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REVIEW

THE ORGANIZATION AND GROWTH OF PRIMARY CELL WALLS OF LUPIN HYPOCOTYL

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Abstract—Models of the primary cell wall are discussed in relation to results obtained by the present authors from studies of the primary cell wall of lupin and mung bean hypocotyls. A structure of the primary cell wall is suggested that differs in several respects from structures already proposed. It has a non-covalent interaction of much of the pectin, hemicellulose and glycoprotein, and a more direct interaction of the glycoprotein and cellulose microfibrils. An idea of scale is introduced through a consideration of degree of polymerization and monomer size of polymers, and of the volume of the cellulose microfibrils. Wall structure is discussed in relation to polymer orientation and elongation of the primary cell wall.

INTRODUCTION

Without the constraint of the cell wall the plant protoplast would be spherical [1]. The importance of the cell wall in determining the expansion and final shape of the plant cell is therefore fundamental, and yet we know little of the structure and molecular mechanism underlying this function. The gross structure of the wall has been known for some time because of the relative ease with which the cellulose microfibrils can be detected, and the relationship between microfibril arrangement and cell growth has been established [2]. Growth occurs at right angles to the predominant direction of cellulose microfibril orientation, and is accompanied by reorientation of the microfibrils [2]. However, the ease with which the inextensible microfibrils can move within the cell wall matrix determines the rate of cell enlargement.

Some authors have already proposed structures for the matrix and have constructed cell wall models [2–6]. The most recent and comprehensive model, that of Albersheim and co-workers [6] will be considered in relation to our results, published previously [7–11]. Other models will not be considered in as much detail, as they are in essence embodied in that of Albersheim *et al.* [6]. Some chemical and biophysical information which must be included in a model of the primary cell wall of lupin hypocotyl will be presented.

MODELS OF THE PLANT PRIMARY CELL WALL

Until recently cell wall models have been concerned only with the organization of the carbohydrate components of the wall. But since the discovery of the arabinosylhydroxyproline-rich glycoprotein extensin in the primary wall [12], and its negative correlation with growth [13], the matrix has become regarded as an extensin–polysaccharide complex.

Two structures of this extensin–polysaccharide complex have been proposed; that of Lamport [5] and that of Albersheim *et al.* [6]. In the first extensin is linked to cellulose microfibrils by an arabinosylhydroxyproline-linked galactan. Discovery of the galactosylserine linkage of cellulose to extensin together with other evidence [14, 15] lead to the model of Albersheim which is outlined in Fig. 1. In this case arabinose oligosaccharide side chains of hydroxyproline are free and the main linkage of the extensin (E) to the wall is through the series of polymers galactan (D) → rhamnogalacturonan (C) → arabinan or galactan (B) → xyloglucan (A), with the xyloglucan being attached to the microfibrils (m) by hydrogen bonds [6]. These hydrogen bonds are the point of movement of matrix and microfibrils and it was initially suggested that these bonds are influenced by pH, itself controlled by an auxin sensitive hydrogen ion pump in the plasmalemma [16].

Another aspect of the Albersheim model that the cell wall matrix polymers are *all* glycosidically linked. Total matrix is therefore regarded as a macromolecular glycoprotein. The only proposed linkage of this macromolecule with the cellulose microfibrils is through the hydrogen bonds of the xyloglucan. On the basis of subsequent work it has been suggested that this model is generally applicable to dicotyledon primary cell walls [17], although the effect of pH in allowing creep of xyloglucan along microfibrils has since been questioned [18]. Changes in xyloglucan metabolism may however reflect the means by which auxin modifies the cell wall to cause elongation [19].

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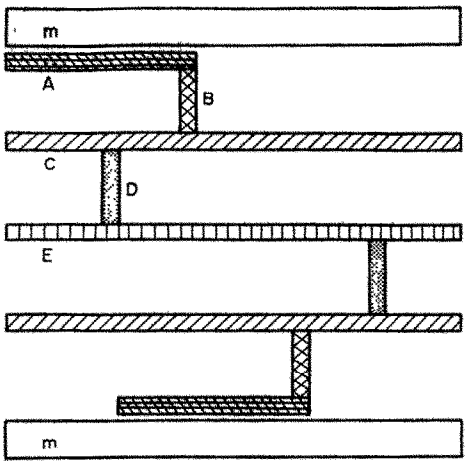

Fig. 1. Polymer sequence in Albersheim model of the primary cell wall of Sycamore. A = xyloglucan; B = arabinan and 4-linked galactan; C = rhamnogalacturonan; D = arabinogalactan; E = extensin; m = cellulose microfibril.

