Cellulases, hemicelluloses and auxin-stimulated growth: a possible relationship

Stephen C. Fry

The plant growth promoter, auxin, may loosen the primary cell wall by increasing the activity of extracellular cellulases—a group of enzymes that cleave hemicellulose chains in the walls of both monocotyledons and dicotyledons. Evidence is reviewed that suggests that these hemicellulose chains tether adjacent microfibrils, and that by cleaving such chains the cellulases facilitate cell expansion. On the basis of this structural arrangement a mechanism for elastic and plastic wall extension is proposed.

Key words—auxin, cellulase, hemicellulose.

S. C. Fry, Dept of Botany, Univ. of Edinburgh, The King’s Buildings, Mayfield Road, Edinburgh, EH9 3JH, UK.

Introduction

Auxin promotes the growth of stems and coleoptiles by loosening epidermal cell walls, making them more susceptible to turgor-driven extension (Cleland 1981, Kutschera 1987). The changes in wall chemistry that underlie loosening are controversial. One problem is that auxin has remarkably similar loosening effects on grass cell walls and on walls of dicotyledons despite the fact that these walls differ markedly in chemical composition (Fry 1988). However, it seems unlikely that grasses and dicotyledons should have evolved fundamentally different mechanisms of auxin action. Here I suggest that the principal effect of auxin in all land plants is an enhancement of cellulase activity and that wall-loosening comes about by the cellulase-catalysed cleavage of load-bearing hemicellulose molecules. I also propose a molecular arrangement of hemicellulose in the cell wall which would put these molecules in a position to limit growth.

Abbreviations—CMC, carboxymethylcellulose; Glcp, glucopyrauose; grasses, the Gramineae including cereals; MLG, mixed-linkage β-(1→3),(1→4)-glucan; XG, xylloglucan.

Auxin treatment increases cellulase activity

Auxin induces the de novo synthesis of cellulase in dicotyledons (Verma et al. 1975). This effect has not been observed in grasses, although grass cell walls do contain a new cellulase of unusual specificity (Hatfield and Nevins 1986); the effect of auxin on levels of this novel protein has not yet been reported.

Cellulases have low pH optima (Verma et al. 1975, Hatfield and Nevins 1986) and so cellulases already present in the wall will be activated by the H⁺ ions which are released into the walls of plant cells treated with auxins (Cleland, 1981). The occasional failures to correlate auxin action with bulk H⁺ secretion (e.g. Kutschera and Schopfer 1985) may be related to the fact that epidermal walls are difficult to sample or perturb owing to the presence of the cuticle. Auxin may, in all responsive tissues, increase the cellulolytic activity of the wall whether or not it induces cellulase synthesis.

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Cellulases more readily attack hemicelluloses than cellulose

A cellulase is an enzyme that hydrolyses the glycosidic linkage \( \beta-(1 \rightarrow 4) \)-\( \beta \)-glucopyranosyl... occurring mid-chain in a polysaccharide:

\[
\cdots \text{d-Glc} \cdots \text{d-Glc} \cdots \text{d-Glc} \cdots \text{d-Glc} \cdots \text{d-Glc} \cdots \text{d-Glc} \cdots \text{d-Glc} \cdots \text{d-Glc} \cdots
\]

Its systematic name is \( \beta-(1 \rightarrow 4) \)-glucan 4-glucanohydrolase (EC 3.2.1.4). Most cellulases are easily assayed by their ability to degrade the viscosity of the soluble model substrate, carboxymethylcellulose (CMC).

Although cellulose is the major wall polysaccharide that contains \( \alpha \)-Glc-\( \beta-(1 \rightarrow 4) \)-\( \alpha \)-Glc-linkages, the principal target of cellulase in healthy plants is, paradoxically, not cellulose. Treatment of isolated cell walls with pure cellulase in vitro leads to negligible erosion of the cellulose (Hayashi and Maclachlan 1984). Most of the cellulose in a plant cell wall is organised into semi-crystalline bundles, microfibrils, which are relatively inaccessible to enzymes.

Primary cell walls contain polysaccharides with recurrent \( \alpha \)-Glc-\( \beta-(1 \rightarrow 4) \)-\( \alpha \)-Glc-linkages in addition to cellulose, viz. the hemicelluloses xyloglucan (XG) and mixed-linkage \( \beta-(1 \rightarrow 3),(1 \rightarrow 4) \)-glucan (MLG). XG makes up about 20% of the primary wall of dicotyledons and occurs in smaller amounts in grass walls. MLG is a major component of certain grass walls, especially in tissues capable of auxin-induced growth (Carpita 1984), but probably does not occur in dicotyledons (Fry 1988).

