) and *Apium graveolens* L. (Apiaceae), as well as ovaries of *Citrus deliciosa* Ten. (Rutaceae) were used. Small pieces of the materials were fixed for 2 h with 2.5% glutaraldehyde and 2% paraformaldehyde in 0.05 M cacodylate buffer, pH 7.0. After washing in buffer, the segments were post-fixed for 3 h with 1% osmium tetroxide, similarly buffered. Tissue dehydration was carried out in an ethanol series and was followed by infiltration and embedding in Spurr's epoxy resin. For light microscopy, semithin sections of resin-embedded tissue were obtained in a Reichert Om U_2 microtome (Reichert AG, Wien, Austria), stained with 1% toluidine blue O in 1% borax and then observed in a Zeiss Axiostar Plus light microscope (Carl Zeiss GmbH, Göttingen, Germany). For transmission electron microscopy, ultrathin sections were cut in a Reichert-Jung Ultracut E ultramicrotome, stained with uranyl acetate and lead citrate and examined with a JEM 2000 FXII transmission electron microscope (Jeol Ltd, Akishima, Tokyo, Japan).

Results

Peroxisomes in chlorenchymatic mesophyll cells usually appear in contact with chloroplasts and mitochondria (Fig. 1A). They are typically surrounded by a single membrane which encloses a dense, fine-granular matrix. Fig.1A was inserted in order to allow structural comparison of the typical leaf microbodies (peroxisomes) with the microbodies of the essential oil-secreting glands described below.

Three types of essential oil secreting glands were studied: peltate oil hairs (ordinarily occurring in members of Lamiaceae), oil cavities (ordinarily occurring in members of Rutaceae) and oil ducts (ordinarily occurring in members of Apiaceae). The peltate hairs are external epidermal glands composed of a multicellular head, a unicellular stalk, and an also unicellular foot (Fig. 1B). The essential oil is biosynthesized in the head cells and becomes released into a cuticular bladder formed at the tip of the head. During the stage of secretion (not during an early or late developmental stage), microbodies appear in the cytoplasm of the head cells (Figs 1C, 1D). Microbodies are globular to ovoid in shape and are measured to have an average diameter of 0.4 μm. They are surrounded by a single membrane and their matrix does not consist of densely-arranged fine granules, as in the case of the typical leaf peroxisomes, but of a loose specky substance with low electron density (Figs. 1C, 1D). This substance forms local aggregations which leave bright irregular interspaces of various size (Fig. 1D). Microbodies exist in a remarkable number and are dispersed without forming close associations with other cell organelles.