RESULTS FROM LUPIN AND MUNG BEAN HYPOCOTYLS IN RELATION TO PROPOSED MODELS

In our work on the extraction of polymers from lupin and mung bean hypocotyls we have obtained several results which suggest that the Albersheim model does not represent the structure of the primary wall of intact lupin [7–11] and mung bean [9] hypocotyls. These results have been published in detail elsewhere and are summarized here, with reference to Fig. 1.

(1) Extraction of the polyuronide from lupin and mung bean hypocotyl wall under conditions which remove and degrade polyuronide [20] extracts little of the wall protein [7,9], as predicted by the model of Albersheim [6]. In Fig. 1 removal of C should release D and E. Although in one experiment [9] 60% of the ^{14}C-proline incorporated for 4 hr into mung bean hypocotyl walls was removed the total unlabelled extractions were much lower, in the order of 10% [21]. It is probable that recently synthesised extensin involved in the incorporation experiments is more easily extracted from the wall than that bound in for longer periods.

(2) 10% KOH at 20–24° will remove hemicellulose without extracting polyuronide [9]. Extractions of hemicellulose (A) (including xyloglucan) should be accompanied by release of both extensin (E) and polyuronide (C), according to the model. Polyuronide is therefore not a component of a polymer bridge between xyloglucan and extensin.

(3) 6M Guanidinium thiocyanate (GTC), which is a powerful chaotropic agent [22], will remove about a third of the 10% KOH soluble hemicellulose (including xylan) from depectinated lupin hypocotyl [11]. If the only linkage between hemicellulose (A) and the microfibrils (m) is via hydrogen bonding of xyloglucan (A), then compounds such as 8M urea or 6M GTC should extract most of the hemicellulose and protein.

(4) Even after prolonged (18 hr, 20°) 6M GTC extraction a further fraction of the wall is extracted by 10% KOH at 0° [11]. Release of this further fraction may require rupture of very alkali labile covalent bonds. The GTC insoluble material includes most of the hemicellulose-A which is however a minor fraction. This latter polymer is over 70% xylose and only 6% glucose and is therefore not a xyloglucan of the type which is claimed to contain all the hemicellulose xylose and to bind the hemicellulose to the cellulose microfibrils. Some 60% of the wall hemicellulose (including that extracted by GTC) may be removed with 10% KOH at 0° without extracting the wall protein.

(5) Extraction of hemicellulose with 10% KOH at 0° does not cause any change in amino acid composition of the walls [11] such as would occur during β-elimination. It is noteworthy that there is no loss of serine although this does occur at room temperature with 10% KOH. Alkaline β-elimination of galactosylserine links is therefore not necessary for extraction of the bulk of wall hemicellulose, which is therefore not dependent on links to the serine for its covalent association with the wall.

(6) 10% KOH at 18–22° releases most of the hemicellulose not soluble at 0°, along with most of the wall protein (as measured by hydroxyproline extraction) [10]. The time course of extraction shows that material initially extracted at room temperature is different to that extracted after 4 hr [10,11]. The latter polymer includes the "1–4 linked hemicellulose-B" fraction of which arabinose is 86% of its monosaccharides [11]. Because arabans with 1–4 linkages have not been reported in plants it is suggested that at this stage extensin and its associated hydroxyproline-linked arabinose oligosaccharides are being released. The galactose level at this point is low, most galactan of the 18–22° 10% KOH soluble polymer having already been extracted. It is therefore unlikely that the arabinose in question is part of a serine-linked arabinogalactan, but rather, is part of the protein of which most is extracted after the bulk of the galactan. A linkage of the protein in addition to that involving the galactan extracted at 18–22° is therefore suggested.

