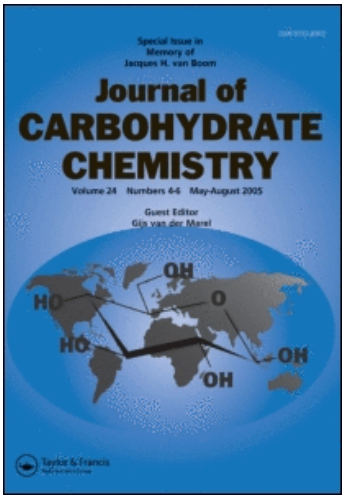

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V. L. Finkenstadt[a]; T. L. Hendrixson[a]; R. P. Millane[a]
[a] Whistler Center for Carbohydrate Research, Purdue University, West Lafayette, Indiana, USA

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MODELS OF XYLOGLUCAN BINDING TO CELLULOSE MICROFIBRILS[1]

V.L. Finkenstadt, T.L. Hendrixson and R.P. Millane*

Whistler Center for Carbohydrate Research, Purdue University,
West Lafayette, Indiana 47907-1160, USA

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ABSTRACT

Molecular modeling is used to investigate the ways in which plant cell wall xyloglucans might bind to the surface of cellulose microfibrils. Binding involving the xyloglucan backbone is found to be sterically restricted. Plausible models are obtained that involve hydrogen bonding between the xylose residues and one kind of cellulose surface. In such a model, the xyloglucan sidechains mediate, as well as modulate, the binding.

INTRODUCTION

The primary cell walls of plants consist of cellulose, hemicelluloses, pectins, and proteins.[2,3] Cellulose is the major component, being 20-30% by dry weight, and the polymer chains are associated in the form of crystalline microfibrils. The hemicelluloses include all the non-cellulosic wall polysaccharides other than pectins. In the dicot and non-graminaceous monocot cell walls, the major hemicellulose components are xyloglucans (XGs).[3] The pectins are a heterogeneous group of polysaccharides including homogalacturonans and rhamnogalacturonans, that cross-link *via* calcium ions to form rigid insoluble gels. Many enzymes and enzyme inhibitors are also present in the cell wall. The primary cell wall controls plant

growth and morphogenesis, and elucidation of its molecular architecture is essential to understanding the molecular mechanisms of growth and development.

Xyloglucans have a cellulosic backbone with about 75% of the glucose residues substituted at O6 with α-D-xylose. Some of the xylose units are substituted at O2 with β-D-galactose, and some of the galactose units are substituted at O2 with α-L-fucose (Figure 1a).[4] Purified XGs associate strongly *in vitro* with purified cellulose microfibrils.[5] They hydrogen-bond tightly to, and coat, the microfibrils *in vivo*, and probably help to keep the microfibrils anchored into the cell wall matrix.[6] However, there is more XG in the primary cell wall than would be required for an uninterrupted monolayer, so that a substantial proportion of the xyloglucan cannot be in contact with the microfibrils,[5] and there is some evidence that an individual XG molecule could be hydrogen-bonded to two different microfibrils.[7] Magnetic resonance studies indicate the presence of both immobile and mobile hemicellulose components.[8] It has therefore been suggested that XG cross-links between microfibrils.[9,10] Cross-linking polymers can be observed directly using special replication techniques and electron microscopy, and these disappear with the removal of fractions rich in glucose and xylose.[11] Furthermore, removal of alkali-extractable polymers results in the microfibrils collapsing together to form bundles of fibers.[11] Lengths of extracted xyloglucans range up to about 7000Å, which is much longer than that of the observed cross-linking polymers (200-400Å).[11] Xyloglucan may also be woven into amorphous regions of the microfibrils.[10] These polymers presumably then play a role in maintaining microfibril spacing by preventing lateral association of the cellulose, which is important for wall porosity and assembly. In fact, it is possible that the assembly of cellulose molecules into microfibrils is directly influenced by the availability of XGs at the assembly sites.[5]

