Fourier Transform Infrared Microspectroscopy Is a New Way to Look at Plant Cell Walls

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ABSTRACT

Highly reproducible Fourier transform infrared (FTIR) spectra from both single onion (Allium cepa) cell walls and their constituent polymers were obtained under a variety of sampling conditions. The specificity of the chemical extraction sequence used in the preparation of the material was confirmed: pectins only are extracted by cyclohexanediaminetetraacetic acid and sodium carbonate, whereas xyloglucans are extracted by increasing concentrations of potassium hydroxide. There was very little contamination of the first potassium hydroxide extract with residual pectin. The low abundance of both phenolics and protein was also confirmed. The first sodium carbonate extraction almost completely removes esters remaining in the cell wall. We have demonstrated that FTIR spectroscopy can detect large conformational changes in pectic polymers on removal from the cell wall and on drying. FTIR spectroscopy provides a powerful and rapid assay for wall components and putative cross-links by identifying polymers and functional groups nondestructively in muro. The availability of microsampling and data acquisition techniques that permit subtraction of the blanket absorption of water make FTIR spectroscopy particularly suitable for studies of cell wall architecture. The use of polarizers with the microscope accessory permits determination of the orientation of particular functional groups with respect to the direction of cell elongation in carrot suspension cells.

Changes in both cell wall composition and structure occur during plant cell cycle, growth, and differentiation, and these changes may, in turn, feed back and influence events within the cell (3). To understand this dynamic relationship between the plant cell and its wall, we need a coherent model of wall architecture at the molecular level. At present we do not have such a conceptual framework largely because we lack appropriate structural methods and suitable probes. Accordingly, much molecular machinery underlying plant growth and development remains obscure (1).

A minimal requirement is to understand how the three relatively invariant cell wall polymer classes, cellulose, hemicellulose, and pectin, are put together in space, and for this we require a simple cell wall system and appropriate methods for uncovering its architectural principles. We have begun our approach to this problem by choosing a chemically simple system, onion (Allium cepa) parenchyma cell wall, and looking at its architecture directly by way of fast-freeze, deep-etch rotary-shadowed replicas (9). This technique has enabled us to define some basic parameters of wall construction. For example, cellulose microfibrils, diameter 5 to 12 nm, are cross-linked by hemicellulose bridges of about 20 to 40 nm long that prevent the lateral association of cellulose. Middle lamella pectins are Ca++ cross-linked, and ester-linked primary cell wall pectins form a network that is coextensive with, but independent of, the cellulose/hemicellulose network. Pectins limit wall porosity, and a neutral galactan is associated with microfibrils throughout extraction. There is no evidence for the existence of a cross-linked extensin network in this tissue.

We have now extended our analysis of this simple system by using FTIR spectroscopy as a probe for the various chemical and conformational changes that occur during sequential polymer extraction from the wall. Our results indicate a wide potential for the use of FTIR microspectroscopy in cell wall research. We show how FTIR can be used as a crude but rapid chemical assay system for walls and polymers to detect conformational changes in wall components and to detect which putative cross-links might be present in the wall. We also show that, in an elongating carrot suspension cell, the orientation of particular functional groups with respect to the long axis of the cell can be determined by the use of polarizers.

The energy of molecular vibration corresponds to the IR region of the electromagnetic spectrum. For IR radiation to be absorbed by a molecule, there must be an interaction of the radiation with an oscillating dipole moment associated with a vibrating bond. The vibration must cause a change in dipole moment for the absorption to occur, and consequently, strong absorption occurs only for asymmetric bonds (16). Different types of functional group absorb in different parts of the spectrum, and thus, IR spectra are used for structural elucidation, and frequency-structure correlation charts are common. Coupling of vibrations can lead to complicated spectra, although an important result of the coupling is to produce "fingerprint" patterns in the region 1200 to 900 cm⁻¹, so that even complex polymers can usually be distinguished. IR spectroscopy has not been used to investigate biological systems until recently because of the severe problem of blanket absorption of water over most of the IR region. However, this has been revolutionized by Fourier transform data acquisition techniques, computer control, and new methods of sample presentation (2).

Abbreviations: FTIR, Fourier transform IR; CWM, cell wall material; CDTA, cyclohexanediaminetetraacetic acid.
Coupling the spectrometer (12) to an appropriate microscope allows selection of a particular area (as small as 10 × 10 μm) in any field of view for microsampling (13). Thus, spectra can be obtained from a defined region of a single cell wall as well as from bulk samples. In the scanning mode, the beam passing through the spectrometer sample compartment is diverted vertically downward to pass through the sample on the stage and then continues to a movable mirror assembly positioned to send it to the IR detector (11).

