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The Structure of Plant Cell Walls

V. ON THE BINDING OF XYLOGLUCAN TO CELLULOSE FIBERS[1]

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BARBARA S. VALENT AND PETER ALBERSHEIM[2]
Department of Chemistry, University of Colorado, Boulder, Colorado 80302

ABSTRACT

Cell wall strength is decreased by both auxin treatment and low pH. In a recently proposed model of the plant cell wall, xyloglucan polymers are hydrogen-bonded to cellulose fibrils, forming the only noncovalent link in the network of polymers which cross-link the cellulose fibers. The decreased strength of the cell wall seen upon lowering the pH might be due to an effect of hydrogen ions on the rate of xyloglucan creep along cellulose fibers. This paper investigates binding of xyloglucan fragments to cellulose. At equilibrium, the per cent of nine- and seven-sugar xyloglucan fragments which are bound to cellulose is sensitive to both temperature and the concentration of nonaqueous solvents. However, neither the per cent of xyloglucan fragments bound to cellulose at equilibrium, nor the rate at which the xyloglucan fragments bind to cellulose, is sensitive to changes in hydrogen ion concentration. These results support the hypothesis that, within the cell wall, xyloglucan chains are connected to cellulose fibers by hydrogen bonds, but these results suggest that this interconnection between xyloglucan and cellulose is unlikely to be the point within the wall which regulates the rate of cell elongation.

The walls of growing plant cells are semirigid structures which are involved in regulating the rate of cell elongation. The nature of the bonds within the cell wall which must be broken in order to permit growth is of great interest to those studying hormonally induced cell elongation. A recently proposed structural model of the plant cell wall (8) has suggested experiments which may identify the critical linkages involved. The model was derived for the walls of suspension-cultured sycamore cells, but appears to hold true for the primary cell walls of a variety of dicots (14; W. D. Bauer, M. Fisher, B. Nusbaum, personal communication).

The model suggests that cellulose fibers slip past each other during cell elongation. The only noncovalent linkage in the network of polymers interconnecting the cellulose fibers is the linkage between xyloglucan and the cellulose fibers themselves (3, 8). Xyloglucan of both bean and sycamore cell walls consists of a β-1,4-glucan backbone with xylosyl residues substituted in a regular pattern along the glucan backbone (3, 14). The linear glucan backbone allows this polymer to hydrogen-bond to cellulose. The fucosyl-1,2-galactosyl disaccharides which are attached to the 2 position of some of the xylosyl residues fold back over the glucan chain protecting the polymer from further aggregation with cellulose or other xyloglucan chains, that is, fucosyl-galactosyl side chains permit only a single layer of xyloglucan to coat each cellulose fiber (3). Some of the xyloglucan chains are covalently attached through their reducing ends to the pectic polymers of the cell wall (8). Xyloglucan polymers hydrogen-bonded to one cellulose fiber are covalently attached to xyloglucan polymers on other cellulose fibers through the pectic polymers. The primary cell wall model therefore is based on a structure which has cellulose fibers covered with a monolayer of xyloglucan molecules; some of the xyloglucan chains are connected to each other by covalent cross-links.

Cell wall extension would occur if the xyloglucan polymers and the cellulose fibers move relative to one another. It was suggested that movement might occur by a nonenzymatically-catalyzed hydrogen bond-creep when several consecutive hydrogen bonds at the end of a xyloglucan chain are broken and then reformed (8). The reformation would only occur at a new position on the cellulose fiber when this reaction was taking place in walls that were under tension (6). In such walls, the xyloglucan polymers could be conceived to move like inchworms along the cellulose fibers. If this mechanism is correct, wall-weakening would result from conditions that enhance the rate of xyloglucan creep.

Evidence in support of the importance of the xyloglucan polymer in cell wall-weakening has come from another laboratory. Labavitch and Ray (personal communication) at Stanford University have looked at the effect of auxin treatment on the turnover or solubilization of radiolabeled cell wall polymers. They found that only a xyloglucan-like polymer of the plant cell wall undergoes a rapid turnover in response to auxin treatment. The turnover is seen as soon after auxin treatment as is cell elongation.