(7) Extraction of the wall with 10% KOH is unable to completely remove either the non-glucose polysaccharides or the protein from the wall. There appears to be a strong association of protein and cellulose microfibrils, and the low level of serine in the alkali resistant protein [11] suggests that galactosylserine linkages are not involved. Certainly a more direct association than through arabinogalactan, polyuronide, polyuronide side chains, and xyloglucan is apparent.

(8) If polymer creep is involved in control of cell elongation then bonds controlling the creep should be at right angles to the direction of elongation. In the Albersheim model and in the similar model of Davies [23] the xyloglucan chains creep along the microfibrils. As the microfibrils are mainly transverse, these models will apply mainly to cell expansion, not elongation, and would predict a relatively larger auxin or low pH stimulation on radial rather than longitudinal expansion. Such is not observed and in fact low pH gave a decrease in radius [24].

MODEL OF LUPIN HYPOCOTYL CELL WALL

The above data suggests that the following general features should be part of any model of the primary cell wall of lupin hypocotyl.

(1) A polyuronide bridge is not involved in binding of extensin into the wall.

(2) A polyuronide bridge is not necessary to the binding of hemicellulose to the wall, and a hemicellulose

bridge is not necessary to the binding of polyuronide to the wall.

(3) Much of the hemicellulose is not covalently bound to the wall or to extensin through galactosylserine links.

(4) A large proportion of the hemicellulose is bound into the wall by very alkali-labile covalent bonds. Weak ester links involving uronic acid residues are a possibility.

(5) Galactosylserine linkages are not necessary for the binding of the hemicellulose mentioned in (4) to the wall.

(6) Much of the wall hemicellulose is bound into the wall by more alkali stable covalent bonds than those broken by 10% KOH at 0°. These are ruptured by 10% KOH at 18–22°. Such hemicellulose polysaccharide may be involved as a covalent bridge between extensin and the microfibrils but represents no more than about 30% of the total hemicellulose.

(7) A fraction of the wall protein and hemicellulose is strongly bonded to cellulose microfibrils. Extensin is covalently bonded to microfibrils or to other polysaccharide itself covalently bonded to microfibrils, or else the covalent bonds of the polymers are so arranged that disentanglement of the polymer complex and microfibrils can occur only if covalent bonds are broken.

(8) Cellulose microfibrils are at right angles to the direction of elongation. Bonds involved in the polymer creep allowing microfibril separation are at right angles to the direction of cell elongation.

A partial model of the primary cell wall of lupin hypocotyl, based on the above points, is given in Fig. 2.

The present work with hypocotyls does suggest that certain sequences of polymers do not occur to a major degree in the wall, but does not actually indicate the position of the bonds that are present. Consequently, the proposed wall structure shown in Fig. 2 depicts the type of networks which might occur. It shows covalent extensin–polysaccharide association (A) with unspecified bonding to the microfibrils, and the pectin or hemicellulose network (B) not involving extensin, but interconnected at either GTC-labile or 0°, 10% KOH-labile junctions.

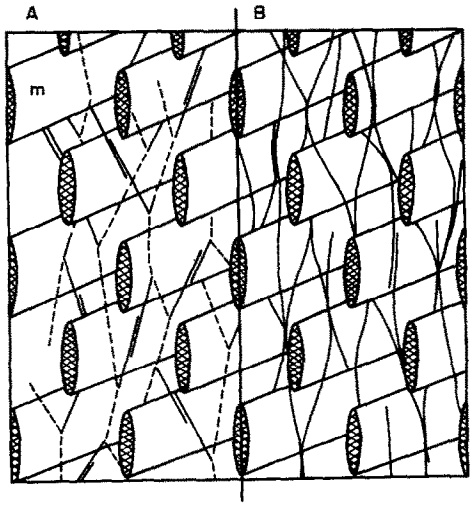

Fig. 2. Partial model of primary cell wall of lupin hypocotyl. A = extensin-polysaccharide network; B = pectin network (A and B drawn separately for convenience); m = cellulose microfibril; ----- = extensin; ——— = polysaccharide; ═══ = junction zone between polysaccharide chains.