The chemical structures of cell wall xyloglucans have been subjected to numerous investigations.[4,12] These indicate that the structure is based on Glc_4Xyl_3 units (Figure 1a). There are variations in the substitution of the xylose residues with β-D-Gal-(1→2) and α-L-Fuc-(1→2)-β-D-Gal-(1→2). The most detailed pictures of the chemical structures of XG have come from examination of the hydrolysis products from glucanase digestion. One particularly detailed study[4] of a XG from rapeseed

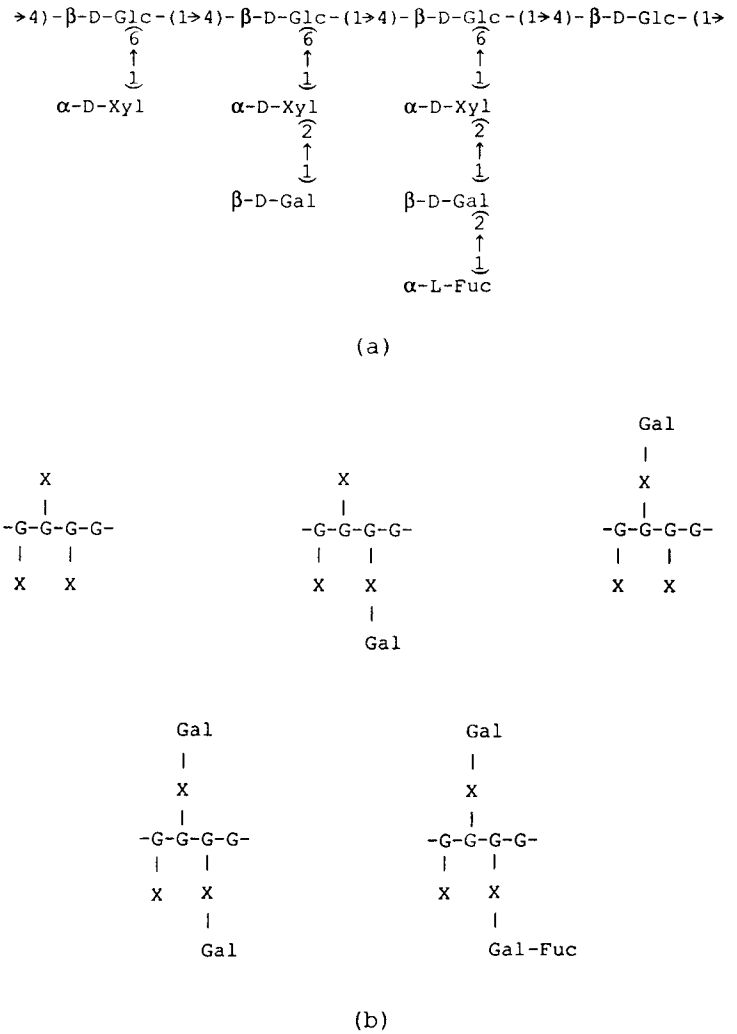

Figure 1. (a) Average structural repeat of cell wall xyloglucan, and (b) hydrolysis products following glucanase digestion.

showed that the polymer is made up almost exclusively of the units shown in Figure 1b. The arrangement of these units is unknown, but is probably random. In a study on the reserve XG from tamarind seeds, partial hydrolysis gave higher molecular weight products, but these were all multimers of the basic Glc_4Xyl_3 units.[13]

X-ray fiber diffraction studies of the three-dimensional structures of both reserve[14,15] and cell wall[16] xyloglucans show that in the solid state, the backbone

adopts a cellulosic conformation. Molecular modeling[15-17] of the sidechain conformations has not been particularly definitive however. As a result of the (1→6)-linkage, there is considerable conformational freedom of the xylose and galactose-xylose sidechains. However, more steric restrictions are introduced as a result of the two adjacent (1→2)-linkages in the fucosylated sidechain. There is considerable opportunity for the sidechains to fold along, and stiffen, the backbone.[15,17] There is also evidence that interactions between the fucosylated sidechain and the backbone tend to favor straightening of the backbone in this region into the cellulose ribbon conformation,[17] although the effects of the disaccharide sidechains were not considered.