MATERIALS AND METHODS

Biological Material

Onion (Allium cepa) CWM is prepared as previously described (9). Cell walls from elongating cells in a carrot suspension culture are similarly prepared.

Extraction Sequence

CWM is sequentially extracted using the method of Redgwell and Selvendran (14) with 0.05 M CDTA (sodium salt, pH 6.5) (Sigma, Poole, UK) at 20°C for 6 h and then 2 h; 0.05 M Na2CO3 and 20 mM NaBH4 at 1°C overnight and then 0.05 M Na2CO3 and 20 mM NaBH4 at 20°C for 3 h; 1 M KOH and 10 mM NaBH4 at 20°C for 2 h and then an additional 2 h; 4 M KOH and 10 mM NaBH4 at 20°C for 2 h and then 4 M KOH with 3 to 4% boric acid for an additional 2 h to leave finally an α-cellulosic residue.

Purification of Wall Polymers

Supernatants containing extracted wall polymers are neutralized to pH 7.0 with HCl, dialyzed against deionized water, and concentrated using an Amicon filter apparatus to a final volume of 2 to 3 mL.

Experimental Conditions

Spectra were obtained on a SpectraTech instrument with microscope accessory. All spectra were obtained at a resolution of 4 cm⁻¹, with microscope aperture dimensions of 100 × 100 μm and 400 interferograms coadded for a high signal to noise ratio. All spectra are highly reproducible. Different areas of the same sample give the same spectrum, and equivalent samples from different experimental runs give the same spectra in all cases. CWM or polymers were dried in a layer on the barium fluoride microscope window (13 mm diameter × 2 mm), and areas with little depth of material were selected in the microscope. The single beam traversing each sample was ratioed with the single beam of the corresponding background. Insertion of polarizers into the path of the IR beam before and after passing through a cell wall sample mounted on the stage of the microscope accessory permits polarized microspectroscopy.

Digital Subtraction

Much information can be gained from digital subtraction of spectra by computer. In the experiments described here, the spectra of components in any mixture are additive: if an unwanted component is present, such as the blanket solvent absorption of water in wet samples, its spectrum can be subtracted from the spectrum of the sample, provided that the component is independent in the mixture and not interacting with another component.

RESULTS

FTIR spectroscopy has been applied to CWM at all stages of extraction and on purified cell wall polymers with a variety of microsampling techniques.

For the chemical species of interest here, the most interesting region of the spectrum lies between 2000 and 900 cm⁻¹. The carboxylic ester group absorbs at approximately 1740 cm⁻¹, amide-stretching bands of protein occur at 1650 and 1550 cm⁻¹, carboxylic acid groups on pectins absorb at 1610 cm⁻¹, phenolics absorb at 1600 and 1500 cm⁻¹, and carbohydrates absorb between 1200 and 900 cm⁻¹. Unfortunately, peaks cannot be unambiguously assigned to particular vibration modes below 1500 cm⁻¹ because many complex vibration modes overlap in this region: bands between 1500 and 600 cm⁻¹ are said to lie in the "fingerprint" region, because this part of the spectrum is unique to a particular compound, but individual peaks cannot be assigned. We have taken advantage of this region to identify polysaccharides by comparison with known standards, without identifying the particular vibrations that give rise to the spectrum. Absorbance readings vary between spectra because the absorbance depends on the thickness of the sample in the path of the beam and this has not been controlled in this experiment.

Onion CWM prepared by grinding in liquid nitrogen was sequentially extracted with CDTA, Na2CO3, and 1 and 4 M KOH according to the method of Redgwell and Selvendran (14). Both the polymers extracted and the residual CWM were examined at each step.

Polymer Spectra

FTIR spectra of extracted polymers were obtained by drying a droplet on a barium fluoride window and selecting an area where the material was of an appropriate thickness. Polymers 1, 2, and 3 (middle lamella pectins extracted by successive CDTA treatments and a primary cell wall pectin extracted by Na2CO3 at 1°C) have a number of features in common; all have peaks at 960, 1015, 1050, 1105, and 1140 (Fig. 1). Polymer 4 (extracted with Na2CO3 at room temperature) when dry lacks the features at 960 and 1140, and the two intense bands are shifted to higher wavenumber (Fig. 2A). Spectra of the alkali-extractable polymers 5, 6, 7, and 8 were alike and were significantly different from the pectin spectra (Fig. 3).