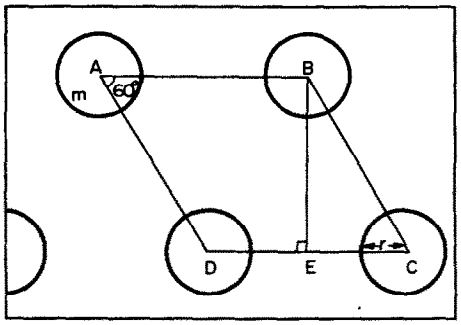

Fig. 3. Calculation of distance between cellulose microfibrils assuming equidistant spacing within wall, and area of one microfibril as 16% of the area ABCD (see text). m = microfibril; r = radius of microfibril (taken as 75 Å).

The suggested organization has been made more relevant to the *in vivo* situation by introducing scale through a consideration of microfibril size and separation, and polymer length, as discussed in the following section.

Microfibril separation and matrix polymer length

The wall is estimated by weight to be 60% water and 40% polymers [2]. The average density of the wall polymers is about 1.5 [25]. The ratio of polymer volume to water volume is therefore 26:60. Thus 30.7% of the wall volume is polymer. In lupin hypocotyl about 40% of the wall polymer is cellulose [7], and as cellulose in contact with water contains 16% water [2] the actual volume occupied by the microfibrils is 16% of the total volume of the wall.

It is necessary to know how far apart the microfibrils are in the cell wall to determine whether a given polymer can link adjacent microfibrils. It is to be expected that in practice some microfibrils will be virtually touching at some points whereas at other places they may be fairly distant from one another.

The greatest minimum distance between microfibrils is therefore the length of a side of the equilateral parallelogram made by joining the centre points of four adjacent microfibrils, minus the diameter of one microfibril (see Fig. 3). The area enclosed by four adjacent microfibrils is the area ABCD minus the area of one microfibril. If the area ABCD is 100 square units the area of the microfibril will be 16 square units.

$$\text{The area of ABCD} = DC \cdot BE$$
$$= DC \cdot 0.5\ DC \tan 60.$$

If the radius (r) of a microfibril is taken as 75Å [26] the cross sectional area of a microfibril, πr^2, which is 16% of the total area ABCD is $16 \times 10^{-2}\ 0.05\ DC^2 \tan 60$ and DC = 358 Å. The greatest minimum distance between microfibrils is therefore

$$358 - 2\,(75) = 208\ \text{Å},$$

and this will also be the average separation.

In practice some microfibrils will be closer than this as the calculation is based on the assumption that polymers are distributed evenly through the cell wall. However the polymers do not have an even distribution, the pectic substances for instance being concentrated in the middle lamella [27]. In many cell walls the microfibrils appear to be arranged in lamellae separated by matrix polymers [2, 28].

The capacity of wall matrix polymers to crosslink or enmesh the microfibrils will depend partly on the length

of the polymers. Pectins are over 500 Å in length [29] and would therefore easily stretch between two microfibrils. Moreover several different pectin chains are capable of forming a network by joining through non-covalent bonds at regions of association termed junction zones [30]. Such a network has recently been demonstrated under the electron microscope [31].

Xylans generally have a degree of polymerization of 150–200 [32]. If the xylobiose unit has similar dimensions to the cellobiose unit (10.2 Å in length) a conservative figure for xylan length would be 500 Å. Xylans have also been shown to be capable of forming junction zones [33].

Estimates of the MW of extensin, based on the size of presumed extensin precursors vary between 35000 [34] and 11000 [5]. The average MW of the amino acids of extensin is about 114, giving a degree of polymerization of 307 in the case of MW 35000, and 97 in the case of MW 11000. Assuming that extensin has a polyproline configuration (as found in collagen) the molecule will increase in length by 3.12 Å per residue [35]. Chain lengths based on the above MWs will therefore be 950 and 303 Å respectively.

Although the above calculations are not based on data derived from lupin hypocotyl cell walls the order of polymer size for this tissue is apparent and it can be appreciated that a very extensive extensin–polysaccharide complex can exist. A series linkage of xylan, pectin, galactan and protein would be capable of stretching over several microfibrils at an average spacing in the wall.

THE CELL WALL UNDER STRESS

Any model relating to the elongating cell wall must take into account large forces responsible for cell extension. The hydrostatic (turgor) pressure (P) of the cell causes a considerable stress on the wall and is the driving force necessary for cell expansion. Stresses on the cell wall can be calculated from a knowledge of pressure in the cell (P), cell radius (r) and cell wall thickness (t), by the following formulae [36].