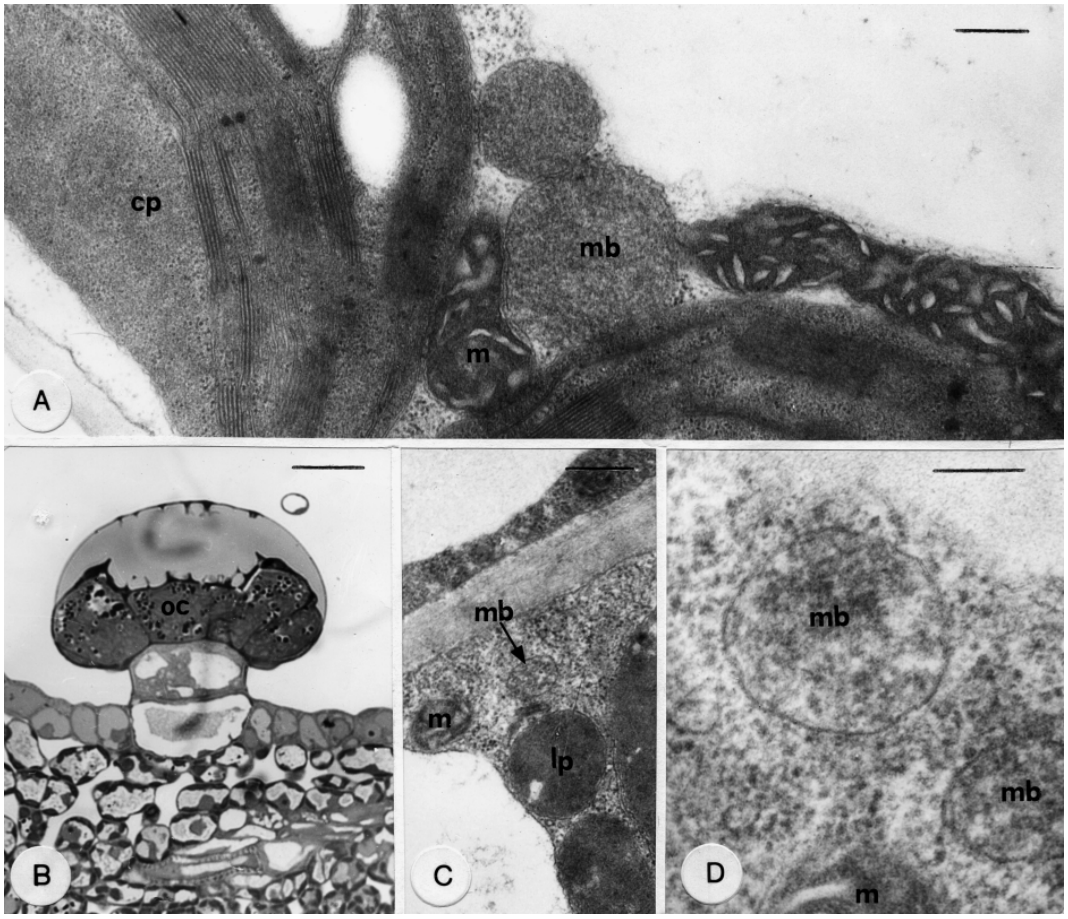

Figure 1A. Ultrastructural appearance of typical mesophyll microbodies (peroxisomes) (mb). They are globular, surrounded by a single membrane and their matrix is densely fine-granular. Microbodies are associated with chloroplasts (cp) and mitochondria (m). Bar = 0.5 µm.

Figure 1B. Secreting peltate oil hair of *Origanum dictamnus*. In the oil cells of the head (oc), biosynthesis of the essential oil takes place. Bar = 20 µm.

Figure 1C. Secreting peltate oil hair of *O. dictamnus*. In the head cells, among other organelles, microbodies (mb) occur. lp = leucoplast, m = mitochondrion. Bar = 0.3 µm.

Figure 1D. Secreting peltate oil hair of *O. dictamnus*. Two microbodies (mb) at high magnification. The microbody matrix is not densely fine-granular as in the mesophyll peroxisomes, but it consists of a loose specky substance with low electron density. Bar = 0.2 µm.

The oil cavities are internal glands localized in the subepidermal parenchyma (Fig. 2A). They are mostly ovoid and bear a central cavity surrounded by successive layers of epithelial cells. The essential oil is biosynthesized in the epithelial cells and becomes released into the central cavity. During the stage of essential oil secretion, in the cytoplasm of the epithelial cells microbodies develop (Figs 2B, 2C). They are relatively numerous, occur isolated or in groups and have an average diameter of 0.4 µm. Microbodies are bordered by a single membrane and their matrix is composed of a loose specky substance of low electron density, as in the case of the *Origanum* microbodies. In their matrix, irregular bright areas locally exist. Microbodies are not in contact with each other or with other organelles.

The oil ducts are also internal glands like the oil cavities. They are, however, not ovoid but tubular in shape and run parallel to the long axis of the organs (stems, roots, leaves). They are composed of a central elongated cavity which is bordered by a one-cell-thick epithelium (Fig. 2D). The essential oil is biosynthesized in the epithelial cells and becomes released into the central cavity. Microbodies appear in the epithelial cells during the stage of essential oil secretion and disappear later during gland aging. They occur free in the cytoplasm and do not develop associations with other cell organelles (Fig. 2E). The ultrastructure and size of the oil duct microbodies are quite the same with those of the peltate oil hairs and the oil cavities.

Discussion

The study of the microbodies in the three types of oil glands (Bosabalidis 1996, Bosabalidis 2002, Bosabalidis & Tsekos 1982) showed that they distinctively differ from the typical green leaf peroxisomes in substructure and size as well. Thus, oil gland microbodies were found to be about three times smaller than leaf peroxisomes (leaf peroxisomes were measured to have a diameter of 1.0-1.5 µm (Gruber et al. 1973), whereas oil gland microbodies of 0.3-0.5 µm). Furthermore, their matrix is not homogenously fine-granular,

