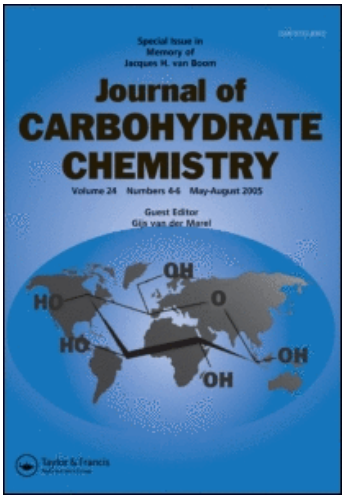

Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Use of the Avidin-Biotin Complex for Specific Immobilization of Xyloglucan Polysaccharides

Bernard Priem[a]; Gérard Chambat[a]; Katia Ruel[a]; Jean-Paul Joseleau[a]
[a] Centre de Recherches sur les Macromolécules Végétales, CNRS, affilié à l'Université Joseph Fourier, Grenoble, France

To cite this Article Priem, Bernard , Chambat, Gérard , Ruel, Katia and Joseleau, Jean-Paul(1997) 'Use of the Avidin-Biotin Complex for Specific Immobilization of Xyloglucan Polysaccharides', Journal of Carbohydrate Chemistry, 16: 4, 625 — 633
To link to this Article: DOI: 10.1080/07328309708007340
URL: http://dx.doi.org/10.1080/07328309708007340

J. CARBOHYDRATE CHEMISTRY, 16(4&5), 625-633 (1997)

USE OF THE AVIDIN-BIOTIN COMPLEX FOR SPECIFIC IMMOBILIZATION OF XYLOGLUCAN POLYSACCHARIDES[1]

Bernard Priem,* Gérard Chambat, Katia Ruel and Jean-Paul Joseleau

Centre de Recherches sur les Macromolécules Végétales, CNRS, affilié à l'Université Joseph Fourier, BP 53, 38041 Grenoble cedex 9, France

Final Form December 12, 1996

ABSTRACT

Several xyloglucan polysaccharides were biotinylated through their galactosyl residues, by combination of partial galactose oxidation and biotinamidocaproylhydrazide coupling. They were shown to easily bind to avidin activated ELISA plates by specific recognition of plant lectins *Ulex europeaus* (UEA-I) and *Ricinus communis* agglutinin (RCA-I). Subsequent chemical reduction of aldehyde groups after biotinylation was shown to improve immobilisation. UEA-I bound to cell wall xyloglucan but not to tamarind seed xyloglucan, due to the lack of fucose in the latter. RCA-I bound to all xyloglucans we tested, however, a higher affinity was observed with seed xyloglucan, suggesting it specifically clusters with the polysaccharide. The usefulness of using biotinylated xyloglucans for antibodies characterization was investigated.

INTRODUCTION

Xyloglucans are plant cell wall hemicelluloses made of a β1,4-glucan backbone substituted by xylosyl, galactosyl, fucosyl, and arabinosyl residues.[2,3] The distribution of xyloglucans in the cell wall has been the focus of interest for many years. One reason for it is that it constitutes an important element in the biogenesis of the primary plant cell wall.[4] At an ultrastructural level, the location of xyloglucans wrapping the cellulosic microfibers is

evidence of their direct involvement in the control of wall expansion. This hypothesis was strenghtened by the recent characterization of the xyloglucan endotransglycosidase (XET), an enzyme that might be involved in the loosening of the xyloglucan/cellulose ties *in vivo*.[5,6] On the other hand, another aspect of the biological importance of xyloglucans comes from the fact that xyloglucan oligosaccharides which are released by enzymatic hydrolysis can act as growth regulators.[7] Several tests for the study of the relationship between activity and structure have been applied. One limitation of the fine biochemical characterization of polysaccharides is that they do not bind to solid matrix (polystyrene plates, activated membranes,...) commercially available and essentially used for protein adsorption. A way to solve that problem was to develop an avidin-biotin based technical approach, consisting of adding biotin groups onto the polysaccharide, providing it a strong affinity for avidin coated material. We describe here a simple method of biotinylation of xyloglucans based on enzymatic oxidation of galactose groups followed by hydrazone formation with biotinamidocaproylhydrazide (BACH). This kind of procedure has been extensively used to biotinylate glycoproteins.[8,9,10] This paper describes an ELISA-plate assay based on the immobilization of a non fucosylated xyloglucan (from tamarind seed) and a fucosylated xyloglucan. Specific lectins labeled with peroxidase were used to attest of the binding. The availability of biotinylated xyloglucan, besides the interest of allowing a new ELISA test assay procedure for xyloglucan-binding proteins, should be useful for studying the metabolism of xyloglucans and associated enzymes.