Tangential stress $\delta t = Pr/t$

and Longitudinal stress $\delta l = Pr/2t$.

Although P is not accurately known it can be estimated from the freezing point depression of the cell contents. A freezing point depression method gives the vacuolar osmotic potential as equivalent to a 0.31 M solution of mannitol. The water potential of the hypocotyl would be just less than zero, and a good estimate of the turgor pressure would be about 6.5 bars [37]. The stress on the wall of a cylindrical cell due to this pressure can be calculated [36] from a knowledge of wall thickness and cell radius.

In the lupin hypocotyl the cell walls which appear to be most important in limiting extension are those of the epidermis and the two to three outer files of cortical cells [38], and in these the ratio of cell radius (r) to wall thickness (t) is 20:1. Approximate equilibrium values for longitudinal stress and tangential stress would therefore be 65 and 130 bars respectively.

In growing cell walls the cellulose microfibrils are oriented mainly in the transverse direction and are thought to resist most of the tangential stress, so that elongation occurs along the cell axis. The cellulose of the lupin hypocotyl has been calculated in this paper to be about 16% of the dry wall volume so that the tangential stress on the microfibrils will be in the order of 1000 bars. Although these forces are high they are not excessive with respect to the calculated properties of the cellulose microfibril. Values for the elastic modulus and breaking strain in the direction of the microfibril are given by Mark [25] and are 1.37×10^4 kg/mm^2 and 753–770 kg/mm^2 respectively. The 1000 bar (10 kg/mm^2) stress on the cellulose would therefore give an elastic extension of less than 0.1% and is only a few percent of the stress required to break the cellulose microfibril.

Although there are fewer microfibrils in the longitudinal than in the transverse direction the orientations are approximate so that the transverse microfibrils will take some longitudinal stress. Longitudinal microfibrils could be important in controlling growth of the intact tissue as bands of such microfibrils have been observed in the epidermis of *Avena* coleoptile [39] and lettuce hypocotyl [40]. There is some evidence that the epidermal layer of a tissue strongly influences its elongation response to auxin [41, 42]. However, peeled sections have been shown [41] to give the usual elongation response to auxin. Cortical cells with their predominantly circumferential arrangement of microfibrils, at least in lettuce hypocotyl [40], provide the main constraint to radial expansion. The main resistance to longitudinal stress may however come from those matrix polymers which can cross-link between the microfibrils in such a way that they resist stress and still allow cell extension. Creep along microfibrils as envisaged by Albersheim *et al.* [6] could apply to the longitudinal microfibrils in the epidermal cell wall but not to the normal expansion of most of the cells, where the microfibril orientation is mainly transverse, and there is little change in cell diameter [23].

Until the microfibrils in Fig. 1 have assumed an angle to the transverse place there will be no shearing of the bonds purported to be involved in creep. A shear component parallel to the direction of creep will then be involved. In the model proposed in this paper the hydrogen bonded regions are in the matrix, and the polymers can undergo shearing of the bonds independent of microfibril orientation, even when the microfibrils are in a plane perpendicular to the axis of elongation (Fig. 4).

Strong forces encountered in the cell wall put many polymers under tension, and this must be taken into account in constructing models of the wall. The model of Albersheim *et al.* [6] may refer to longitudinal microfibrils of cells at zero turgor pressure but is not likely to hold for elongating cells under tension.

The orientation of cellulose microfibrils has been widely studied [2], but little is known of the orientation of matrix polymers in higher plants, a question which is clearly very important to any consideration of their role in control of elongation. Visualization of pectin with heavy metal stains [31] raises the possibility of employing such methods on preparations of whole cell wall.