XG (Fig. 1a–d) has a cellulose-like backbone, but differs from cellulose in that 60–75% of the glucose residues carry \( \alpha \)-xylose and other short side chains (Fry 1988, 1989a). Cellulases can chop the backbone of XG at any non-xylosylated glucose residue. Since hemicelluloses are less crystalline than cellulose, and are thought to coat the cellulose microfibrils, cellulases are better and quicker able to attack the former (Hayashi and Maclachlan 1984).

MLG is an unbranched chain composed entirely of glucose residues, of which about 70% are \( (1 \rightarrow 4) \)-linked and 30% \( (1 \rightarrow 3) \)-linked. MLG consists mainly of the repeating units \((1 \rightarrow 3),(1 \rightarrow 4),(1 \rightarrow 4) \)- and \((1 \rightarrow 3),(1 \rightarrow 4),(1 \rightarrow 4),(1 \rightarrow 4) \)-. These units can be hydrolysed by plant cellulase (Wong et al. 1977). MLG also contains rarer, cellulose-like units such as \((1 \rightarrow 3),(1 \rightarrow 4),(1 \rightarrow 4),(1 \rightarrow 4),(1 \rightarrow 4),(1 \rightarrow 4),(1 \rightarrow 4),(1 \rightarrow 4) \)-. These ‘soluble cellulose’ units, which will be rigid molecular rods contrasting with the flexibility of the rest of the MLG molecule (Buliga et al. 1986), may occur regularly along the polysaccharide chain, about every 50–80 glucose residues (Hatfield and Nevis 1986). The novel cellulase recently discovered in grasses seems to have a particularly high activity on the cellulose-like domains of MLG, which it attacks at a higher rate even than CMC. MLG is thus very probably the natural substrate of this enzyme.

In conclusion, auxin induces and/or activates enzymes (‘cellulases’) that attack major hemicelluloses of both grasses and dicotyledons.

### Auxin induces xyloglucan-breakdown

The effect of auxin on cellulase activity is paralleled by effects on the breakdown of hemicelluloses in vivo. The best-established example is the induction of XG breakdown, which has been observed both in dicotyledons and in grasses (Fry 1989a). The effect can be seen as nicking (a small reduction in the molecular weight of a large proportion of the wall-bound XG), sloughing (liberation of a small proportion of formerly wall-bound XG into solution) and degradation (complete disappearance of a small proportion of XG from the plant, presumably by hydrolysis to monosaccharides). The finding in vivo of XG-oligosaccharides (Fry 1986b) also suggests breakdown of the polysaccharide.

Since the backbone of XG is a \( \beta-(1 \rightarrow 4) \)-glucan with no other sugar residues reported except as side chains, mid-chain cleavage must require the action of a cellulase. Thus, wherever we see XG degradation, a cellulase must be at work.

### Auxin induces MLG-breakdown

Auxin promotes the breakdown of MLG, presumably to free glucose (Sakurai and Masuda 1978). It seems that the initial, and rate-limiting, event is attack by an endo-hydrolase (Hatfield and Nevis 1986): this enzyme cuts the MLG molecule, thereby exposing a new non-reducing terminus that can then be rapidly attacked by an exo-hydrolase to liberate glucose. The endo-hydrolase may be the cellulase that attacks the cellulose-like \( \beta-(1 \rightarrow 4) \)-domains of the MLG molecule. Antibodies directed against either the exo-glucanase or the MLG-specific cellulase block the action of auxin on growth (Nevis et al. 1987).

The auxin-induced breakdown of MLG to the monosaccharide is probably only indirectly related to the action of auxin on wall-loosening. Glucose production may be a symptom of the more important, preceding step – endo-attack of MLG.

How could the endo-attack of either MLG or XG promote growth?

### Biophysics of growth

In stems and coleoptiles, the epidermis limits the expansion of the whole organ (Sachs 1882, Kutschera 1987). The internal tissues (pith, cortex, etc) become turgid by the osmotic uptake of water and tend to expand; the epidermis tends to resist this expansion by exerting a back-pressure. Growth (irreversible increase in volume) is the outcome of the two opposing forces: it requires

Physiol. Plant. 75, 1989

533
the plastic (irreversible) deformation of the epidermal wall. To some extent, the organ can be regarded as a 'cell' and the epidermis as its 'wall'.