RESULTS AND DISCUSSION

Biotinylation of Xyloglucans. *(i) Xyloglucan of blackberry* (FXG) - This sample was selected as a typical fucose-containing cell wall xyloglucan. It was purified from the cell suspension culture medium of blackberry.[11] The sugar molar composition was as follows: Man, 1.0; Ara, 2.0; Fuc, 3.5; Gal, 11.0; Xyl, 39.0; Glc, 43.5. Treatment with galactose oxidase was continued until the number of aldehyde groups formed reached a constant value. The corresponding yield of the oxidation was of 33 % of the total galactose residues. The rate of biotinylation was measured after BACH treatment and ultrafiltration and was estimated to be of 2.8 nanomoles of biotin equivalent per 100 μg of polysaccharide, which represents a degree of biotinylation of galactose of 3.8 percent in the oxidized FXG. This sample was called B-FXG (biotinylated fucosylated xyloglucan).
(ii) Xyloglucan of tamarind seed (GXG) - This sample was chosen as a fucose-free, galactose-containing xyloglucan.[12,13] The sugar composition was as follows: Ara, 2.1;

Xyl, 37.8; Gal, 17.3; Glc, 42.8. Contrary to the blackberry xyloglucan and in order to keep a large proportion of unoxidized galactosyl residues, the enzymatic oxidation was controlled and stopped when aldehyde groups became detectable by a colorimetric assay. Percentage of oxidation was estimated to 9.5 percent of the total galactose residues. The rate of biotinylation was measured after BACH treatment and ultrafiltration and was estimated to 1.1 nanomole of biotin equivalent per 100 μg of total polysaccharide. This corresponds to a degree of biotinylation of galactose of 0.95 percent in the oxidized galactose. This sample was called B-GXG (biotinylated galactosylated xyloglucan).

Coating of B-XGs on microtitration plates. In order to show that biotinylated xyloglucans could bind onto microtitration plates, two plant lectins labeled with peroxidase were used. The lectin of *Ulex europeaus* UEA-I was chosen in order to detect fucosylated xyloglucan because of its high affinity for α-L-fucose, and the lectin of *Ricinus communis* RCA-I was chosen in order to detect galactosylated xyloglucans because of its high affinity for β-D-Gal. A first experiment was conducted for a direct ELISA plate assay, by detection of biotinylated xyloglucans on avidin-coated plate. Both unreduced B-XGs and $NaBH_4$ reduced B-XGs were assayed in order to evaluate the utility of the reduction upon the test. As shown in Figure 1, UEA-I agglutinin binding was observed after B-FXG loading. A very good correlation was found between the amount of loaded material and the absorbance response for both reduced and unreduced B-FXGs.

An optimal amount was reached around 0.5 μg, and then a decrease of absorbance was observed with the unreduced form of B-FXG, but not with high amount of its reduced form. According to our data, reduction improves the immobilization of the B-XGs, despite the fact that the lectin does not bind galactose. This effect may be due to a stabilization of hydrazone bound by reduction. B-GXG was tried in the assay but no UEA-I binding could be detected.Conversely, RCA-I agglutinin binding was observed for all B-XGs (Figure 2). Reduced polysaccharides provided higher binding, in agreement with the fact they contain more galactose after reduction (*gal*-CHO reduction) . The amount of material necessary to saturate the plate was higher for B-FXG than for B-GXG, in agreement with the fact that B-FXG had three more biotinylated galactose residues. This resulted in the binding of a larger number of B-GXG molecules at saturation of avidin sites in the plate. In other words, the more biotinylated galactose per XG molecules, the less total XG molecules will bind avidin sites. In conclusion of these experiments, reduced B-XGs will be chosen for the next experiments at their optimal loading amounts.