Low pH stimulates cell wall-weakening *in vitro* (11–13) as well as *in vivo* (4). The characteristics of acid-induced cell elongation are similar to those induced by the growth hormone auxin (11, 12) and, in fact, stimulation of elongation of pea internode segments by auxin is accompanied by a decrease in the pH of the incubation medium (9). Thus, if hydrogen ions can be shown to alter the strength or rate of synthesis of bonds within the cell wall, these bonds may be those that control the rate of cell elongation.

In this paper, an examination is made of the possibility that low pH weakens the cell wall by enhancing the rate of xyloglucan creep along the cellulose fibers. Intact xyloglucan polymers cannot be used in this work because their binding to cellulose fibers is strong and, for experimental purposes, irreversible. Therefore, the effects of pH and temperature on the binding of xyloglucan to cellulose was investigated using nine- and seven-sugar xyloglucan fragments. These fragments have a four resi-

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[2] To whom correspondence should be addressed.

due β-1,4-glucan backbone and are capable of forming, at most, three hydrogen bonds with cellulose (3).

MATERIALS AND METHODS

^{14}C-Labeled xyloglucan was prepared from the extracellular polysaccharides of suspension-cultured sycamore cells according to the method of Bauer *et al.* (3). To prepare nine- and seven-sugar ^{14}C-xyloglucan fragments (Fig. 1), the purified radiolabeled xyloglucan polymers were treated exhaustively with *Trichoderma viride* cellulase (≃1 unit/mg of polysaccharide) for 48 hr at room temperature in 50 mM sodium acetate buffer, pH 5.2; 0.01% thimerosal was added to prevent bacterial growth. The xyloglucan fragments produced by this treatment were lyophilized, dissolved in 2 ml of distilled H_2O, and fractionated on a Bio-Gel P-2 column (1.5 x 112 cm) equilibrated in distilled H_2O at 50 C (3).

For the equilibrium-binding studies, the ^{14}C-labeled fragments were added to 100 mg of Whatman CC-41 microgranular cellulose powder in 2 ml of solvent. The suspension was stirred for 30 min and then left for 2 hr while the cellulose settled out. Samples of the supernatant fluid were counted using a Beckman LS-250 liquid scintillation system and Beckman D scintillation fluid.

Experiments to determine the rate of binding of ^{14}C-labeled xyloglucan fragments to cellulose were done by adding the nine-sugar fragment to 200 mg of Whatman CF-11 cellulose (medium length fibers) suspended in an aqueous acetone solution to give a final volume of 4 ml of 80% acetone. The reaction mixtures were titrated to the desired pH at 2 C after addition of the cellulose and then cooled to −20 C. Addition of the labeled fragments did not significantly change the pH reading. One hundred μl aliquots were taken from the rapidly stirred solution using a micropipet. The tip of the pipet was covered with fine-meshed nylon to prevent the cellulose from plugging the pipet. In this way, samples could be obtained within 0.5 min of the addition of the xyloglucan fragments.

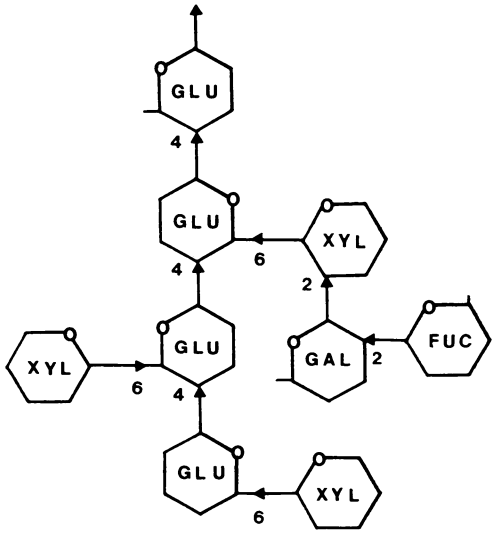

FIG. 1. Structure of the nine-sugar xyloglucan fragment. Recent, unpublished results indicate that the fucosyl-galactosyl side chain is attached to the first xylosyl residue. The seven-sugar fragment lacks the fucosyl-galactosyl side chain. These oligosaccharides are obtained by the methods of Bauer *et al.* (3). The β-1,4-linked glucan backbone of the oligosaccharides allows them to hydrogen bond to cellulose.

