

# Investigation of macromolecule orientation in dry and hydrated walls of single onion epidermal cells by FTIR microspectroscopy

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## Abstract

Polarised infrared spectra from the wall of a single epidermal onion cell were obtained using a Fourier transform infrared (FTIR) microscope. The use of a newly constructed hydration cell allowed studies of both composition and architecture of intact walls of single hydrated plant cells. By comparing spectra taken with infrared light polarised perpendicular, or parallel, to the long axis of the cell, orientations of macromolecules in dry and hydrated cell walls were investigated. It was observed that bands associated with pectin were stronger with polarisation perpendicular to the direction of the cell elongation. On the other hand, bands associated with cellulose were more intense with polarisation parallel to the direction of cell elongation. These results show that in dry and hydrated cell walls, not only was there a net orientation of cellulose, but also of pectin. The implication of this is that pectin, which was previously thought to play no structural role in cell walls may, in fact, contribute to the mechanical and structural properties of the cell network. Such results are likely to have a tremendous impact on the formulation of definitive models for the static and growing cell wall. © 1997 Elsevier Science B.V.

**Keywords:** Onion epidermis; Cell wall; Single cell; Hydration; Fourier transform infrared microspectroscopy

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## 1. Introduction

Plant cells exhibit directional growth, or elongation, with cells expanding to form cylindrical shapes rather than expanding isodiametrically to become inflated spheres. Since it is cell walls that limit the expansion capabilities of the cell, the internal organisation of cell wall components ought to play an important role in the underpinning mechanism of plant growth and development. Cell walls are structurally

complex with their key components—cellulose, hemicellulose, pectins, proteins and phenolics—forming a network of complicated architecture. Our knowledge on the molecular level about cell wall architecture is, however, still very limited [1].

Infrared spectroscopy is a useful tool for the study of molecular structure and conformation. By employing polarised light, infrared spectroscopy can be used to study molecular orientations, since, if molecules are oriented, the intensities of certain infrared signals depend on the angle between the direction of the electric field vector of the incoming infrared radiation and the direction of the changing dipole moment of

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the molecules. Infrared polarisation studies on plant cell walls have previously been carried out using conventional infrared spectroscopy on large and multiple layers of dry samples such as internodal cell walls of algae [2], coleoptile cell walls of oats [3], and parenchyma cell walls and epidermal cell walls of pea stems [4,5]. The results have shown that the orientations of molecules differ according to the type of cell. In such experiments, the results of polarisation can be affected both by the heterogeneity of the cells, and by possible changes in polymer conformation caused by the drying process. In contrast, the development of an infrared microscope has opened the possibility of obtaining spectra from *single* cell walls [6]. By combining this instrument with a newly designed hydration sample it is possible to study cell walls in conditions more similar to those found *in vivo*. Based on previous investigations of the walls of single elongated carrot cells and walls of different cell types from carrot seedlings [7], the work presented here is an infrared polarisation study of single onion cells under different hydration conditions.

## 2. Experimental

Infrared spectra were collected using a Fourier transform FTS-60 spectrometer with a Spectra-Tech IR-Plan microscope attachment [6]. A single polariser (KRS5, Graseby-Specac, UK) was used to produce linear polarised infrared light. The polarisation direction was determined using a stretched polyethylene film with well-known polarisation behaviour [8]. The hydration cell was fitted with upper and lower windows of barium fluoride, and a demountable chamber with two inlets. The two inlets were connected to an atmosphere of controlled humidity which can be circulated as necessary through the cell (a full description of the cell will be published). A strip of the epidermal layer from a fresh onion bulb (Var. Jumble) was prepared by carefully washing and scraping off the viscous material adhered to one side of the epidermis which was then placed flat on the lower barium fluoride window of the hydration cell.

Spectra were obtained from the centre of one of the epidermal cells with incident light polarised either

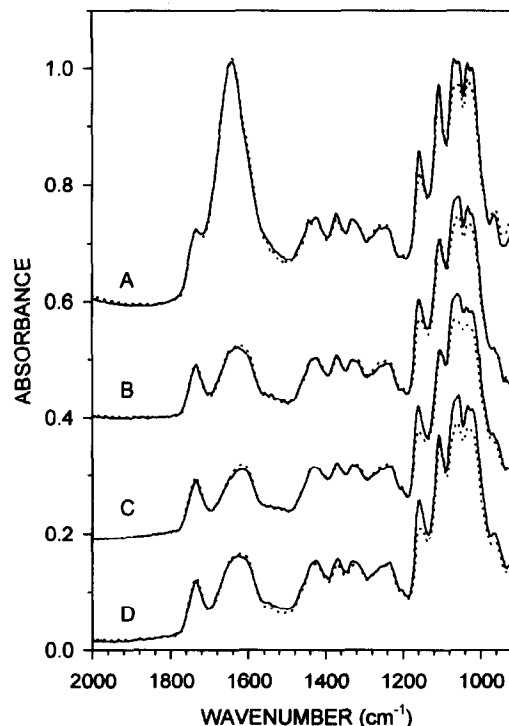


Fig. 1. Polarised infrared absorption spectra of the wall of an onion epidermal cell under different humidity conditions: (A) when the sample was still very wet after being washed; (B) when the sample was in 98% relative humidity atmosphere; (C) after the sample was dried; (D) after rehydration in 98% relative humidity atmosphere. The dotted lines represent spectra taken with the light polarised perpendicular to the long axis of the cells and the solid lines represent spectra taken with the light parallel to the long axis of the cells.