Test of Competition with Lectins. These experiments were carried out to get a better idea of the relative specificity of both lectins for the structural features of xyloglucans. A comparison was done between the polysaccharides and their corresponding oligosaccharide subunits, in order to assess the possible size effect of the polymers. As

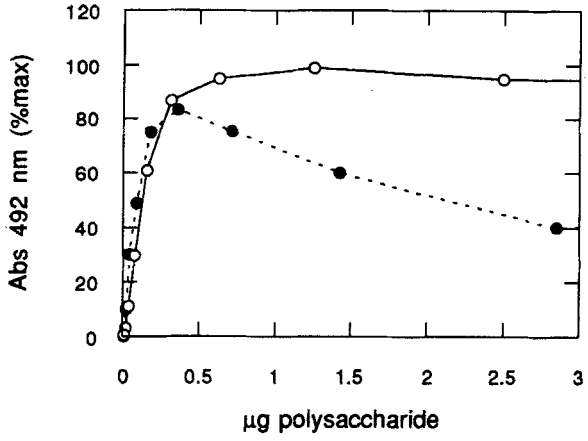

Figure 1. Binding of UEA-I agglutinin to biotinylated fucosylated xyloglucan. Biotinylated samples were added at variable amounts on avidin coated plate as described in 'Material and Methods'. Unreduced (●) and reduced (○) forms. The reduction step was done after biotinylation in order to reduce galactose-aldehyde groups which did not react with the biotinylated hydrazide (BACH). Data are expressed as a percentage of highest absorbance, compared to control well without xyloglucans. Each value is the mean of four replicates.

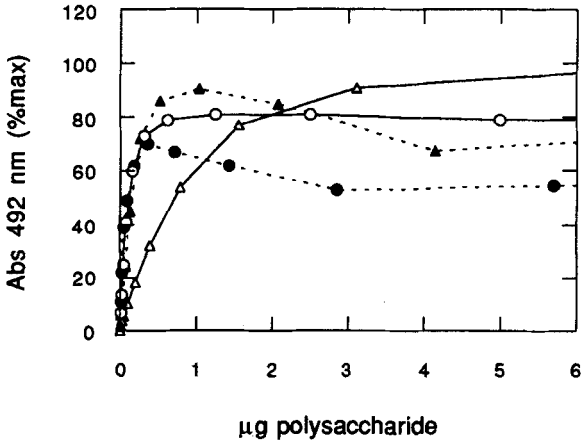

Figure 2. Binding of RCA-I agglutinin to biotinylated xyloglucans. B-FXG (●, unreduced; ○, reduced) and B-GXG (▲, unreduced form; Δ, reduced form;) . Data are expressed as a percentage of highest absorbance, compared to control well without xyloglucans. Each value is the mean of four replicates.

shown on figure 3, it can be seen that UEA-I has a lower affinity for its oligomer subunit than for FXG.

The improvement (several fold) of affinity occurring with the polymer could be due to the fact that the same molecule of polymer contained several fucosyl residues, resulting in a multi-site effect, a well known phenomenon commonly observed with lectins or antibodies. At the contrary, RCA-I behaviour was very different according to the nature of the xyloglucan competitor: the IC 50 % of native GXG was about 1000 fold higher for the polymer compared to the subunit oligomer (Figure 4). Such increase of affinity with multivalent epitope suggests the lectin clusters with the seed xyloglucan, a phenomenon which can be observed with tetrameric lectins when there is a good fit between intramolecular distances of two binding sites and the lectin.[14,15] Indeed, RCA-I exhibits a tetrameric quaternary structure, contrary to UEA-I and RCA-II agglutinins.[16,17] The fact this effect is not seen with FXG suggests a different repartition of the galactosyl groups along its main constituting chain compared to those of GXG. It is known that seed XGs and cell wall XGs exhibit structural differences. Interestingly, nonasaccharide of tamarind XLLG containing two galactose, exhibit a lower affinity for the lectin than galactose itself. Since the two galactose residues of the oligosaccharide are known to be contiguous, it may be that the lectin can bind only one of those residues.

Xyloglucan Antibodies. Biotinylated xyloglucan was assayed to characterize a rabbit antibody raised with blackberry xyloglucan (FXG).

