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Author(s): Stoddart, B.

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The Structures of Plant Tissues and the Effects of
Drying and Fluid Preservation Upon Them
- Bob Stoddart

1. A CAVEAT

Biochemical knowledge, both of plants and animals, has largely been obtained, in the first instance, by the detailed analysis of a limited range of tissues from a few convenient and readily procured species. Comparisons with further tissues and species have then followed, but there is still a general limitation on the range of species and tissues analysed. This is a problem when considering the biochemical changes that may occur in natural history collections during their preparation and storage, for they will contain specimens for which biochemical analyses *in vivo*, or immediately after tissue death have never been performed and for which extrapolation from other species may be misleading. This is certainly the case for botanical specimens, where information is strongly biased towards higher plants, terrestrial plants, species easily obtained in bulk and species of commercial importance. Much more is known about the macromolecules in the walls and cells of plants important to the foodstuffs and the pulp and paper industries, for example, than is known of their counterparts in many algae, bryophytes or inconspicuous and non-commercial wild flowers. What follows largely refers to the tissues of terrestrial plants and algae of the *Characeae* and is unavoidably based upon analyses of a restricted range of species and their tissues. Hence xerophytes and higher plants adapted for, for example, life immersed in water, which are seriously under-represented in biochemical analyses, may have important differences from the 'average' in the properties of their tissues and the lower algae are certainly very substantially different from higher plants.

2. CELL WALL STRUCTURE: GENERAL

Plant cells have walls, which are domains of extracellular matrix surrounding the individual cells and under their control. The properties of the cell walls largely determine the mechanical properties of plant tissues and will preserve much of the shape and appearance of the tissue, even after the cells are dead. Thus the gross appearance and much of the histological structure of a specimen of plant tissue will be preserved in its cell walls and these walls will also provide a protective environment for the nucleic acids and proteins of the cells within and may limit leaching of some of the lipid.

Most of the dry mass of a 'typical' plant cell wall is attributable to carbohydrate, either in the form of polysaccharide or glycoprotein glycans. The rest of the dry mass derives mostly from protein, almost entirely as glycoprotein, mineral, chiefly calcium salts and silicates, and a variable amount of plant phenolics in the form of lignin. Other components are minor. In life, however, water can be a substantial proportion of the mass of a plant cell wall, especially if the wall is physiologically in a highly aqueous environment.

3. CELL WALL MOLECULES

Terrestrial plants and the higher algae have walls which are usually described as being composed of cellulose, hemicellulose and pectins, together with minerals and lignin. These fractions are defined by the methods of their extraction and none of them consists of a single type of macromolecule, nor can they be compared exactly between tissues and species.

A. CELLULOSE

The cellulose fraction consists of cellulose (which is often defined as a β-1, 4 glucan, but normally contains a small content of covalently linked mannose) plus small amounts of structurally distinct glucomannan and galactoglucomannan.

B. HEMICELLULOSE AND PECTINS

The hemicellulose fraction is largely composed of xylans, arabinoxylans and glucuronoxylans, with variable amounts of related polymers. The pectin fraction is extremely complex and contains rhamnogalacturonan, arabinogalactans and larger polymers containing all of these sugars with smaller contents of xylose, mannose, glucuronic acid, apiose (sometimes with KDO and aretic acid) and having a variable degree of

methyl esterification, acetylation and – Omethyl content.

Pectins are best considered as a family of modular polysaccharides in which the individual, major modules are homogalacturonan (or polygalacturonan PGA), rhamnogalaturonan (RGA which is a copolymer of galacturonic acid and torhamose) and rabinogalactan (AGa, which resembles the arabingalactan of cell walls). It is usually considered that the 'backbone' of pectins consists of regions of PGA and RGA, spliced end-to-end, with the AGa forming branches, attached to the RGA regions. However, there is evidence that some pectins, especially that of soy bean, can carry PGA attached to AGa, so producing a structure in which the AGa may be part of the 'backbone' and PGA may be largely present in side chains. These two structural models are not necessarily mutually exclusive: there could be a range of structural types possible with 'classical' pectins and soy bean pectins representing extreme forms. It should be noted that many (but not all) plant gums resemble pectins or parts of pectins and are likely to represent evolutionary variants of them, or the products of incomplete or unbalanced assembly.

In addition to the major 'modules' of pectins above, there are smaller branches within pectins, which include xyloglucuronan and rare sugars such as apiose, KDO (ketodeoxsyoctulosonic acid) and aceric acid, as well as small quantities of mannose and (unusually) glucose. The locations and roles of these sugars have largely become evident only recently and, in some cases, are still uncertain.