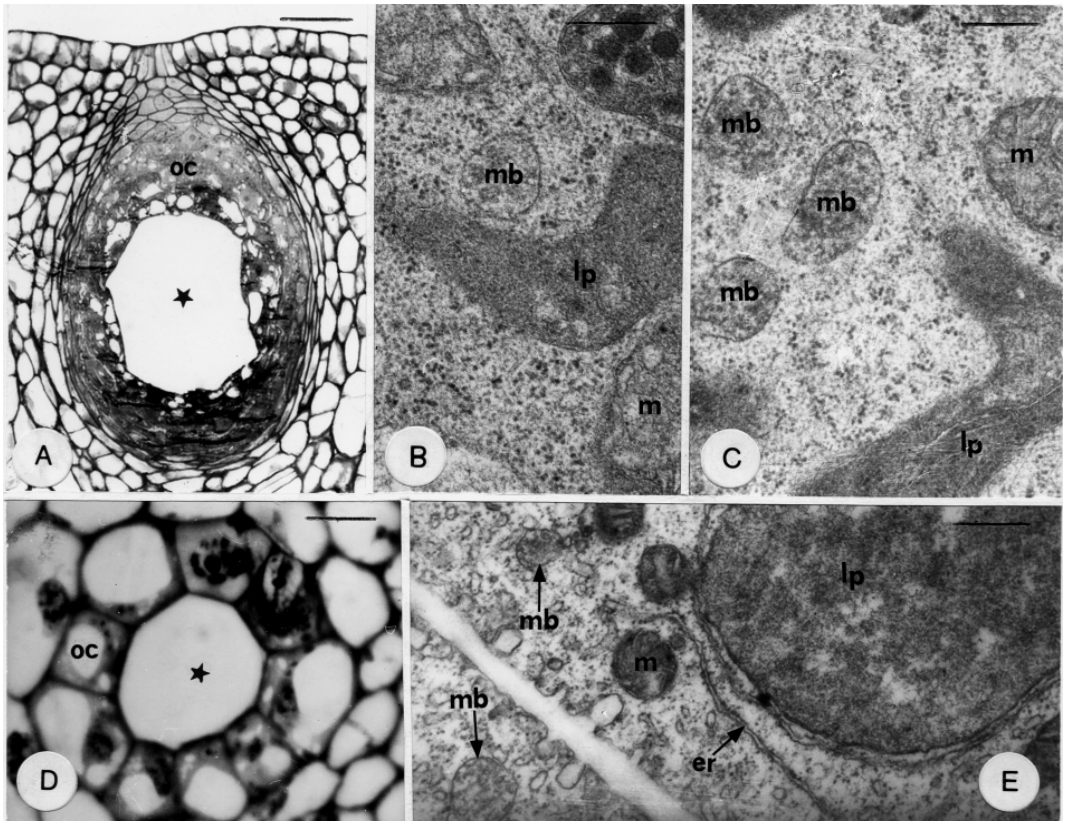

Figure 2A. Secreting oil cavity of *Citrus deliciosa.* The gland is ovoid and consists of a central essential oil-accumulating lumen (asterisk), surrounded by successive layers of epithelial oil cells (oc). Bar = 30 μm.

Figure 2B. Secreting oil cavity of *C. deliciosa*. Partial view of an active oil cell. mb = microbody, lp = leucoplast, m = mitochondrion. Bar = 0.3 μm.

Figure 2C. Secreting oil cavity of *C. deliciosa.* Assembly of three microbodies (mb) in the cytoplasm of an oil cell at the stage of essential oil secretion. Bar = 0.3 μm.

Figure 2D. Secreting oil duct of *Apium graveolens.* The duct appears in cross-section composed of a central cavity (asterisk) bordered by a single layer of epithelial oil cells (oc). Bar = 15 μm.

Figure 2E. Secreting oil duct of *A. graveolens.* In the cytoplasm of the epithelial cells microbodies (mb) and mitochondria (m) occur. Endoplasmic reticulum elements (er) are prominent and often surround plastids (lp). Bar = 0.4 μm.

but composed of dispersed patches. In the relevant literature on structure and secretion of oil glands, microbodies have been often reported to occur in the secretory cells. However, in all of the cases just a mention on their presence has been made without any reference to their structural similarities or dissimilarities to the typical leaf peroxisomes, as well as to their possible function related or not to the biosynthesis and secretion of the essential oil. Oil glands in which microbodies have been observed in the secretory cells during the stage of secretion are reported to be the oil ducts of *Artemisia campestris* and *Heracleum sphondylium* (Schnepf 1969, Ascensão & Pais 1988), the oil cavities of *Porophyllum lanceolatum* (Rossi Monteiro et al. 1999), the idioblastic oil cells of *Marchantia* sp. (Galatis & Apostolakos 1976), the peltate oil hairs of *Humulus lupulus, Primula* sp., *Pityogramma chrysoconia* and *Ribes sanguineum* (Wollenweber & Schnepf 1970, Schnepf & Klasova 1972, Tsekos 1974, Oliveira & Pais 1990), etc.

Most of the literature reports on microbody origination converge to the view that microbodies arise from the endoplasmic reticulum on the basis of ultrastructural, cytochemical and biochemical observations (Schnepf 1969, Berger & Schnepf 1970, Lanyon-Hogg et al. 2010). In the endoplasmic reticulum elements, specific enzymes participating in essential oil biosynthesis have been cytochemically identified (Gershenzon et al. 1989, Turner & Croteau 2004). Under the above regards, the presence of essential oil-biosynthetic enzymes within the microbodies might have a reasonable basis. As peroxisomes lack DNA, their function is determined by the import from the cytosol of specific metabolic enzymes (Lanyon-Hogg et al. 2010). On the other hand, enzymes involved in terpene biosynthesis have been found to occur in the cytosol (Bick & Lange 2003). The association of the microbodies with the cytosol of the secretory cells in the oil glands studied might provide a ground of interpretation for a possible presence of terpene biosynthetic enzymes in the microbodies.

Though the above interpretations involve a great deal of speculation, they actually provide some points of explanation (documented by TEM micrographs) as concerns the possible role of the oil gland microbodies in essential oil biosynthesis and secretion. Supportive to these interpretations are the appearance of the microbodies during the stage of essential oil secretion, their relatively high number and also their divergence from the typical peroxisomes as concerns their structure and size. In association with our structural studies, the real role of the gland cell microbodies in essen-

tial oil biosynthesis would be effectively clarified by studies of biochemists/cytochemists in which the presence of terpene-biosynthetic enzymes would be identified on isolated microbodies. If results will be positive, oil gland microbodies will not be any longer classified under the type of "non-specialized" microbodies.

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