Cutin synthesis: A slippery paradigm

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Despite its biological importance, the mechanism of construction of cutin, the polymer matrix of plant cuticles, has not yet been elucidated. Recently, progress on lipid barrier formation of polymers such as cutin and suberin has been recently reviewed by Pollard *et al.*[1] In their review the authors state that the ubiquitous cutin is the least understood of the plant extracellular polymers and that major questions about cutin structure and its macromolecular assembly remain to be resolved. At the time this paper was being published our research group has developed a new hypothesis on plant cutin synthesis.[2]

Aerial parts of higher plants are covered by a continuous extracellular layer, the cuticle. The main function ascribed to the cuticle is to minimize water loss, to limit the loss of substances from plant internal tissues, and also to protect the plant against physical, chemical, and biological impacts.[3] Cuticles of higher plants are chemically heterogeneous in nature, basically consisting of a wax fraction, soluble in common organic solvents, and an insoluble cuticular matrix, which forms the framework of the cuticle. This cuticular matrix is mainly formed by the biopolymer cutin, a high-molecular weight polyester composed of various interesterified C_{16} and C_{18} hydroxyalkanoic acids. Removing waxes and cutin from isolated cuticles yields some residual material, predominantly polysaccharides, that represents the portion of the epidermal cell wall to which the cuticular membrane was attached.[3]

For many years the scientific community has been trying to unravel the metabolic pathway of cutin and wax synthesis, with a very limited success. Even with the recent use of *Arabidopsis* as a plant model, the exact mechanism of cutin synthesis remains elusive despite the myriad of genes identified related to epidermal lipid development. Our research approach on plant cutin synthesis integrates previous data on plant cuticle and cutin fine structure throughout development,[3] self-assembly studies of the hydroxylated fatty acids that constitute the cutin biopolymer,[4] and chemical *in vitro* synthesis of cutin, which was done for the first time in our laboratory.[5] Self-assembly properties of lipid molecules present at the interface are gaining interest in the last years and giving fresh new answers to aspects related to molecular arrangement of plant waxes and the synthesis of complex plant barrier polymers such as suberin and sporopollenin.[6,7]

The special chemical properties of cutin monomers, whose chemical structures can be found in the review by Pollard *et al.*,[1] are responsible for the formation of nano self-assembled vesicles. These lipid vesicles formed by cutin monomers, namely *cutinsomes*, yield prime cutin by chemical autopolymerization.[2] This ability to polymerize is a consequence of the striking and emergent physicochemical properties of *cutinsomes*. In our work, we proposed that the accumulation of *cutinsomes* at the outer part of plant cell wall, in the first stages of cuticle formation, could lead to vesicular fusion and further polymer development. Several observations at early stages of cutin development indirectly support this hypothesis: the long-time observed nanoscopical osmophilic vesicles migrating through the cuticle of many plants,[3] and the analysis of AFM topography of tomato fruit cutin.[2] Interestingly, the mechanics of nano cutin vesicles formation agree well with the general model of cutin assembly shown in Fig. 4 of Ref. 1, where the authors indicate the putative existence of oleophilic droplets or lipid vesicles transporting cutin monomers or oligomers.

The polymerization mechanism of cutin proposed excludes the involvement of any protein, which means a non-genomic control in the last step of the cutin synthesis pathway. This implies that the emerging role of acyltransferases as putative condensing enzymes for cutin formation in *Arabidopsis* epidermis[1,8] is, answering the question raised by the authors, very doubtful. It is also our opinion that the cutin of *Arabidopsis* cuticle is atypical, as it is clearly indicated in the review, and that this plant is not a good model for cutin formation but a very limited one. On the other hand, nobody has reported yet the existence, in any plant, of a protein directly and clearly involved in the polymerization of cutin monomers. We could even wonder if this protein really exists indeed.

The assembly of cutin polyester is being investigated using different approaches. Molecular engineering of this polymer with monomers of other lipid polymers such as suberin has been applied with limited advantage,[8] especially if *Ara-*

