

Chapter 1

Chemistry and Structure of Cuticles as Related to Water and Solute Permeability

From the very beginning of life on earth, all living organisms established protective interfaces between themselves and the aqueous or gaseous environment. In all cases these interfaces are of lipid nature. The first unicellular organisms developed cell membranes of phospholipids separating the cytoplasm from the surrounding aqueous environment. Phospholipids are major constituents of cytoplasmic membranes of contemporary organisms. Later in evolution, multicellular organism with specialised tissues and organs appeared, and the mainland was conquered successfully by plants and animals. Since the water potential of the atmosphere is always strongly negative, there is a constant loss of water from living organisms to the atmosphere. In order to survive and avoid desiccation, land-living animals and plants had to cope with this situation. With terrestrial higher plants, the evolutionary answer to this challenge was the development of a cuticle about 500 million years ago. Insects and mammals are also protected by cuticles or skins. Their cuticles have similar functions, but they differ in chemistry and structure from the plant cuticle (Andersen 1979; Rawlings 1995).

The plant cuticle is an extracellular polymer membrane which covers all primary organs such as stems, leaves, flowers and fruits. In contrast to most synthetic polymer membranes, which are mostly homogeneous in structure and composition, plant cuticles are polymer membranes characterised by a pronounced heterogeneity in both chemical composition as well as fine structure. A functional analysis of barrier properties of plant cuticles requires detailed information on chemistry and structure. It is one of our major objectives to relate chemistry and structure of cuticles to water and solute permeability. We have evaluated the literature in an attempt to find the information necessary for relating permeability of cuticles to chemistry and structure.

Using the terminology of engineering, cuticles can be classified as composite membranes. They are composed of two chemically distinct fractions, the polymer matrix membrane (MX) and soluble cuticular lipids (SCL), often called cuticular waxes. For unambiguous chemical analysis and for measuring permeability, cuticles are isolated either chemically or enzymatically (Schönherr and Riederer 1986). The method of choice is enzymatic isolation at room temperature using pectinase

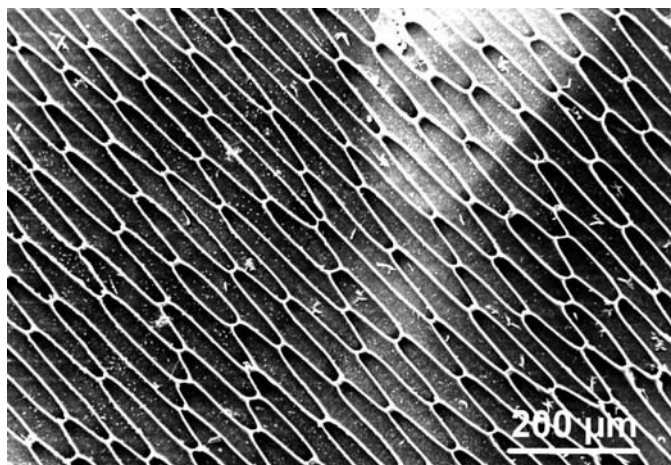


Fig. 1.1 Scanning electron micrograph of the morphological surface of a cuticle isolated with pectinase from an inner *Clivia miniata* leaf. Cuticular pegs, protruding between anticlinal cell walls, reveal the pattern of the epidermal cells

(Sect. 9.1). This avoids heat and treatment with chemicals which might cause hydrolysis or other chemical reactions. Pectinase digests the pectin layer interposed between cuticles and the cellulose wall of the epidermis. Occasionally a pectinase/cellulase mixture has been used, but the benefit of including cellulase has never been clearly demonstrated. Even when isolated using pectinase alone, the inner surfaces of the cuticular membrane look clean and cellulose residues are not detectable (Fig. 1.1).