Molecular-level information on the mode of binding of XGs to cellulose microfibrils is of interest as it relates to the physiochemical and functional properties of the cell wall, including the strength of the cellulose-xyloglucan network, the regulation of cell wall expansion by hydrolysis and/or breaking of hydrogen bonds, and the relationship to other cell wall macromolecules such as pectins.[2] The affinity of xyloglucans for cellulose has led to the proposal that the xyloglucan backbone adopts a cellulose-like conformation and that the backbone hydrogen bonds to the microfibril surface.[14,16-18] There are some difficulties with this proposal however. Referring to Figure 1, both the sidechains and hydroxyl groups available for hydrogen bonding are positioned equatorially on both edges of the XG backbone. Therefore, the sidechains should prevent the available hydroxyl groups on the backbone from approaching a cellulose surface close enough to facilitate hydrogen bonding. We present here the results of molecular modeling studies on this and other possible modes of XG binding to a cellulose microfibril surface.

METHODS

Molecular modeling and refinement was performed using the linked-atom least-squares procedure.[19] This method utilizes repulsive, quadratic non-bonded inter-atomic potentials that are matched to Buckingham potentials, and quadratic attractive hydrogen bond potentials. Although these potentials are crude, they are sufficient to allow exploration of sterically feasible conformations and interactions of the

molecules, and allow steric optimization of complex polymers and assemblies in a reasonably efficient manner.

Cellulose surfaces were generated based on the cellulose I crystal structure.[20] The cellulose molecular structure[20] was used for the xyloglucan backbone. The monosaccharide, disaccharide and trisaccharide sidechains were constructed with standard $^4C_1$ pyranose ring geometries,[21] and were attached to the backbone to represent the different units shown in Figure 1b. The molecular axes of the cellulose and XG backbone were parallel (or antiparallel). The geometry of the cellulose surface and the conformation of the XG backbone were fixed. Variable parameters were the conformation angles (ϕ,ψ,χ) at the sidechain (1→6)-linkages and (ϕ,ψ) at the two (1→2)-linkages, the conformations of the XG primary hydroxyl groups, and three translations and one rotation (about the XG molecular axis) that define the position and orientation of the XG molecule relative to the cellulose surface. The angle χ at the (1→6)-linkage is defined as the conformation θ(C5-C6-O6-C1) that can occupy one of the three staggered domains centered at $\chi \simeq 60°$, -60°, 180°.

Different conformations and juxtapositions of the XG relative to the cellulose surface were explored by first refining the XG conformation in isolation, with χ restrained to a desired domain. The distance between the XG molecule and the cellulose surface was then gradually reduced step-wise, with the XG conformation and orientation being rerefined at each step, including the effects of the XG-cellulose interactions. Sterically acceptable conformations and juxtapositions were considered to be those for which all the nonbonded interatomic distances are longer than the short limits defined by Ramachandran et al.[22] Plausible models of XG-cellulose binding were considered to be those that satisfy the above short-contact criteria and that involve at least four hydrogen bonds (with O·····O distances between 2.60 and 3.00 Å) per XG repeat unit, between the XG and the cellulose surface. Models that did not satisfy these short-contact and hydrogen bond criteria were rejected.