C. CELL WALL PROTEINS

The proteins of plant cell walls are a heterogeneous population. Many of them are both very highly glycosylated and of unusual amino-acid composition being particularly rich in glycine and hydroxyproline and sometimes low in their content of aromatic amino-acids. Several are rather insoluble in water and many are unusually resistant to proteolysis. They tend, therefore, to be associated, during extraction, with the 'cellulose' and 'lygnin' fractions, which are the least soluble fractions of cell walls. Several cell wall proteins are thought to be linear and triple-helical, but their organisation in the intact wall is largely unknown. Some cell wall proteins are probably carbohydrate-binding proteins, or lectins, which may be interacting with glycans of the cell membranes.

D. OVERALL STRUCTURE

Cellulose forms the major fibrillar component of the wall and is a large contributor to its tensile strength. Xylans are considered to be associated with the outside of cellulose fibrils and to cross-link them by forming a kind of three-dimensional 'chicken wire' structure. The pectins act as a kind of in-fill to this and are often visualised as a kind of hydrated 'plaster' to the cellulose and xylan 'laths', though their physical chemistry suggests a more complex behaviour than just this and they are clearly vital to the mutual adhesion of the walls of individual cells.

E. TYPES OF BONDING

Plant cell walls contain a wide assortment of chemical bonds, which vary greatly in their bond strengths and chemical stability. Covalent bonds are of many kinds, of which those that form the covalently linked backbones of the polysaccharides are the major contributors to the gross integrity and shape of most plant tissues, but those of proteins are also important. Chains of cellulose and xylan are relatively stable and do not show appreciable breakage either in dry specimens or under the conditions found in fluid-preserved specimens, even where the preservative fluid has become acidic over time, so long as its pH remains above 3.5 and no peroxidation occurs. Below that pH slow hydrolysis of glycosides is possible. Pectins and the arabinogalactans show more variation in stability among the glycosides that they contain. Arabinofurranoside linkages are labile and will tend to hydrolyse appreciably below pH 5.0, over time and at ambient temperature. The rate of hydrolysis increases rapidly as the pH falls or the xylofuranosides, will behave in a similar way. In contrast, glycosides of non-esterified uronic acids are very stable and resist acid hydrolysis, so that the sugar ring may degrade or fragment in strong mineral acid before the glycoside breaks. Hence galacturonan chains are very unlikely to undergo hydrolysis even in old, acidified, preservative fluids and the same will apply to terminal glucuronic acid in xylans or callose. However, methyl esterfied galacturonans are liable to degrade by a different mechanism at any pH above about 8 to 8.5. This mechanism is that of a β-elimination reaction, in which the glycoside on the non-reducing terminal side of a methyl esterified residue of galacturonic acid breaks to form an unsaturation between carbons 4 and 5 of the esterified uronic acid.

Enzymatic catalysts of this type of reaction can occur and some fungi can produce pectin transeliminases.

Relatively weak covalent bonds occur between mineral and polysaccharide in cell walls. Boronate esters form bridges between apiosyl branches on polygalacturonic acid and are thought possibly to do the same with KDO and aceric acid. By analogy with other sugar boronates, they are likely to be reasonably stable at high pH, but to hydrolyse in mild acid and to be unstable and, possibly rearrange near neutrality. Silicate esters are likely to be even less stable and their study is extremely difficult, because of the ubiquity of silicates in water exposed to glass, or to plastic containing silicate filler, and the lack of any convenient radioisotope of silicon. Calcium ions can interact electrostatically with carboxyl groups of uronic acids, but can also coordinate with the hydroxyl groups of sugars and this may well be the more important interaction in the sequestration of calcium within plant cell walls.

It is unclear how far electrostatic interactions, for example between carboxyl groups of uronic acids and amino- or guanido-groups on proteins contribute to the stability of plant cell walls. In an aqueous environment, especially with salts present, such interactions may be limited and effective only over very short distances or in very specific local environments from which water is excluded or within which it is ordered. Exclusion of water, for example by lignification, will have a very large effect and will increase the effective bond strength of any electrostatic interaction: the dielectric constant of the environment may be massively altered.

Hydrogen bonds are extremely abundant in plant cell walls. Some form directly between sugars, as in cellulose, while others occur between glycan and protein, within proteins, or between glycan and/or protein and lignin. In some cases, there is a hydrogen-bonded interaction involving a molecule of water as a bridge. Such water is 'structural water' and is not readily exchangeable with bulk water, being removable only by severe, forced desiccation. Water itself is highly hydrogen-bonded and tends to form domains: this is a major consideration in thinking about how the removal or addition of water will affect the properties of stored plant tissues.