We shall refer to isolated cuticles as cuticular membranes (CM), while the term “cuticle” is reserved to cuticles still attached to epidermis and/or organs. Cuticles cannot be isolated from leaves or fruits of all plant species. CM which can be obtained by enzymatic isolation have been preferentially used for chemical analysis, because this avoids ambiguities concerning the origin of the materials (waxes, cutin acids) obtained by extraction and depolymerisation. If enzymatic isolation of cuticles is not possible, air-dried leaves must be used. In these cases, there is a risk that some of the products obtained by solvent extraction or depolymerisation may have originated from other parts of the leaf.

1.1 Polymer Matrix

CM can be fractionated by Soxhlet extraction with a suitable solvent or solvent mixtures. The insoluble residue is the polymer matrix (MX), while the soluble lipids (waxes) can be recovered from the solvent. Chloroform or chloroform/methanol are good solvents, but many others have been used which do not quantitatively extract high molecular weight esters or paraffins, especially when used at room temperatures (Riederer and Schneider 1989).

Leaf CM [*Citrus aurantium* (bitter orange), *Hedera helix* (ivy), *Prunus lauro-cerasus* (cherry laurel)] preferentially used in transport experiments have an average mass of $250\text{--}400\mu\text{g cm}^{-2}$ (Schreiber and Schönherr 1996a), although CM thickness can vary between 30 nm (*Arabidopsis thaliana* (mouse-ear-cress)) and $30\mu\text{m}$ (fruit CM of *Malus domestica* (apple)). Specific gravity of CM is around 1.1 g cm^{-3} (Schreiber and Schönherr 1990), and using this factor the average thickness of these leaf CM can be calculated to range from about 2.3 to $3.7\mu\text{m}$.

If the MX is subjected to hydrolysis in 6 N HCl at 120°C , an insoluble polymer is obtained. This polymer has the consistency of chewing gum, and an elemental composition very similar to a polyester of hydroxyfatty acids (Schönherr and Bukovac 1973). It is considered to be pure cutin. The aqueous HCl supernatant contains a complex mixture of carbohydrates, amino acids and phenols, but only amino acids have been determined quantitatively (Schönherr and Bukovac 1973; Schönherr and Huber 1977). Some cuticular carbohydrates and phenolic substances have also been characterised (Marga et al. 2001; Hunt and Baker 1980). Polarised light (Sitte and Rennier 1963) and thermal expansion (Schreiber and Schönherr 1990) indicate the presence of crystalline cellulose. It is not known if polar solutes obtained by acid hydrolysis are simply trapped in the cutin as polysaccharides or polypeptides, or if they are covalently attached to cutin. Phenolic acids contained in the MX of ripe tomato fruits are released by ester hydrolysis, but it is uncertain if they were linked to cutin or to other constituents of the MX (Hunt and Baker 1980). Riederer and Schönherr (1984) have fractionated CM of leaves and fruits from various species (Table 1.1).

The mass of the CM per unit area varies widely among species between $262\mu\text{g cm}^{-2}$ (*Cucumis* (cucumber) fruit CM) and $2,173\mu\text{g cm}^{-2}$ (*Lycopersicon* (tomato) fruit CM). The wax fraction varies even more and is smallest with *Citrus* leaves (5%) and largest with *Pyrus* (pear) cv. Bartlett abaxial leaf CM (45%). The average weight fraction of the MX is 76%, with cutin and polar polymers amounting to 55% and 21% respectively. Variation among species in the fraction of polar polymers and cutin is much smaller than in mass per area of CM or in weight fraction of waxes (Table 1.1).

1.2 Cutin Composition

Cutin is insoluble in all solvents, and its composition can be analysed only following depolymerisation. MX obtained by solvent extraction of CM are usually used for these studies. This has been the standard analytical approach in the past decades for analysing the chemical composition of cutin. Cutin obtained after acid hydrolysis of polar polymers has never been analysed, and for this reason we do not know if the same cutin monomers are liberated when starting with MX or with cutin. Depolymerisation has been performed using ester cleaving reactions. In early studies, cutin was hydrolysed using methanolic or ethanolic KOH and hydroxyfatty acids were obtained by acidifying their potassium salts. After transesterification of

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