RESULTS

Figure 2 shows diagrammatically some of the kinds of surfaces (a, b, c) that could occur on a cellulose microfibril, based on the native (cellulose I) crystal

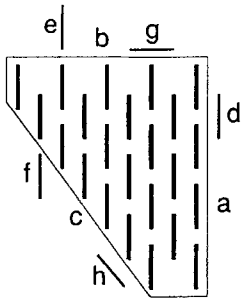

Figure 2. Schematic diagram of cellulose molecules (thick lines) in the native crystalline form, showing some of the surfaces (a–c) that could occur. Some possible positions of xyloglucan molecules (d–h) on these surfaces are shown by the thin lines. The view is along the molecular axes.

structure,[20] viewed along the molecular axes. These include surfaces along the principal cell edges that contain faces (a) and edges (b) of the cellulose molecules, and surfaces along the cell diagonal (c). Note that the angle (γ) between the unit cell edges is shown as 90° in Figure 2a for simplicity only. The correct value (γ=96.5°) was used in the actual modeling. There are many ways in which a xyloglucan molecule might bind to these surfaces. It could meander randomly over the cellulose surface, but tight binding will be facilitated if the interactions are *cooperative*. Cooperativity is achieved only if the cellulose and xyloglucan backbone axes are parallel (or antiparallel), and this is the kind of model we consider here. The different positions of the XG on the cellulose surface shown as d-h in Figure 2 include face-to-face (d), edge-to-edge (e and f) and face-to-edge (g and h) arrangements. We describe here results obtained from modeling the arrangements d, e and g.

The first model of XG binding to a microfibril surface considered was the edge-to-edge arrangement shown as e (or f) in Figure 2. This is important to assess one kind of model that would involve hydrogen bonding between the XG backbone and the cellulose surface as described in the introduction. Refinements of such models with χ in each of the three different conformational domains showed that hydrogen bonding between the XG backbone and the cellulose was impossible in all cases because of steric interference by the sidechain. Edge-to-edge arrangements of this kind are therefore untenable.

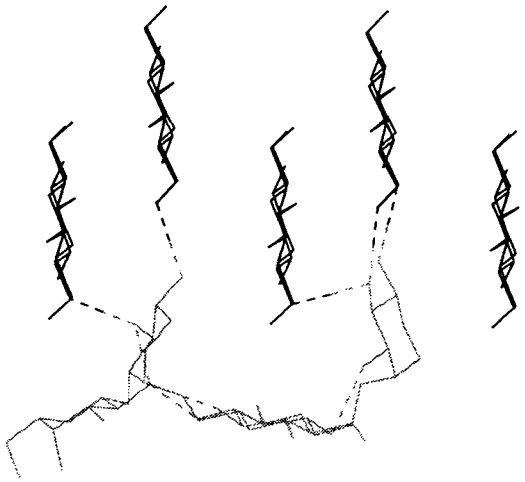

Figure 3. One of the models of a xyloglucan molecule (grey) bound to a cellulose (black) surface, as described in the text, viewed along the molecular axes. Broken lines denote hydrogen bonds.

The second kind of model examined was the face-to-face arrangement shown as d in Figure 2. Although a variety of conformations, positions and orientations of the XG were explored, none were found that led to sterically acceptable models that involved a sufficient number of hydrogen bonds (as defined above) between the XG backbone and the cellulose.

The third kind of model considered was one in which the face of the XG backbone lies across the microfibril surface formed by the edges of the cellulose molecules (g in Figure 2). The idea here is that the xylose units may be able to fold around and hydrogen bond to the edges of the cellulose molecules. Models of this kind were examined by exploring each conformational domain for χ on each of the two edges of the XG backbone, together with the position and orientation of the XG molecule on the microfibril surface. Two plausible models of this kind, with xylose sidechains only, were found. One of these involves χ in the -60° domain on one edge of the XG molecule and χ in the +60° domain on the other edge. This model, viewed along the molecular axes, is shown in Figure 3, where the cellulose molecules are shown in black and the XG molecule in gray. The xylose residues are directed towards the surface and nestle into, and hydrogen bond to, the cellulose crystal

structure. The xylose residue on the left of the Figure has the χ in the -60° domain and that on the right has it in the +60° domain. There are 7 or 8 (depending on the linkage conformations relative to the XG sequence) hydrogen bonds (broken lines in Figure 3) between the xylose units and the cellulose surface per Glc_4Xyl_3 unit in this model. A similar model is obtained if the linkage with χ in the -60° domain is changed to the 180° domain. Similar models are also obtained with two different translations of the XG molecule, relative to the cellulose molecules, along its molecular axis. These models are for parallel orientation of the XG and cellulose molecules, but preliminary results indicate that similar models can be built with the XG and cellulose molecules oriented antiparallel.