Hydrogen bonds have considerable potential for rearrangement, especially after the removal of the relatively tightly bound structural water and many will only very slowly revert to their former pattern on rehydration. This process, termed 'curing' makes the rehydration of severely dried specimens difficult and slow and lies at the bottom of problems of mechanical disruption that can occur if such rehydration is not performed slowly and very carefully.

4. CELL WALL FORMATION AND ASSEMBLY

After a plant cell has undergone mitosis, a new cell wall, flanked by new cell membranes, develops between the two daughter nuclei in the position of the equator of the spindle. It forms by the fusion of vesicles that move up between microtubules and, in its earliest form, it consists of a single layer of highly acidic material termed the cell plate. This is flanked, on either side, by cell membranes which are new plasmalemmata. Both the cell plate and the membranes extend at their edges by the accretion of new vesicles, the direction of the growth being determined by or reflected in the cytoskeleton. Eventually they fuse with the pre-existing wall and plasmalemma and the daughter cells become separated. There is evidence for the presence of acidic rhamnogalacturonan in the cell plate, but cellulose and most of the hemicellulose materials appear to be absent.

Before the cell plate has reached the pre-existing walls, a deposition of cell wall material begins at its centre, inward of the cell plate on each side, but external to the new cell membranes. This material is primary cell wall and contains pectins, hemicellulose and cellulose, in addition to glycoproteins and minerals. The primary cell wall, like the cell plate before it, grows outward to fuse with the walls of the parent cell. At this stage, the cell wall shows both elasticity and plasticity, being capable of extension and remodelling by the living tissue. It remains capable of structural and metabolic change and is dynamic. Its polysaccharides can undergo a variety of structural changes which can alter their physical properties and so enables the cell within, which controls this, to adapt and show differentiation of function. Locally, the membranes of adjacent cells may remain in contact, usually via desmosomes or hemi-desmosomes and at these points the wall is discontinuous and a distinctive polysaccharide, callose (a β-1, 3 glucan), is laid down around, but not across, the desmosome pits. It can also be deposited as a wound response. At this stage, a cell can undergo growth and extension and further cell division, or it may undergo secondary cell wall deposition, which is associated with its eventual differentiation and death.

Secondary cell wall deposition (or secondary thickening) involves the deposition of a new layer of cell wall internal to the primary wall. This new wall is generally much thicker than the primary wall and may be deposited in a spiral or reticulated pattern. It is rich in cellulose and contains some xylan, but the deposition of pectin eventually ceases. Cell wall plasticity is lost, but some elasticity remains, though the wall becomes more rigid. The remodelling of the wall draws to an end and finally the deposition of lignin begins, usually at the corners of the cells. This polymeric phenol forms by a free radical mechanism and may also be produced as a response to injury. It greatly reduces the permeability of the cell wall to water. In xylem, the cells die as their lateral walls lignify, while their end walls are degraded, so that vascular channels (xylem vessels) form. In phloem, sieve tubes form, with a little cytoplasm remaining within them in communication, via the plasmodesmata, with their companion cells. Thus, though the cells may be dead or moribund, their walls continue to serve functions in mechanical support and in providing the vascular systems of vascular plants.

5. PROPERTIES OF THE MOLECULESIN RELATION TO CELL WALLS

A. GENERAL

Cellulose is the major tensile element in plant cell walls. Cellulose fibres are longer than the individual molecules of cellulose and there are regions where the molecules overlap, which may be associated with their mannose content. The individual molecules are arranged in as antiparallel fashion, which maximises the opportunity for hydrogen bonding between them, so that they bind to each other rather than to water and form a strong, inextensible, non-slipping array. When water is further excluded by lignin, the effective strength of these hydrogen bonds is increased by about an order of magnitude so that wood is strong. In secondary wall the degree of polymerisation of the individual cellulose molecules is much greater than in the primary wall and the fibrils of cellulose are much more markedly aligned.

Xylan chains also tend to interact with each other and with cellulose by hydrogen bonds and they are conformationally similar to cellulose. However, xylans can contain appreciable amounts of arabinose, galactose, glucuronic acid and, rarely, other sugars and they show variability in this, for example, between angiosperms and gymnosperms, which can affect their properties by making their chains bend. In the *Rosaceae* the xylan chains are known to branch, but it is unclear how far this occurs in other orders.