The molecular mechanism behind auxin induced wall elongation has yet to be clarified. There are uncertainties in wall structure and in the meaning of rheological measurements on the wall [43]. Lowering of the minimum yield stress [44] or increased extensibility [42] could be involved. There is also a lack of evidence for the nature of agents responsible for wall loosening. Albersheim *et al.* [6] considered cleavage and reformation of non-covalent bonds to be the basic mechanism of cell elongation, while earlier theories were more con-

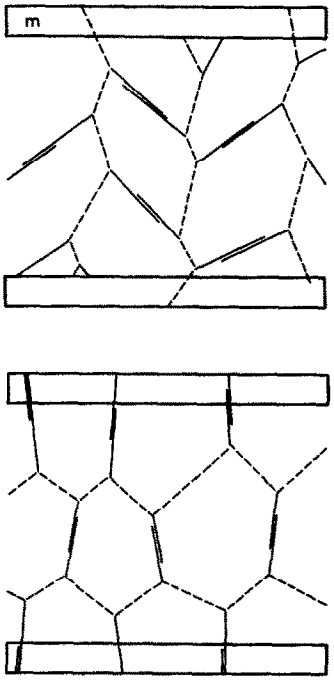

Fig. 4. Arrangement of junction zones (possible regions of creep) of matrix polysaccharides where extensin is either perpendicular (A) or parallel (B) to the cellulose microfibrils. m = microfibril; ----- = extensin; ═══ = junction zone between polysaccharides.

cerned with hydrolytic enzyme activity [45]. Rapid responses to auxin are too rapid to involve synthesis of carbohydrases [43] although activation of enzymes cannot be ruled out [46]. Auxin induced lowering of pH could activate hydrolytic enzymes such as wall glycosidases [47, 48] although the aldonolactone inhibitors of glycosidase activity did not inhibit growth of lupin hypocotyls [49]. In addition, normal auxin induced growth of lupin hypocotyls occurred in the absence of any detectable drop in pH, and had different characteristics to that induced by low pH [50]. The activity of wall carbohydrases alone is insufficient to cause cell elongation at constant turgor pressure [51]. It is important now that the site and *in vivo* action of the wall hydrolases be identified. Cleavage of extensin does not appear to be involved as there is no detectable protease activity in wall preparations or in digests of walls prepared with protease-free cellulase and pectinase [20].

A role for extensin in wall biosynthesis has been suggested [52] on the basis of an auxin stimulated incorporation of a hydroxyproline rich fraction into *Avena* coleoptile cell walls in the presence of carbohydrate substrate. Auxin also causes an increase in addition of wall polysaccharide by intussusception [53]. This implies that if the wall or that part of it involving extensin is an extended polymer, covalent bonds must be capable of forming in the free space of the wall, that is, probably away from the polymer activating processes. The dimensions of polymers and the separation of cellulose microfibrils considered earlier in this paper suggest that if all primary wall polymers are glycosidically linked, for intussusception to occur, polymer units must link covalently in the wall matrix. Given that intussusception occurs, and that ATP driven polymer activation does not occur in the wall, it is difficult to see the matrix being a macromolecule rather than a mass of separate units.

The idea of bond formation throughout the wall is however attractive as it enables the covalent bonding of the whole wall at any one time to be under metabolic control and to play a direct part in changes in wall properties.

Recent work has shown that the glycosylation of hydroxyproline with arabinose-oligosaccharides occurs within the cytoplasm [54]. There is now evidence [55, 56] that hydroxyproline is linked to polysaccharides in some plants, probably by a similar system to that involved in attachment of the arabinose oligosaccharides. Formation of galactosylerine linkages has been suggested to occur in the wall [34]. However, little is known of the fabrication of an extensin–polysaccharide complex from polymers. Should it be possible to follow the growth of such a complex, a great deal will be learnt of the structure of the cell wall.

Albersheim *et al.* [6] have made an unparalleled contribution to our understanding of polymer structure and bondings in the primary plant wall but their model does not appear to accommodate bonds of the labilities encountered in our studies, and which should be fitted into a more comprehensive model than proposed in the present paper or previously. The alkali fractionation studies of the present authors, while providing less information, do suggest that linkages other than glycosidic bonds are involved in the cohesion of matrix polymers, and that certain polymers are not glycosidically interconnected. An accurate cell wall model must eventually take into account stresses on the cell wall, orientation of wall components, detailed structure of wall polymers and the nature of bonds between components. Our knowledge of primary cell wall structure is at the moment too inadequate to allow the models proposed now to be other than working hypotheses.

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