In addition to examination of cell walls or polymers in the solid state, bulk samples of polymers were also run in the liquid state using a liquid cell in the spectrometer sample compartment. Liquid state spectra were identical with solid state spectra except in the case of polymer 4 (a primary cell wall pectin extracted with Na2CO3 at room temperature), which now resembled other pectins (Fig. 2B). On drying a droplet of the solution from the liquid cell onto a microscope window, the solid state spectrum (Fig. 2A) was recovered. Polymer 4 is thus distinguished from the other pectins in undergoing conformational changes on drying.

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Figure 1. FTIR spectra of dried pectins extracted from onion cell walls by CDTA for 6 h (A), CDTA for an additional 2 h (B), and then an additional extraction with Na₂CO₃ at 1°C overnight (C) have most of their major spectral features in common.

Controls

Two purified and well-characterized polymer samples, kindly provided by Dr. A. Darvill (Complex Carbohydrate Research Center, Athens, GA) and Dr. M. J. Gidley (Unilever, Colworth, UK), were used as controls; the first, a purified xyloglucan; the second, low mol wt citrus pectin obtained by acid hydrolysis. The envelope of the xyloglucan spectrum (Fig. 4C) closely resembles the spectra of polymers 5, 6, 7, and 8. Xyloglucan is extracted even at the first Na₂CO₃ extraction and is the major component in this fraction. The purified pectate blocks gives a spectrum with the same features as polymers 1, 2, and 3 (Fig. 4A) as well as polygalacturonic acid (Sigma) (Fig. 4B): the polygalacturonic acid backbone of pectins must contribute most to their spectra. FTIR provides a rapid chemical assay for the specificity of extraction, confirming, for example, that pectins are removed only in the first half of the extraction sequence and hemicelluloses are removed in the second.

Extractants

Spectra were taken of cell walls at each stage of extraction. Because these samples have small amounts of contaminating extractants present, spectra of all extractants were obtained. Sodium carbonate, sodium borohydride, and boric acid have no peaks in the carbohydrate region (1200–900 cm⁻¹), but potassium hydroxide has a single peak, and the chelating agent CDTA (sodium salt) has many peaks in this region that could contribute to the CWM spectra. Where appropriate, the spectra of CDTA and KOH have been digitally subtracted from the CWM spectra.

Cell Wall Spectra

The carboxylic ester peak at 1740 cm⁻¹ in unextracted CWM (Fig. 5D) reduces on treatment with CDTA and vanishes completely after the first Na₂CO₃ extraction, confirming that the first Na₂CO₃ extraction completely deesterifies the cell wall. The spectrum of the extracted polymer also shows no absorbance at 1740 cm⁻¹, showing that this is not due merely to extraction of ester-containing components.

The carbohydrate region, 1200 to 900 cm⁻¹, shows little obvious variation during the course of extraction (Fig. 5). Although some cell wall sugars have distinguishing spectral features, many of the bonds, such as C-C, C-H, and C-O are common to all sugars. In purified polymers, distinguishing groups are enriched and characteristic peaks can be seen, but in the whole and partly extracted cell walls, these are obscured by the overlap of the bands of the various polymers.

However, digital subtraction of each successive extraction step from the previous one shows the removal of unique components at each step (Fig. 6).

Pectin Conformation

The difference between the spectrum of a cell wall before and after an extraction step might be expected to be equivalent to the spectrum of the polymer extracted. However, a series of digital subtractions of each successive extraction step from the previous one were generated from the first half of the extraction sequence (Fig. 6) but did not resemble the spectra of the pectins extracted at each step (Figs. 1 and 2). We have considered only the first half of the extraction procedure because direct visualization of the remaining material in the electron microscope (9) shows no disruption to...
the remaining cell wall matrix on removal of pectin, i.e., pectin behaves as a coextensive but independent network in the onion cell wall. On the other hand, removal of xyloglucans does result in reorganization of the remaining matrix, which could then contribute to the generated difference spectrum.