When an organ has been fully turgid for a long time, the epidermis will have stretched almost as far as is possible under the prevailing turgor and will not undergo further expansion unless load-bearing bonds in the epidermal wall are broken (Cleland 1981). Auxin probably induces the breakage of such bonds.

What are the cleavable, load-bearing wall bonds? Microfibrils are neither degraded during growth nor are they extensible. Therefore, cell growth requires that existing wall microfibrils move apart (——→ ———) and/or past one another (——→ ———), rather than break (——→ = + =) or stretch (——→ ———). The cleavable, load-bearing bonds can thus only be the 'strings' that interconnect the microfibrils. Could these be hemicelluloses?

Modelling the position of hemicelluloses in wall architecture

To discuss how the cellulase-catalysed cleavage of hemicelluloses might loosen the wall, we need a reliable picture of how hemicelluloses are held in the cell wall. Unfortunately this is lacking. What is known is that the backbones of a proportion of the hemicellulose molecules are bound to the surfaces of microfibrils by numerous hydrogen-bonds (Hayashi et al. 1987, MacKay et al. 1988).

Individual hemicellulose molecules are long enough to hydrogen-bond to two or more microfibrils at the same time. In MLG the rigid, cellulose-like β-(1→4) \( \alpha \)-zones will act as semi-independent rods (length ca 5 nm) spaced at intervals (ca 30 nm) along the more flexible β-(1→3), (1→4)-linked MLG chain [total contour length ca 500 nm (calculated from Wada and Ray 1978)]. Since a β-(1→4) \( \alpha \)-rod is predicted to hydrogen-bond along the surface of a microfibril (diameter ca 4 nm) much more strongly than the intervening flexible parts of MLG, the MLG molecule will in effect be a set of tethered holdfasts. Each β-(1→4) \( \alpha \)-zone of a newly-secreted MLG molecule will seek out its own stretch of microfibril to which to bind. It is most improbable that all the β-(1→4) \( \alpha \)-zones along a single MLG molecule will 'choose' the same microfibril.

There has been some reluctance to accept MLG as a true structural component of the wall because it is a highly flexible molecule: it has been irreverently described as molecular putty! However, the recent discovery of its β-(1→4) \( \alpha \)-zones supports the idea of a (hydrogen-bonding) structural rôle.

Individual XG molecules (backbone contour length 150–1500 nm) can probably also hydrogen-bond to two or more microfibrils at the same time. New XG molecules, which are synthesised intracellularly, are presented to the accreting face of the cell wall by the vesicle-ful, in random orientation. Primary cell walls probably contain somewhat more XG than would be required to coat all the microfibrils with a monolayer (Hayashi et al. 1987): there will thus be competition for binding sites. A new XG molecule may often find one microfibril with one end of its backbone and a different one with its other end. There is some experimental evidence for this arrangement (Hayashi and Maclachlan 1984).

A factor tending to ensure that an XG molecule that initially binds two microfibrils continues to span them (rather than coming to lie along a single microfibril) is the presence, in the space between adjacent microfibrils, of pectin and extensin molecules. These polymers will, by physical entanglement, (a) minimise the...
Fig. 2. Postulated behaviour of hemicellulose molecules during cell expansion. You are inside the cell, looking out at what is initially the inner face of the cell wall, i.e. the wall is in the plane of the paper. Each picture shows a pair of neighbouring microfibrils (\ldots\ldots\ldots\ldots\ldots) and some (a small minority) of their associated hemicellulose chains (\_\_\_\_\_\_\_), which are hydrogen-bonded to microfibrils at junction zones (\__\_\__\_\__\_\__).

(a) Young, newly-deposited wall layer: hemicellulose chains slack; (b) Slightly older: some hemicellulose chains have been stretched taut by turgor-driven cell expansion; (c) Older; continued cell expansion is unzipping some junction zones; (d) Elasticity: if turgor is relieved at stage (c), junction zones re-form and the wall layer returns to state (b). Rubber elasticity of the hemicellulose may cause further reversion to state (a); (e) Creep: if turgor is maintained, junction zones cannot re-form and other, unattached hemicellulose chains take the place of the stretched chain, thereby rendering state (c) permanent; (f) Wall loosening: if turgor is maintained and the stress-bearing hemicellulose chains are chopped by cellulase (*), permanent microfibril separation (= wall growth) is enhanced.