The antibody was able to bind both B-FXG and B-GXG, on the base of the regular ELISA plate assay (data not shown). Assays were made onto streptavidin ready-to-use coated plate, due to the low titer of our antibody resulting in higher background. Indeed, those plates were found to provide lower background. As can be seen in Figure 5, the binding on B-FXG could be reversed by competition with native XGs. Our data shows that FXG and GXG are comparable competitors, despite the fact FXG was used for the antibody making. This result was in agreement with the fact it could bind both B-XGs.

CONCLUSION

The technique of biotinylation of xyloglucans we developed herein was shown to be successful based on their detection onto avidin or streptavidin coated plates using specific lectins or antibody. Contrary to a similar technique used for asialylo-GP biotinylation, a supplemental step of reduction of unreacted aldehyde groups was done in order to recover most of the native feature of the polymer, treatment which appeared to have a stabilizing effect on the immobilization.

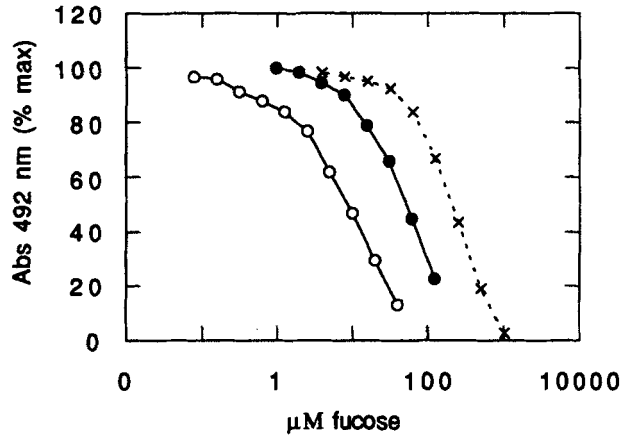

Figure 3. Competitive inhibition of the binding of UEA-I to biotinylated blackberry xyloglucan. Biotinylated blackberry xyloglucan (B-FXG, 0.5 µg per well) was immobilized on avidin-coated microtitration plate as described in 'Material and Methods'. The peroxidase-labeled lectin was added both with variable amounts of the competitor. Competitors were : ○, native blackberry xyloglucan; ●, oligomer of blackberry xyloglucan XXFG or $Glc_4Xyl_3GalFuc$; ×, α-L-fucose. Data are the averages of four replicates.

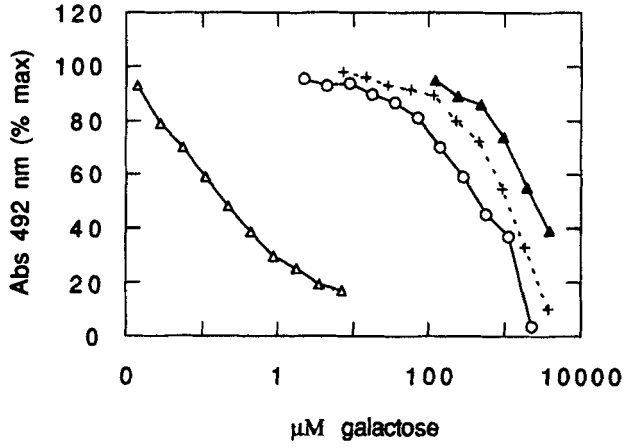

Figure 4. Competitive inhibition of the binding of RCA-I to biotinylated tamarind seed xyloglucan. Biotinylated tamarind seed xyloglucan (B-GXG, 5 µg per well) was immobilized as described in 'Material and Methods'. The peroxidase-labeled lectin was added both with variable amounts of the competitor. Competitors were : Δ, native tamarind seed xyloglucan; ○, native blackberry xyloglucan; +, α-D-galactose; ▲, oligomer of tamarind seed XLLG or $Glc_4Xyl_3Gal_2$. Data are the averages of four replicates.