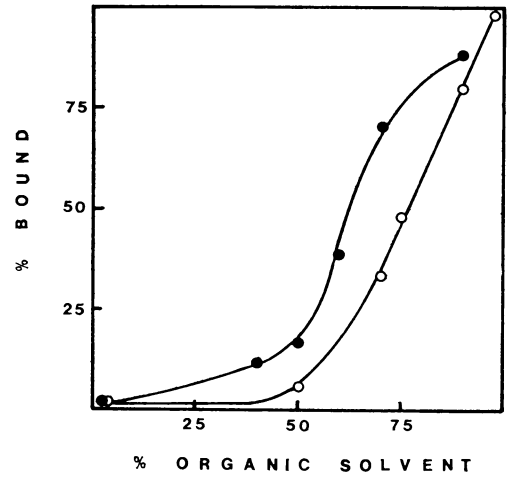

FIG. 2. Effect of organic solvents on the binding to cellulose of xyloglucan fragments at 2 C. ^{14}C-Labeled xyloglucan fragments were added to suspensions of 100 mg of Whatman CC-41 microgranular cellulose in a total of 2.0 ml. The per cent of the xyloglucan fragments bound at equilibrium was determined by the counts per minute remaining in the aqueous acetone (●) or aqueous ethanol (○) supernatant solutions.

RESULTS

The nine- and seven-sugar fragments (Fig. 1) isolated following endoglucanase hydrolysis of xyloglucan do not bind to purified cellulose in water (3), indicating that portions of the xyloglucan chains within the wall can lift off the cellulose fibers and, thereby, initiate creep of the xyloglucan chains. The short xyloglucan fragments can be made to bind to cellulose by adding organic solvents such as acetone and ethanol to the suspension. The per cent of xyloglucan fragments bound to cellulose at equilibrium increases rapidly with increasing concentrations of acetone or ethanol when the per cent of the organic solvent is greater than 50% (Fig. 2).

The effect of temperature and pH on the bonding of xyloglucan fragments to purified cellulose was studied using these aqueous-organic solvent systems. Control samples, in which cellulose was left out and which were centrifuged to remove any precipitate, indicated that the results obtained were due to binding and not the precipitation of the xyloglucan fragments. The nine- and seven-sugar fragments are soluble in the aqueous solutions of ethanol and acetone used.

Effect of Temperature on the Binding of Xyloglucan Fragments to Cellulose. Raising the temperature weakens the cell wall in intact tissues (10, 12). If our hypothesis is correct, the rate of xyloglucan creep should increase as temperature increases. The effect of temperature on the binding of the nine-sugar fragment to cellulose in 60% acetone is illustrated in Figure 3. The per cent of the xyloglucan fragment bound to cellulose at equilibrium decreases from 33% at 2 C to 11% at 45 C. A similar effect is seen for the binding of the seven-sugar xyloglucan fragment to cellulose in 65% acetone (Fig. 4). The percent of the fragment bound to cellulose at equilibrium decreases from 60% at 2 C to 38% at 45 C. The per cent of the fragment bound to cellulose also decreases when the temperature is lowered below 2 C. The reason for this decreased binding at very low temperature is not understood.

Effect of pH on the Percent of Xyloglucan Fragments Bound to Cellulose at Equilibrium. Cell walls of frozen-thawed etiolated pea stem segments at pH 5 are weakened as compared to similar walls at pH 7 (R. Cleland, D. L. Rayle, personal communications; see also (1)). The effect of pH on the binding of

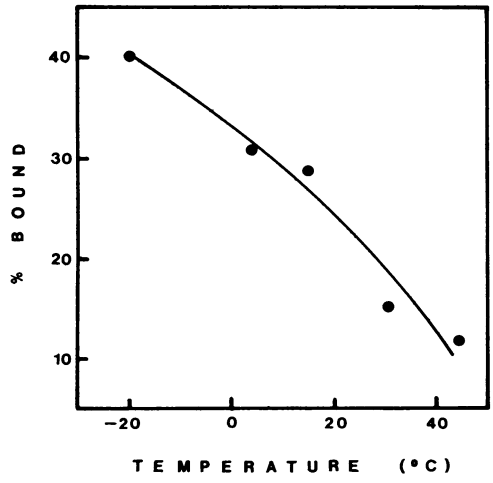

FIG. 3. Effect of temperature on the binding of the ^{14}C-labeled nine-sugar xyloglucan fragment to cellulose in 60% acetone. The labeled fragments were added to suspensions containing 100 mg of Whatman CC-41 microgranular cellulose to give a total volume of 2.5 ml of 60% acetone. After a binding period of 30 min, the cellulose was allowed to settle and 1-ml aliquots of the supernatant solutions were taken for counting.