The pectins are of particular importance in the maintenance of the integrity of plant tissues, because they are largely responsible for the adhesion *between* the cell walls, while cellulose and xylan are important for the cohesion *within*
the cell walls. In particular, the long acidic galacturonan segments of rhamnogalacturonans appear to be capable of strong association between adjacent molecules. Again, this is largely through hydrogen bonding between adjacent polyuronide sequences, though their pattern is somewhat different from those of cellulose, in that the usual configuration of the α-1, 4 galacturonan sequences in solution is not that which gives maximal hydrogen bonding, but, if stretched by about 20%, the chains can change conformation and maximise their inter-chain hydrogen bonding. The larger pectins that contain large branches of arabinose, galactose and other sugars appear to be less likely to associate, except where there are long, uninterrupted chains of non-esterified galacteronic acid, but they have a greater tendency to hydrate, with readily exchangeable water, at high relative humidity. Rhamnogalacturonans can show a different behaviour with water, in that they often take up 'structural' (ie almost non-exchangeable) water with high efficiency even at low relative humidities. The behaviour of pectins is further complicated by the sensitivity of their physical chemistry to their degree of methyl esterification and its pattern. What is clear, is that the distribution of water within plant cell walls will be highly sensitive to relative humidity, with the pectins probably accounting for a greater proportion of the water regain than their dry mass fraction within the wall might seem to imply and that they may well retain a very high proportion of the structural water within the wall even at low relative humidity (eg in dried herbarium specimens).

B. HYSTERETIC EFFECTS AND WATER REGAIN

If samples of plant tissues, their walls or the molecules that compose them are exhaustively dried, for example over calcium hydride, and are then allowed to equilibrate with atmospheres of known, defined relative humidity, at given temperatures, and are weighed, graphs of water regain against relative humidity can be plotted. Similarly, starting samples equilibrated with 100% relative humidity (ie saturation) can then be

equilibrated at lower relative humidities and similar graphs derived. For cellulose, the two curves thus obtained are close to each other and resemble similar curves for chitin and several other polysaccharides. The separation of the curves (hysteresis) is modest, but is still a little more pronounced than for most proteins. The water regain at high relative humidity is also modest, suggesting that exchangeable water is not a particularly marked feature of highly hydrated cellulose and that structural water is not present in large quantities. With the pectins, the picture is very different. Native homogalacturonan, especially if of low methyl ester content, shows marked hysteresis and a large water regain at high relative humidity. This implies that structural water is a notable feature of homogalacturonan, while both types of pectin, and especially those rich in arabinogalactan, can take up considerable amounts of exchangeable water. This tendency for water to associate with pectic polysaccharides is also reflected in their large 'partial specific volumes', which are measures of the amount of space occupied by a hydrated molecule when in aqueous solution. Carbohydrates in general show larger partial specific volumes than proteins and the pectins have some of the largest values of all. They also often show highly non-ideal behaviour in solution, for example in studies of their viscosity, which reflect interactions between and/or within molecules of the pectins which alter greatly with their dilution.

C. DEHYDRATION AND REHYDRATION

The long-term drying of plant material, as in herbarium specimens, leads to the 'curing' of cell wall polysaccharides (see above) and their becoming very insoluble and difficult to rehydrate. Elasticity is lost and the tissue becomes brittle. Rehydration is often possible if it is performed very slowly, through graded alcohol or glycerol solutions or by slowly raising the relative humidity around the specimen, but care must be taken to minimise differential swelling and attendant mechanical stress and to avoid the risk of bacterial or fungal growth as rehydration occurs. Thick specimens are particularly difficult to rehydrate, since the process will begin at the surface and may well proceed some way before water penetrates into the deeper layers, hence increasing the risk of mechanical failure. It should also be remembered that dehydration can similarly cause stresses and mechanical failure and that this may only be apparent when rehydration occurs in some specimens. Such failures are usually visible in larger dried specimens, especially in wood, particularly along natural cleavage planes such as medullary rays. Fracturing of this type in seasoned timber is termed 'shaking' and is a common feature of old timbers in buildings.

In general, pressed and dried herbarium specimens are sufficiently thin to rehydrate quite well, but it is seldom desired to rehydrate them. However, 'potted' specimens, kept under preservative fluids, may be much thicker and are likely to present problems if they leak and dry out. Mechanical failure may well have occurred before their condition is seen, especially if they are in closely packed, stored, reference collections that are only inspected occasionally and are kept – for good reason – in the dark. The restoration of such specimens can prove difficult and merely replacing the preservative fluid, without considering how to rehydrate with the minimal induction of stress, may be unwise and harmful. In some cases, where some damage has already occurred, it may be better either to dry the specimen completely or to infiltrate it with a different type of preservative. Even where a specimen has obviously fractured, the individual cells may remain well preserved and most of the cell-cell adhesion may remain except along the fracture planes. The proteins and nucleic acids of the tissue may be well preserved and some of the more robust enzyme activities may still be detectable. Such specimens should not be discarded without careful thought.