We can generate an additional set of spectra by digital subtraction of spectra of extracted pectins from their respective wall difference spectra (Fig. 7). In algebraic terms, if cell wall fraction A → extracted polymer B + remaining cell wall fraction C give rise to spectra a, b, and c, respectively, then by digital subtraction, spectrum a − spectrum c should be equivalent to spectrum b. However, as can be seen in Figure 6, this is not the case, and a new spectrum, spectrum d, is generated; thus, spectrum a − spectrum c = spectrum d. If we now subtract polymer spectrum b from spectrum d, then we can generate another spectrum, spectrum e, i.e., spectrum a − spectrum b − spectrum c = spectrum e (Fig. 7).

If there is no change in the spectrum of polymer B on extraction from the cell wall, then spectrum e would be a null spectrum. The fact that it is not (Fig. 7) indicates that a change has occurred in polymer B on extraction. Spectrum e for the first extraction step (Fig. 7A) is unique, but subsequent spectra (Fig. 7B) from the difference in wall material before and after the second, third, and fourth extraction steps, minus the respective spectra of the pectins removed in these steps, are very similar (Fig. 7B) when the inevitable loss in signal to noise ratio is considered: these spectra have very well-defined peaks.

The spectra in Figure 7 may represent the change that occurs in the molecular environment of the polymer when it is extracted from the wall into solution. Changes in the molecular environment of the polymer could stem from at least two sources, chemical modifications to the polymer and changes in polymer conformation upon extraction from the wall. Control experiments have shown that chemical modifications to polymers in vitro have a characteristic but small effect on the spectrum of the polymer. The principal chemical change in pectins, for example, upon extraction from the cell wall is deesterification. Remethylesterification of pectins adds characteristic methyl band stretches to the spectrum, but these can only be detected by digital subtraction of the deesterified pectin spectrum from the remethylesterified pectin spectrum (data not shown). The effect is very weak compared with the massive differences to be accounted for between the wall difference spectra and the extracted polymer spectra. Although it is possible that there is a contribution to the spectrum because of conformational changes in the

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**Figure 3.** Spectra of alkali-extractable polymers from onion cell walls are significantly different from those of extracted pectins and have two large peaks in common at 1020 and 1043 cm⁻¹. A, polymers extracted from depectinated CWM with 1 M KOH for 2 h; B, a second 1 M KOH-extractable fraction; C, a first 4 M KOH-extractable fraction; D, a second 4 M KOH-extractable fraction.

**Figure 4.** A, The spectrum of purified citrus pectate blocks, a kind gift of Dr. M. J. Gidley, Colworth, UK, confirms the identity of polymers extracted in the first half of the extraction sequence. B, Polygalacturonic acid (Sigma) has a very similar spectrum to the extracted onion cell wall pectins (Fig. 1) and to purified citrus pectate blocks (Fig. 4A) and must give rise to the spectral features observed in these. C, Purified xyloglucan from sycamore suspension cells, a kind gift of Dr. A. Darvill, Complex Carbohydrate Research Center, Athens, GA, has similarities in the envelope shape with the alkali-extractable polymers from onion.
remaining matrix, the spectra indicate a precise and reproducible change, not consistent with a global reorganization in the matrix. Also, images of CWM before and after removal of pectin obtained by the fast-freeze, deep-etch, rotary-shadowed replica technique show that the cellulose/hemicellulose network is not disrupted by the removal of pectin (9), and the spectra of the extracted CWM (Fig. 5) do not show a significant change in their spectral envelope.

The spectra in Figure 7, therefore, most probably represent the change in conformation as the polymer is released from the packing constraints in the wall into its free conformation in solution. The similarity of spectra in Figure 7B suggests that even heterogeneous pectins adopt the same conformation in the wall, whereas polymer 1 is in a different conformation, possibly induced by the presence of Ca$^{2+}$ in the middle lamella. It is also clear from the behavior of the last-extracted pectin (Fig. 2) and from data on carrageenans (4, and our unpublished data) that conformational changes in polymers have a very large influence on spectra in terms of absorbances, about 2 orders of magnitude greater than that of chemical modifications.

**Polarized Microspectroscopy**

A carrot cell-suspension culture contains a mixture of cell shapes from round to extremely elongate. The process of elongation is accompanied by, or perhaps is a result of, changes in the orientations of cell wall polymers. Although it is easy to determine by birefringence in the polarizing microscope that cellulose microfibrils have become oriented perpendicularly to the axis of elongation of the carrot cell, the orientations of matrix components, in this case protein, pectin, and xyloglucan, are very difficult to determine.