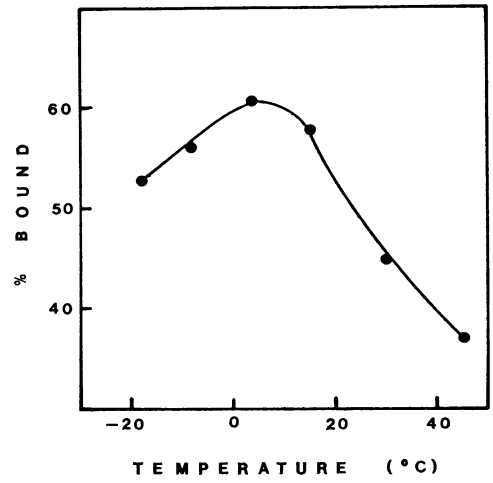

FIG. 4. Effect of temperature on the binding to cellulose of the ^{14}C-labeled seven-sugar xyloglucan fragment in 65% acetone. The fragments were added to suspensions of 100 mg of Whatman CC-41 cellulose to give a final volume of 2 ml of 65% acetone. After a binding period of 30 min, the cellulose was allowed to settle and 1-ml aliquots of the supernatant solutions were taken for counting.

the nine- and seven-sugar fragments to cellulose was examined, although the pH values must be interpreted loosely since they were obtained in aqueous solutions of acetone and ethanol. However, the pH values reported are roughly proportional to aqueous pH values and can be used as a relative measure of hydrogen ion concentration (2). The pH measurements were obtained using a Beckman 39030 ceramic junction combination electrode (silver-silver chloride internal element) with a Beckman Research pH Meter, standardized with Beckman pH 7 buffer.

Sixty-nine per cent of the nine-sugar xyloglucan fragment is bound to cellulose after equilibrium is reached in 70% acetone at 2 C. The per cent of xyloglucan fragment bound at equilibrium is the same over a wide range of hydrogen ion concentrations (Table I). The same result is obtained when the binding studies are done in aqueous ethanol.

Effect of pH on Rate of Binding of Xyloglucan Fragments to Cellulose. Although the degree of binding of the xyloglucan fragments to cellulose at equilibrium is not altered by changes in the concentration of hydrogen ions, it remained possible that the rate at which xyloglucan fragments bind to and come off cellulose is accelerated by hydrogen ions. The rate of attachment of the nine-sugar xyloglucan fragments to cellulose in 80% acetone was found to be too fast to measure at room temperature. At −20 C, a rate of disappearance of labeled fragments from the supernatant solution can be measured, but, even at −20 C, the reaction is more than 50% complete in 5 min. A typical time course for the binding of the fragment to cellulose in 80% acetone at −20 C is presented in Figure 5. No significant change in the time course has been detected over a wide range of hydrogen ion concentrations. Thus, the rate of

Table I. *Effect of pH on the Binding to Cellulose of ^{14}C-labeled Seven-Sugar Xyloglucan Fragments*

Suspensions of Whatman CC-41 cellulose in acetone solutions were titrated with HCl and NaOH. The pH values given here should be used only as an indication of relative hydrogen ion concentration (see text). After addition of the labeled seven-sugar fragments, each reaction contained 100 mg of CC-41 Whatman cellulose in 2 ml of 70% acetone at 2 C. The reactions were allowed to come to equilibrium (2.5 hr) before assay of the radioactivity in the supernatant solution.

pH	Bound	
	cpm	%
1.9	1110	69
1.9	1114	70
2.6	1078	67
2.6	1132	71
5.0	1066	67
5.0	1086	68
6.7	1094	68
6.7	1080	67