6. FIXATION AND FLUID PRESERVATION

Plant tissues are rather less likely to be subjected to fixation than soft animal tissues, partly because they retain their form relatively well on dehydration. More often, they are subjected to pseudo-fixation by dehydration in alcohol or alcohol-based fluids. However, some will have been fixed by true fixatives such as formalin or glutaraldehyde and the chemical mechanisms of action of these will not be materially different between plant and animal tissues. Proteins will have been cross-linked, primarily, but not exclusively, by way of amino- and guanido- groups of lysine and arginine respectively and most of these cross-links will be stable. A few will slowly reverse over time, if the specimen is not then kept in a preservative fluid containing a little aldehyde. As in animal tissues, true fixatives will cross link proteins so restricting molecular motions and leading to some degree of shrinkage. In plants, this is usually less marked than in animal tissues, because of the higher density of protein in most extracellular matrices of animals and the largely non-reactive structural 'skeleton' of carbohydrate in plant cell walls. Nevertheless, fixation may generate stresses in plant tissues, causing failure, and may impede attempts at rehydration in fixed specimens that have subsequently dried out.

In botanical collections, the most common preservative fluids tend to be alcohol-based, including alcohol itself, and fluids containing aldehydes, phenols or arsenites are unusual. Some plant specimens, if in fluids with a high water content, may tend to swell over time and their surfaces may become friable or may split. If alcohol is present, plant lipids may be leached out, especially if some of them are surface-active, such as saponins. Pigments, such as chlorophylls, phenolics and carotenoids, can also leach and the chlorophylls may lose magnesium and decolourise. If the fluid can oxidise and become acidic the risk of demetallising tetrapyrroles, such as chlorophylls, is increased and if transition metals, such as ferric iron, leach into the fluid or arise exogenously from mounts or caps of jars, for example, they may react with phenols in the tissue to produce dark staining.

7. ALGAL AND FUNGAL TISSUES

With the exception of algae of the *Characeae,* such as *Chara* and *Nitella* spp., algal walls have a different composition from those of the higher plants. Though they usually contain cellulose (a few contain chitin, below)they generally lack xylans and pectins, but possess other unrelated polysaccharides which, in some cases, are sulphated. They form a very diverse group, but share a common capacity for great hydration and, physiologically, normally exist at close to 100% saturation with water. On drying, some may 'cure' severely, but many algae can be dried and rehydrated very successfully. Heating, however, can lead some algal walls to a form of irreversible denaturation in which they show some shrinkage and become more leathery in texture. Overall, there is insufficient chemical information, except for those species of importance to the foodstuffs industry, and too much diversity in algal cell walls to generalise very far. This is a field in which more analysis would be highly desirable. The same is true of the higher fungi, where the analytical information on the composition and structure of their cell walls is seriously limited.

Fungal collections, for reasons largely of tradition, are usually held in the botanical sections of natural history collections. Like plants, the larger fungi have appearances which are largely determined or reflected by their cell walls, but the resemblance does not go far beyond this. Fungal walls have more in common, chemically, with animal cell surfaces than with plant cell walls and, even so, they are very much in a category of their own.

While some lower fungi (eg.chytrids and the *Hyphochitridiomycetes*) have cellulose in their walls, most often have chitin instead: this is a polymer of 2 deoxy, 2 acetamido-D-glucose (N-acetyglucosamine) which is largely analogous to cellulose. A few fungi appear to contain both, but the higher hyphal fungi have chitin. This forms a distinct layer in their walls adjacent to the cell membrane. Outside this is a thin layer of protein and, outside that, a much thicker layer of glucan and mannan, the latter being a highly elaborated form of the 'high-mannose' glycoprotein glycans found in all eukaryotes. In some yeast, such as *Saccharomyces cevevisiae* chitin is less abundant, a thick layer of glucan adjoins the membrane and a layer of mannan lies outside the glucan, but slightly interpenetrates it. In other pleomorphic yeasts, such as *Candida albicans*, hyphal and yeast forms are only marginally different from each other. Thus fungal walls differ with the growth form and species of the fungus and are very unlike plant cell walls. Many show other saccharides attached to the mannan and in some hyphal fungi the septa are chemically different from the lateral walls. Again, chemical information on the species found in natural history collections is seriously inadequate.

Fungal specimens are usually either dried or preserved in alcohol. Dried specimens generally show less shrinkage than in plant tissues, though a few, such as *Daldinia concentrica* are inclined to split along cleavage planes. The layers of the wall generally do not separate. Alcohol preservation generally works well, though extraction of pigment sometimes occurs.