However, the use of the microscope accessory permits insertion of polarizers into the path of the IR beam before and after passing through the cell wall sample. An area of cell wall is delimited by the double-bladed apertures in the microscope and then spectra of that area are obtained with the polarizers aligned first parallel and then at right angles to the long axis of the cell. Figure 8, A and B show the spectra obtained from an elongate carrot suspension cell wall with the polarizers aligned parallel and perpendicular to the long axis of the cell, respectively. Although these spectra look very similar, digital subtraction of the spectrum in Figure 8A from that in Figure 8B shows that particular functional groups are preferentially oriented. Provisional assignments are an ester band at 1740 cm$^{-1}$ and amide bands characteristic of proteins at 1650 and 1550 cm$^{-1}$; the bands at 1490 and 1600 cm$^{-1}$ are possibly phenolics. These bands and the bands in the carbohydrate region of the spectrum indicate that the vibrational modes are oriented transversely to the long axis of elongated cells (Fig. 8C). On round carrot cells the subtraction of parallel and perpendicularly polarized spectra, at any angle of the polarizers, results in a null spectrum.

**DISCUSSION**

A new arsenal of biophysical technologies is now available with which to assault the cell wall: $^{13}$C cross-polarization-magic angle spinning NMR, Raman and FTIR spectroscopies, electron energy loss spectroscopy, and fast-atom bombardment MS, but with some exceptions (7), relatively little has
so far been achieved with them. The first advantage of FTIR microspectroscopy is the ability to sample individual cell walls rather than a bulk specimen. The specificity of chemical analysis of a single cell type is thus guaranteed.

Polymeric components of the cell wall can be extracted from purified plant cell walls by many different techniques, including the use of enzymes, chelating agents, alkali, and organic solvents (17). There are two main problems with the cleavage approach to cross-links. Because polymers may be held in the wall by more than one type of cross-link, failure

**Figure 6.** Digital subtraction spectra of successive extraction steps of onion CWM show distinct carbohydrate features to be removed at each step. A, CWM after extraction with CDTA for 6 h subtracted from the spectrum of unextracted CWM; B, CWM after extraction with CDTA for a further 2 h subtracted from the spectrum of CWM after a first CDTA extraction step; C, CWM after extraction with Na$_2$CO$_3$ at 1°C subtracted from the CDTA-extracted CWM; D, CWM after extraction with Na$_2$CO$_3$ for a further 3 h at room temperature subtracted from the spectrum of CWM after the first Na$_2$CO$_3$ extraction step.

**Figure 7.** Digital subtraction spectra generated by subtracting the spectrum of the polymer removed at each extraction step from the corresponding spectrum (in Fig. 6) of the difference between the spectra of CWM in successive extraction steps. A, The spectrum generated from the difference between CWM before and after the first extraction step, minus the spectrum of the polymer removed by CDTA in this extraction step, is distinct. B, Subsequent difference spectra generated from the difference in CWM before and after the second, third, and fourth extraction steps, minus the respective spectra of the pectins removed in these steps, show remarkable spectral similarities.

**Figure 8.** A, Spectrum of an area of an elongated carrot suspension cell with polarizers aligned parallel to the long axis of the cell. B, Spectrum of the same area of cell with polarizers aligned perpendicular to the long axis of the cell. C, The digital subtraction spectrum generated from the difference between A and B shows an ester peak at 1740 cm$^{-1}$, carbonyl peaks at 1650 and 1550 cm$^{-1}$, possibly phenolic stretches at 1490 and 1600 cm$^{-1}$, and carbohydrate peaks at 1160, 1117, and 1065 cm$^{-1}$ to be preferentially oriented transversely to the long axis of the cell.
of a cleaving agent to solubilize a particular polymer does not prove that the targeted cross-link was not partly responsible for holding the polymer in the wall, and where the specificity of action of the cleaving agent is not unique, release of the polymer cannot be taken as evidence that the cross-link existed in the wall. FTIR provides a nondestructive chemical assay for wall components and may be used to detect the presence of any putative cross-links because it detects the presence of specific bonds in muro.

Once extracted, chemical analysis of wall components involves hydrolysis of polysaccharides to monosaccharides and their subsequent separation as volatile derivatives (11). The problem with conclusions drawn from sugar analysis is that they give only an indication of which polymers might be present by analyzing building blocks rather than constructs. Secondary, tertiary, and quaternary levels of polysaccharide structure are lost, and even knowledge of the sugar composition in isolated fractions gives no indication of the heterogeneity of the polymer population. An awareness of the increasing range and complexity of cell wall polysaccharides demands that any chemical analysis of wall components should be complemented by methods for analysis of the polymers in muro. FTIR spectroscopy can provide a fingerprint that corresponds to a specific intact polysaccharide rather than its component monosaccharides, and a data base containing such fingerprints can then be used to confirm the presence of a particular polysaccharide in a complex mixture.