Once the models with monosaccharide sidechains had been optimized, we explored the implications of the presence of disaccharide and trisaccharide sidechains by adding the galactose and fucose-galactose units in turn. The feasibility of adding a galactose residue to the xylose units that bind to the cellulose surface depends on the particular model and the conformational domain at the (1→6)-linkage. In all cases however, a galactose residue could be accommodated on one xylose unit, and in some cases on both. For example, for the model shown in Figure 3, a Gal-Xyl sidechain can be accommodated without steric difficulty where the (1→6)-linkage is in the -60° domain (as shown in Figure 3), but not in the +60° domain.

The conformational properties of the trisaccharide sidechain are quite different however. Addition of a fucose residue to models containing disaccharide sidechains leads to serious steric compression in all cases. This is a result of over-short interatomic contacts between the fucose residue and the XG molecule, rather than between the XG and the cellulose surface, however. Therefore, the fucose residue prevents the trisaccharide sidechain from adopting a conformation that allows it to bind to the cellulose surface in this way.

DISCUSSION

Although it has been known for some time that xyloglucans associate strongly with cellulose microfibrils, both *in vitro* and *in vivo*, practically nothing is known of the molecular basis of this behavior. Earlier proposals that regions of the xyloglucan

backbone may be free from sidechains along one edge[14,18] are unlikely to be correct in view of more recent data indicating that the chemical structure is based almost exclusively on Glc_4Xyl_3 units. It is generally assumed that the XG backbone adopts the cellulosic ribbon conformation to make it geometrically compatible with cellulose, but this alone does not explain how such a heavily substituted molecule can bind so strongly to cellulose.

The results presented here show that the XG sidechains prevent direct edge-to-edge association of the XG backbone with a cellulose surface. The results also indicate that direct face-to-face associations involving the XG backbone are unlikely. However the results do show that binding may actually be mediated by the xylose substituents without involving the XG backbone, with the galactose and fucose units modulating this binding. Such modulation may be necessary to reduce the likelihood of a whole XG molecule associating with a single microfibril. This would then favor cross-linking between different microfibrils, which appears to be important for the functional properties of the cell wall matrix. Interleaving of xylose sidechains between cellulose chains has been suggested previously, although not investigated in any detail.[18]

The implications of the models described here for binding of a XG molecule to a cellulose surface are as follows. Sidechains can bind to the surface if they have one of a number of the "favorable" conformations at the (1→6)-linkage, and if the XG molecule is correctly positioned and oriented relative to the surface. This occurrence over a few XG residues could nucleate a binding site, propagating the interaction over additional XG residues. At each step of the propagation, a number of possibilities exist. (1) If the sidechain (1→6)-linkage is in a favorable conformation and it has the appropriate structure, then binding occurs, strengthening the association of this XG segment with the cellulose surface. (2) If the (1→6)-linkage is in an unfavorable conformation then either (a) the sidechain is directed away from the surface and does not contribute to the binding, but allows the following residue the opportunity to bind, or (b) the sidechain is directed towards the surface, forcing the XG backbone away from the surface and terminating the binding of this segment.

Clearly there are many potential ways that xyloglucans might bind to the surface of a microfibril. The models described here illustrate examples of how the

heavily substituted xyloglucans might bind to one of these surfaces, and how the sidechains may modulate this binding. Other arrangements are plausible and should be investigated. For example, there are a number of diagonal surfaces (e.g. c in Figure 2) that could form on the microfibril that have exposed hydroxyl groups and may facilitate binding (e.g. h in Figure 2).

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