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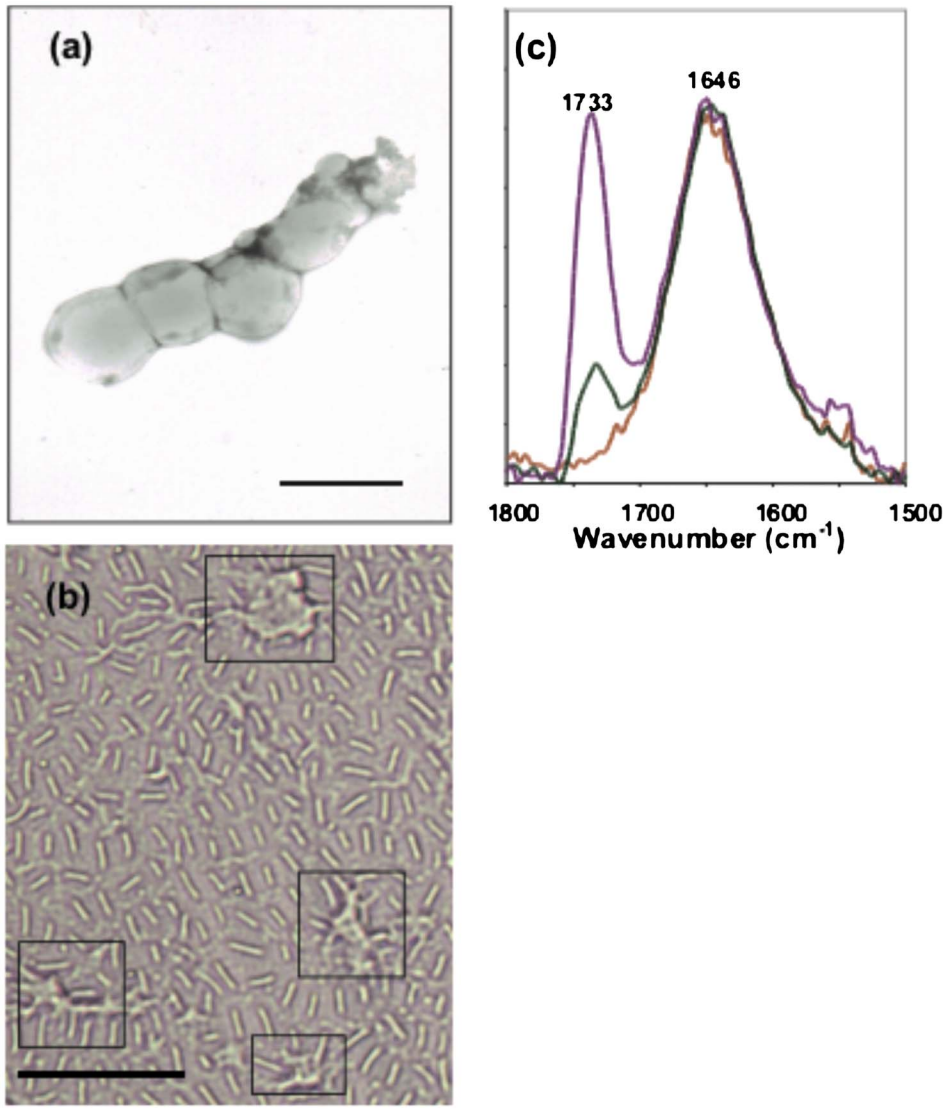

FIG. 1. (Color online) (**a**) TEM image of aggregated nanovesicles of the cutin monomer 9,10,16-trihydroxyhexadecanoic acid. The method of preparation is described in Ref. 2. Scale bar indicates 500 nm. (**b**) Light microscopy photograph of the outer surface of a film of regenerated cellulose used as a membrane in a diffusion experiment with vesicles of the cutin monomer following the method described in Ref. 9. The cellulose membranes were fixed into small methacrylate diffusion chambers with an inner diameter of 7 mm. They were filled with a dispersion of *cutinsomes* in water and closed by the cellulose film which was in contact with the solution. The diffusion chambers were placed into a closed chamber at 25 °C and relative humidity of 35% for 4 days. Vesicles form elongated particles throughout the surface which sometimes collapse in polymerized domains (see squares). Scale bar indicates 25 μm. (**c**) Attenuated total reflectance infrared spectra of cellulose control (orange), and the outer (green) and inner (purple) faces of the above mentioned cellulose membrane. Absorption at 1733 cm^{-1}, assigned to the stretching of CO functional group in an esterified chemical environment and indicative of polymerized *cutinsomes*, can be observed in both faces. Band at 1646 cm^{-1} appears in all samples and is assigned to the hydroxyl bending of water adsorbed on cellulose.

bidopsis is used as a model. On the other hand, cutin assembly or, even better, interaction of *cutinsomes* with plant cell wall components such as cellulose, hemicellulose, and pectin could yield essential information on this subject. This is a topic we are currently working on in our laboratory. As an example we include in this communication data on the interaction between *cutinsomes* and regenerated cellulose films (see Fig. 1 and figure legend). From here it can be concluded that generation of large microscopic domains of cutin polyester on a bed of cellulosic material is possible as the sole result of physic-chemical interactions. The major challenge for this hypothesis would be to demonstrate the presence of these special lipid vesicles *in vivo*, a question we are presently trying to address. Nonetheless, our model of cutin synthesis does not exclude a genetic control at the monomer level or even a further involvement of enzymes in later stages of cutin synthesis. Therefore, all the hypotheses on the construction of this unique biopolymer remain in full play; they might complement each other, be convergent, or be divergent, but they should fulfill the words of the great Argentinean writer Jorge Luis Borges, "*reality may avoid the obligation to be interesting, but hypotheses may not*."

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