FTIR spectra can confirm the specificity of extraction procedures. For example, only pectins are removed by CDTA and Na$_2$CO$_3$, and xyloglucans are removed in the second half of the relatively mild chemical extraction sequence used in these experiments, although it may not be possible to obtain such clean separation of wall components from other, more complex, wall types. FTIR spectra did not show the characteristic amide-stretching bands of proteins (1550 and 1650 cm$^{-1}$) in the cell walls, and there are no phenolic stretches present, consistent with previous demonstrations that onion cell walls are unusually low in both protein and phenolic components (14).

The computer models of Walkinshaw and Arnott (15) have shown that pectate chains are sterically capable of packing in quite a number of ways. However, the number of conformations of pectin in muro is limited by the available volume of the wall; pectin gels formed by adding Ca$^{2+}$ to extracted wall pectins have much greater volume than the volume of the wall from which they were extracted. Primary cell wall pectins are extremely long polymers (300–500 nm), and the constraints of the wall (itself only 100 nm wide in onion [10]) makes it likely that pectins adopt specific conformations in muro and may be oriented in particular directions. The first extracted polymer, held in the middle lamella by Ca$^{2+}$ cross-links, has a distinct conformational change on removal from the wall (Fig. 7A). In the electron microscope, these pectins are rigid rods unlike subsequently extracted pectins (7), and a layer of rigid rods can be visualized in the middle lamella between two cell walls.

Polymer 2 is released by chelating agents from the wall but is at least partially methylesterified and has a different sugar composition from polymer 1. Polymers 3 and 4 have almost identical changes in conformation (Fig. 7B) on extraction into solution. By sugar analysis, polymers 3 and 4 are rhamnogalacturonans rather than homogalacturonans, although with slightly different sugar composition. Polarized IR spectra suggested that pectins have an oriented structure in pea epidermal cell walls with their molecular chains oriented preferentially parallel to the direction of cell elongation (5).

The spectrum of polymer 4 illustrates both the specificity of extraction, because only the second Na$_2$CO$_3$-extractable pectic fraction undergoes a conformational change on drying, and the heterogeneity in the pectin population. In many different tissues, the composition of the wall pectins changes during development and differentiation of the constituent cells. Onion cell wall pectins are a highly heterogeneous group of molecules by sugar analysis and anion exchange chromatography (14), appearance in the electron microscope (9), length measurements, and affinity for antibodies that recognize different pectic epitopes (10).

The use of polarizers with the microscope accessory can give information about the orientation of specific functional groups. In elongated carrot cells, it appears that esters, protein bands, and possibly phenolic stretches, as well as carbohydrate stretches, are oriented transversely to the long axis of the cell. Although it is not possible to say in which direction the polymers are aligned from this without much more detailed knowledge about polymer conformation than is currently available, it is clear that the matrix polymers as well as the cellulosic microfibrils must be very precisely aligned. This has implications for cell wall models. Only one cell wall model (8) includes an architectural role for proteins in an extended network that is oriented with respect to the cellulose. Mechanisms for cell wall elongation must involve massive architectural rearrangements of all cell wall components.

A recent study has used FTIR microsampling to investigate the distribution of carboxylic groups from pectins and amine groups from proteins and correlated these features to the banding pattern in the enormous cell walls of Chara corallina (6). It is certain to be only the beginning of many applications of FTIR to questions in cell wall research.

IR spectroscopy has long been a powerful and rapid assay technique for the chemist, and now FTIR can provide an equivalent service for the biochemist to determine sample composition and purity. It will prove useful for in vitro reconstitution studies of cell walls, in which interactions and conformational changes can be monitored and the presence of particular bonds assayed. We shall use FTIR to map changes in wall composition during elongation and differentiation and between tissue types, and the technique offers the opportunity, by using polarizers, of studying polymer orientation. The presence of putative cross-links in muro, e.g. diterfic cross-links or uronyl esters, could be directly detected by a nondestructive assay, thus setting constraints on models of wall architecture.

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LITERATURE CITED