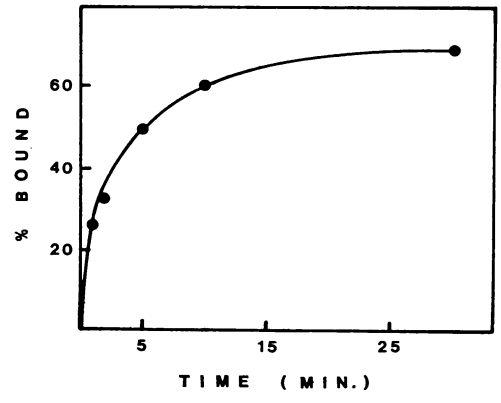

FIG. 5. The rate, at −20 C and in 80% acetone, of binding to cellulose of the ^{14}C-labeled nine-sugar xyloglucan fragment. Two hundred mg of Whatman CF-11 cellulose (medium length fibers) were suspended in 3.75 ml of aqueous acetone solution and adjusted to the desired pH at 2 C. The solutions were then cooled to −20 C. At zero time, 250 μl of water containing 1.3 × 10^4 cpm of the ^{14}C-labeled nine-sugar xyloglucan fragment was added to give a suspension of 200 mg of cellulose in a total volume of 4 ml of 80% acetone. For radioassay, 100-μl aliquots were taken from the rapidly stirred suspension using a micropipet with its tip protected with fine meshed nylon.

binding of xyloglucan to cellulose appears to be insensitive to changes in hydrogen ion concentration.

DISCUSSION

Bauer *et al.* (3) have presented evidence in support of the hypothesis that hemicelluloses bind strongly to cellulose and that this attachment is mediated through hydrogen bonds. In particular, they studied the bonding between xyloglucan, the hemicellulose of the primary cell walls of dicots, and cellulose. The data presented in this paper support the hypothesis that xyloglucan chains are connected to cellulose fibers by hydrogen bonds. Fragments of xyloglucan are induced to bond in a stable manner to cellulose by reducing the tendency of these fragments to hydrogen bond with their solvent. This was accomplished by the addition of organic solvents to the normal aqueous environment of these carbohydrates (Fig. 2). In addition, it is demonstrated that increasing the temperature decreases the amount of xyloglucan fragments bound to cellulose (Figs. 3 and 4). This finding also supports a hydrogen-bond mode of attachment between xyloglucan and cellulose.

The fact that the xyloglucan fragments used in these studies are not measurably attached to cellulose in a physiological environment is in agreement with the suggestion (8) that pieces of the xyloglucan chains may lift up from the cellulose fibers and that such unattached regions can lead to movement of the xyloglucan chains along the cellulose fibers; this proposed type of movement was called xyloglucan creep. None of the results presented in the present paper discount the possibility that xyloglucan chains creep along the surface of the cellulose fibers.

If xyloglucan creep is the rate-limiting step in elongation growth, then there is considerable evidence that the rate of creep should be accelerated at pH 5 as compared to pH 7 (5, 7, 9, 11). One would not expect that such limited changes in hydrogen ion concentration would alter the number of hydrogen bonds that exist at equilibrium between xyloglucan and cellulose because the equilibrium value will be altered only if hydrogen ions are actually added to or removed from one of the reactants or products. Since the ionization of a sugar hydroxyl is negligible below pH 12, and since the protonation of the oxygen atom of a sugar hydroxyl is negligible above pH 1, hydrogen ions would not be added to or subtracted from xyloglucan or cellulose under the conditions used in the present studies. This prediction is supported by the finding that changing the pH from about 2 to 7 does not alter the amount of xyloglucan fragments bound to cellulose at equilibrium (Table I).

Although the number of hydrogen bonds between xyloglucan and cellulose is not affected by physiological changes in pH, it was possible that hydrogen ions participated in the making and breaking of the hydrogen bonds between xyloglucan and cellulose, that is, hydrogen ions might have acted as a catalyst. If this were the case, the rate at which the hydrogen bonds between these molecules are made and broken would be sensitive to physiological pH changes. For example, if the concentration of hydrogen ions is rate-limiting, then, at pH 5, the rate of both synthesis and degradation of these bonds would be 100 times faster than at pH 7. And if the rate of synthesis and degradation of these bonds limits the rate of xyloglucan creep, then at pH 5 the rate of creep would be 100 times faster than at pH 7. However, our results demonstrate that changing the pH from about 2 to 7 has no measurable effect on the rate at which hydrogen bonds are formed between xyloglucan fragments and cellulose (Fig. 5). This suggests to us that the accelerated rate of growth observed at pH 5, as compared to pH 7, does not result from accelerating the rate of xyloglucan creep.

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