parallel or perpendicular to the long axis of the cell. Each pair of single beam spectra for the two polarisation directions was taken on the same spot of the sample. For each sample spectrum, a corresponding background spectrum was acquired at the same polarisation conditions. Typically, an area of  $30\ \mu\text{m}$  (cell width)  $\times$   $100\ \mu\text{m}$  was selected for collecting infrared spectra. The spectral resolution used was  $8\ \text{cm}^{-1}$  and 64 interferograms were co-added. Four humidity conditions were used: (A) when the sample was still very wet after being washed; (B) when the sample was in an intermediate humidity state in the vapour atmosphere of  $\text{K}_2\text{SO}_4$  salt solution (98% relative humidity); (C) after the sample was dried by the drying agent  $\text{P}_2\text{O}_5$ ; (D) after rehydration in the vapour atmosphere of  $\text{K}_2\text{SO}_4$  solution.

### 3. Results and discussion

The four pairs of infrared spectra are shown in Fig. 1. The dashed lines represent spectra taken with the light polarised perpendicular to the long axis of the cells, while the solid lines represent spectra taken with the polarisation parallel to the long axis of the cells. Large differences in intensity were observed in the carbohydrate fingerprint region between 1200 and 950  $\text{cm}^{-1}$  and smaller differences in intensity were observed in the longer wavenumber region between 1200 to 1800  $\text{cm}^{-1}$ . To study the differences in more detail, the difference spectrum of each pair was obtained by subtracting the spectrum taken with the perpendicular polarisation from the one with parallel polarisation. The four difference spectra so obtained together with an infrared spectrum of cellulose (E) are depicted in Fig. 2. The carbohydrate fingerprint regions of all the difference spectra match very well to the fingerprint region of the cellulose spectrum.

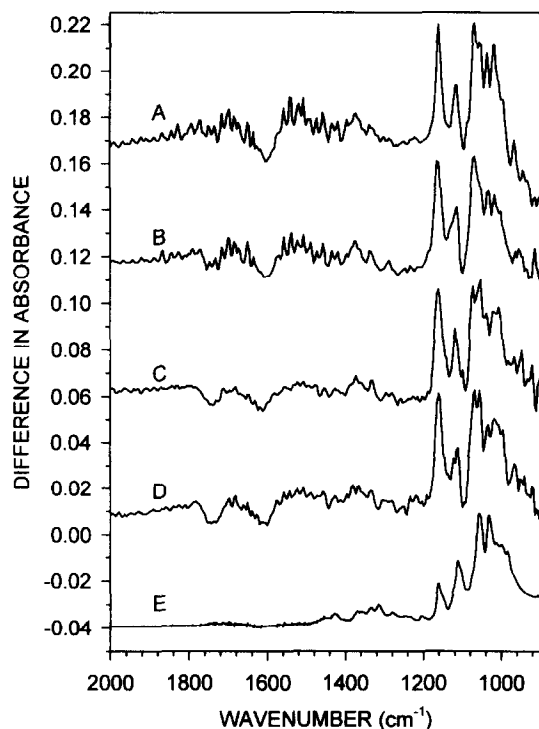


Fig. 2. Difference spectra obtained by subtracting the spectra taken with the perpendicular polarisation from the one with parallel polarisation. A, B, C and D indicate the same conditions as in Fig. 1 respectively. E is the infrared spectrum of cellulose.

Since the signals at 1070, 1115 and 1165  $\text{cm}^{-1}$ , which represent cellulose backbone vibrations of the polymer chain, are more intense with the parallel polarisation, it suggests that cellulose in the epidermal cells is arranged parallel to the long axis of the cells [9]. The signals at 1740 and 1605  $\text{cm}^{-1}$  represent the vibrations of ester groups and carboxylate groups in pectin respectively. They are negative in the difference spectra, showing that the signals of the carbonyl groups from the pectin are stronger in the perpendicular polarisation. Although the conformation of pectin in cell walls is not well understood, we can nevertheless conclude that pectin is also aligned in the epidermal cell walls. The absence of the ester band at 1740  $\text{cm}^{-1}$  in the difference spectrum A for the wet sample could be caused by overlap with the strong water band centred at 1640  $\text{cm}^{-1}$ . The observed polarisation effects therefore appear to be the same for the four humidity conditions.

### 4. Conclusion

The investigation has shown that infrared microscopy, which can probe chemical information about the walls of single cells with sampling dimensions down to 20  $\mu\text{m} \times 20 \mu\text{m}$ , is an ideal method for study of cell wall architecture. By combining the FTIR microscope with a specially designed hydration cell, we have shown that there is a net orientation of both cellulose and pectin in the walls of onion epidermal cells. Furthermore, the orientations of the cell wall polymers are not affected by the hydration or drying conditions in the wall of onion epidermal cells.

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