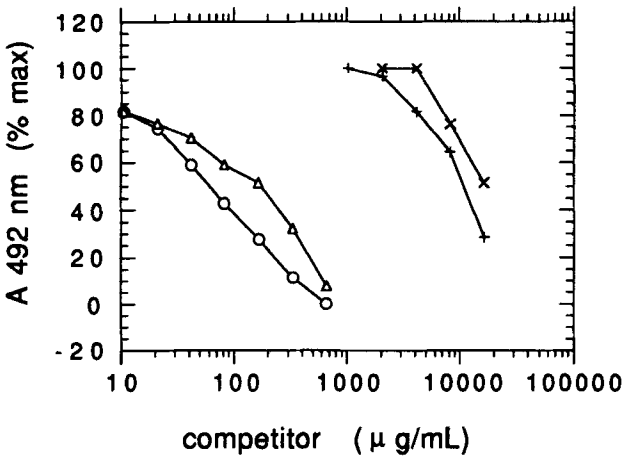

Figure 5. Competitive inhibition of the binding of anti-XG antibody to biotinylated blackberry xyloglucan. Biotinylated blackberry xyloglucan (B-FXG, 0.5 μg per well) was immobilized on streptavin-coated plate. Rabbit antibody, issued from repetitive injections with native blackberry xyloglucan, was added with both competitors. Anti-rabbit secondary antibody coupled to peroxidase was used for detection. Competitors were: O, native blackberry xyloglucan; Δ, native tamarind seed xyloglucan; +, D-galactose; ×, L-fucose. Data are the averages of two replicates.

Despite the fact there are other reports describing immobilization of xyloglucans[18] or related neoglycoproteins,[19,20] biotinylation not only appears as an alternative procedure, but should be useful for other studies involving xyloglucans.

EXPERIMENTAL

Chemicals. Biotinamidocaproylhydrazide, galactose-oxidase (from *Dactylium dendroides*), peroxidase-labeled *Ulex Europaeus* lectin (UEA-I), *Ricinus communis* lectin (RCA-I), egg-avidin (affinity purified grade) was from Sigma. Soluble tamarind (*Tamarindus indica*) seed XG (non-fucosylated) was prepared as described.[12,13] Soluble blackberry XG (fucosylated) was purified from the extracellular medium of a 40 days old suspension culture.[11] For both tamarind and blackberry XGs, only the fraction retained on 3 kd cut-off ultrafiltration membranes (Amicon system) was used in the present work.

Xyloglucan Biotinylation Procedure. The biotinylation technique was based on the oxidation of terminal galactosyl residues by galactose oxidase, followed by incubation with a hydrazine derivative of biotin (BACH), reacting to galactose-aldehyde

groups *via* hydrazone linkage formation. 50 Mg of XG were incubated with 50 µL of galactose oxidase (250 $U.mL^{-1}$), 50 µL of horse radish peroxidase (1 $mg.mL^{-1}$), completed to 5 mL with an acetic acid-Na buffer (0.1 M, pH 5.5). The reaction was performed at room temperature under stirring. The formation of aldehyde groups resulting from galactose oxidation was followed by the estimation of the reducing aldehydes with a ferricyanide based reagent.[21] 1 mL of reagent (250 mg of K-ferricyanide; 10 g of Na_2CO_3, in a final volume of 1 L with H_2O; adjusted to pH 12 with NaOH) was added to 50 µL of the oxidized polysaccharide. After 5 min at 100 °C, the solution was cooled and its absorbance was measured at 420 nm. The extent of the oxidation was controlled by stopping the mixture in a boiling water bath for 5 mn in order to inactivate enzymes. 100 µL of a 20 $mg.mL^{-1}$ BACH solution were added to the mixture, the resulting mixture was stirred overnight at room temperature. On the next day, the biotinylated-XG was ultrafiltrated on 3 kd cutt-off membrane against H_2O. The rate of biotinylation was estimated by the 4-hydroxyazobenzene-2-carboxylic acid-based assay.[22] Samples were kept frozen until use.

ELISA Plate Assay. *Plates:* tests with lectins were performed in Nunc plates, Maxisorb type; tests with antibody were made in ready-to-use streptavidin coated plates (Combiplate 8, LabSystems). The composition of the cited buffers is as follow: *coating buffer*, 0.1 M carbonate/bicarbonate/Na buffer, pH 9.2; *PBS buffer*, 0.1 M Na-phosphate pH 7.2, 150 mM NaCl; *washing buffer*, PBS plus 0.05% Tween 20; *blocking buffer*, PBS plus 1 % BSA ; *carrier buffer*, PBS plus 0.1 % BSA. All buffer contained 0.01% thimerosal as a preservative agent.