Possible behaviour of hemicellulose during elastic and plastic extension

Consider the career of a particular hemicellulose molecule. It is synthesised intracellularly, packaged into a vesicle, and deposited at the innermost, newly-accreting face of the cell wall. Here it forms hydrogen-bonded junction zones, at intervals along its length, with two or more newly-synthesised, naked microfibrils (Fig. 2a). At this stage, the parts of the hemicellulose molecule that lie between the microfibrils are slack. With time, these parts are stretched taut by continued cell expansion and soon come to bear the burden of turgor (Fig. 2b). [A layer of wall is load-bearing mainly when it is about \(\frac{1}{6}\) to \(\frac{1}{4}\) of the way along its journey from the plasma membrane to the outermost (oldest) part of the wall (Richmond 1983).] Continued cell expansion now requires something to 'give'. As the proportion of the turgor borne by our hemicellulose molecule becomes unbearable, some of the hemicellulose/microfibril hydrogen bonds at the ends of each junction zone will be broken, the microfibrils will be able to move a little further apart (\_\_\_\_\_\_\_\_) and the cell will continue to expand (Fig. 2c). From here on, there are three options:

(1) If turgor is relieved soon afterwards (e.g. by plasmolysis), spontaneous re-formation of the hydrogen bonds will pull the microfibrils together again (Fig. 2d). Also, the tendency of MLG (and, less so, XG) molecules to adopt an entropically favourable coil rather than to be a taut rod will tend to pull adjacent microfibrils together. The wall extension in Fig. 2c will thus have been elastic (reversible).

(2) If turgor is maintained, some of the microfibril surface sites vacated by the stretched hemicellulose molecule will be occupied by zones of other hemicellulose molecules that had not yet found partners to which to hydrogen-bond (Fig. 2e). (Promotion of hemicellulose secretion might hasten this 'squatting' process and thereby promote growth. Other newly-secreted matrix polymers might act as wedges and also prevent the return of the stretched hemicellulose.) If turgor is then finally relieved, our hemicellulose molecule will find that it is unable to return to its original sites owing to the presence of other molecules. The wall extension has thus been rendered irreversible. This could be a molecular basis for viscoelastic creep, often observed in stretched cell walls.

(3) If the hemicellulose occupies the key structural position envisaged, it is easy to imagine that the cellulases that cleave it will promote plastic (irreversible)
extension (Fig. 2f), and that an auxin-induced increase in the amount or activity of cellulosases will increase the growth rate. In pea stem cell walls, cellulase has been localised to the inner 1/2 to 1/4 of the wall (Bal et al. 1976), the important portion with respect to the control of growth. Cellulases could achieve bond breakage with least loss of wall strength if they acted principally as transglycosylases (Fry 1988), rather than as simple hydrolases, so that a new bond was formed for every one broken. Since transglycosylation results in no change in molecular weight of the wall polysaccharides, it would not readily be detected. A major unanswered question in plant growth is whether wall hemicelluloscs are subjected to transglycosylation in vivo.

Relation to other control points in wall growth

The plant needs ways of both loosening and tightening its walls, since it has to be able to increase or decrease its growth rate to suit the environment. I have suggested elsewhere (Fry 1986a, 1988) that cell growth may be retarded, e.g. upon treatment with growth inhibitors, by the peroxidase-catalysed crosslinking of phenolic side chains on wall polysaccharides and glycoproteins. That hypothesis is not at odds with the present one; growth is so important that there are likely to be several control points. Thus, tight cross-linking of matrix polymers via phenolic coupling could override or limit any loosening of the wall caused by cleavage of hemicelluloscs. In addition, accurately steered phenolic cross-linking of wall polymers could possibly make molecular loops (Fry 1989b) that would strap hemicellulose chains onto microfibrils at particular points and inhibit the ‘unzipping’ mode of cell expansion proposed in Fig. 2e. As if to ensure the independence of loosening and tightening mechanisms, plants have avoided putting phenolic side-chains on the cellulose-substrates (XG and MLG), and have restricted them to arabinoxylans, pectins and extensin (Fry 1988).

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References


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536 Physiol. Plant. 75, 1989
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