(i) Wells were activated with 100 µL of a 10 $\mu g.mL^{-1}$ avidin solution in coating buffer overnight at 4 °C. This step was missed for the tests with antibody. (ii) After two washes with PBS buffer, 200 µL of blocking buffer was added and left for 30 min at room temperature. (iii) After 4 washes with the washing buffer, 100 µL of biotinylated-XG in PBS was added and left for 2 h at room temperature. (iv) After 4 washes with the washing buffer, 100 µL of peroxidase labeled lectin (2 $\mu g.mL^{-1}$) diluted in PBS buffer was added and left for 2 h at room temperature. For competition assays, competitors were added to the wells before addition of the lectin. (v) After 4 washes with the washing buffer, 100 µL of peroxidase substrate OPD was added. The coloration was allowed to develop for 10-15 min at room temperature, and the reaction was stopped by addition of 50 µL of HCl 2N. Absorbance was read at 492 nm against appropriate controls ($avidin^+/XG^-$ and $avidin^-/XG^+$).

Chemical Reduction of Uncoupled Oxidized Galactose. Biotinylated xyloglucans were dissolved in a 0.1 HCO_3Na buffer adjusted to pH 9 with NaOH. $NaBH_4$ was added at the concentration of 10 $mg.mL^{-1}$. After 4 hours at room temperature, the reaction was stopped by lowering the pH with acetic acid to about 4.5. Salts were eliminated by extensive dialysis against distilled water.

REFERENCES

1. Presented at the *XVIIIth International Carbohydrate Symposium*, Milan, Italy, July 21-26, 1996.
2. S.C. Fry, *J. Exp. Bot.*, **40**, 1 (1989).
3. T. Hahashi, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, **40**, 139 (1989).
4. M.C. McCann, B. Wells and K. Roberts, *J. Cell Sci.*, **96**, 323 (1990).
5. S.C. Fry, R.C. Smith, K.F. Renwick, D.J. Martin, S.K. Hodge, and K.J. Matthews, *Biochem. J.*, **282**, 821 (1992).
6. K. Nishitani and R. Tominaga, *J. Biol. Chem.*, **267**, 21058 (1992).
7. S.C. Fry, S. Aldington, P.R. Hetherington, and J. Aitken, *Plant Physiol.*, **103**, 1 (1993).
8. G. Avigad, D. Amaral, C. Asensio, and B.L. Horecker, *J. Biol. Chem.*, **237**, 2736 (1962).
9. J.L. Guesdon, T. Ternynck, and S. Avrameas, *J. Biol. Chem.*, **27**, 1131 (1979).
10. E.A. Bayer, H. Ben-Hur, and M. Wilchek, *Anal. Biochem.*, **170**, 271 (1988).
11. J.P. Joseleau, N. Cartier, G. Chambat, A. Faik, K. Ruel, *Biochimie*, **74**, 81 (1992).
12. P. Kooiman, *Rec. Trav. Chim.*, **80**, 849 (1961).
13. W.S. York , H. van Halbeek, A.G. Darvill, P. Albersheim, *Carbohydr. Res.*, **200**, 9 (1990).
14. R.T. Lee and Y.C. Lee, *Carbohydr. Res.*, **101**, 39 (1982).
15. R.T. Lee, K.G. Rice, N.B.T. Rao, Y. Ichikawa, T. Barthel, V. Piskarev, and Y.C. Lee, *Biochemistry*, **28**, 8351 (1989)
16. I. Matsumoto and T. Osawa, *Biochim. Biophys. Acta*, **336**, 329 (1969).
17. M. Wilchek and T. Miron, *Biochem. Int.*, **4**, 629 (1982).
18. Y. Sone and Y. Fujikawa, *J. Nutr. Sci. Vitaminol.*, **39**, 597 (1993).
19. Y. Sone and K. Sato, *Biosci. Biotechnol. Biochem.*, **58**, 2295 (1994).
20. J. Puhlmann, E. Bucheli, M.J. Swain, N. Dunning, P. Albersheim, A.D. Darvill, and M.Hahn, *Plant Physiol.*, **104**, 699 (1994).
21. W.S. Hoffman, *J. Biol. Chem.*, **120**, 51, (1937).
22. N.M. Green, *Biochem. J.*, **94**, 23c (1965).