

Enzymatic functionalization of cellulosic fibres for textile and other applications: xyloglucan as a molecular anchor

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doi:

Abstract: The inherent recalcitrance of paracrystalline cellulose in biofibres to chemical functionalization, which limits the performance and applications of these biofibres, was investigated by a biomimetic approach, which circumvents the challenges inherent in direct chemical methods for cellulose modification. The unique ability of the plant enzyme xyloglucan *endo*-transglycosylase (XET) to produce chemo-enzymatically functionalized xyloglucan (XG) polysaccharides was harnessed together with the inherently strong interaction of XG with cellulose to surface-anchor the XG derivatives. An overview of the use of XG-XET technology in the functionalization of diverse cellulosic substrates is presented. Specific examples are given to highlight the potential of the system for future applications in textiles, pulp and paper, and biomedicine.

Key words: xyloglucan, xyloglucan *endo*-transglycosylase, cellulose, surface modification, biocomposites, biomimetics, functionalization.

11.1 Introduction

Increasing demand on global resources for the production of materials has long sustained interest in diverse applications of biofibres, ranging from paper and packaging to construction materials and textiles. Although synthetic, petrochemical-derived fibres sometimes have distinct performance advantages over their natural counterparts, wood pulp, cotton, and bast fibres are often desired, or even preferred, in many industrial and consumer products. In some cases, this is trivially because of economic considerations; (ligno)cellulosic biofibres are often cost-competitive with respect to synthetic alternatives. On the other hand, biofibres possess unique material and tactile properties that are often favoured by consumers, in addition to the general perception that these plant-derived materials are both natural and sustainable.

The physical/chemical surface modification of cellulosic fibres is very often performed to improve functional properties for specific applications

following liberation from the plant tissue source. This may involve selective removal of components, such as associated lignin, hemicelluloses and extractives, by pulping and bleaching chemicals to improve the optical properties of the final paper or textile product. Realisation of the full potential of cellulosic fibres, however, requires the addition of chemistry to the fibre surface in many applications. Some common examples include the application of strength-building compounds, retention aids, hydrophobic molecules, and inorganic fillers during papermaking, as well as the binding of dyes, optical brighteners, and sizing agents to textile fibres and fabrics. Clearly, the need for surface functionalization extends to high performance products based on cellulose fibres, such as 'smart' textiles, electronic paper, cellulosic biocomposite materials, and biomedical devices.

Indeed, a wide range of classical chemical methods have been developed to modify cellulosic fibre surfaces, either through physical adsorption or direct covalent attachment of molecules. Although powerful, the covalent derivatization of cellulose has certain limitations. Cellulose polysaccharide chains exist as insoluble, paracrystalline aggregates, which are characterized by low reactivity of the tightly hydrogen-bonded hydroxyl groups. Further, extensive reaction of these same hydroxyl groups leads to disruption of cellulose crystallinity, chain degradation, and, ultimately, a loss of fibre strength (Sassi and Chanzy, 1995; Sassi *et al.*, 2000; Klemm *et al.*, 2005). In some cases, reactions may require non-aqueous media, thus necessitating a solvent exchange or drying step, which may result in altered cellulose morphology or be technically impractical on a large scale. These concerns are particularly relevant for cellulosic hydrogels, such as bacterial cellulose or microfibrillated cellulose/nanocellulose suspensions, which are emerging as promising new biomaterial templates (Bodin *et al.*, 2006, 2007a, 2007b; Henriksson *et al.*, 2008; Iwamoto *et al.*, 2005; Nakagaito and Yano, 2005; Yano *et al.*, 2005).

With the potential of cellulosic fibre functionalization in focus, a number of years ago our laboratory began work on a biomimetic approach to circumvent the inherent challenges of direct chemical approaches. In this approach, the strong interaction of cellulose with the cell-wall-matrix polysaccharide xyloglucan (XG) was harnessed, together with the unique catalytic properties of an endogenous plant transglycosylating enzyme, xyloglucan *endo*-transglycosylase (XET). The subsequent sections will provide an overview of some of the basic biochemistry of XG and XET in the context of the plant cell wall, and highlight how this system has been appropriated, in conjunction with organic chemistry, to install a range of functional groups on cellulose fibres. The practical application of this system has a strong foundation in the historical use of native XG as a sizing agent textile and paper industries.

11.2 Xyloglucans: a family of functional plant polysaccharides

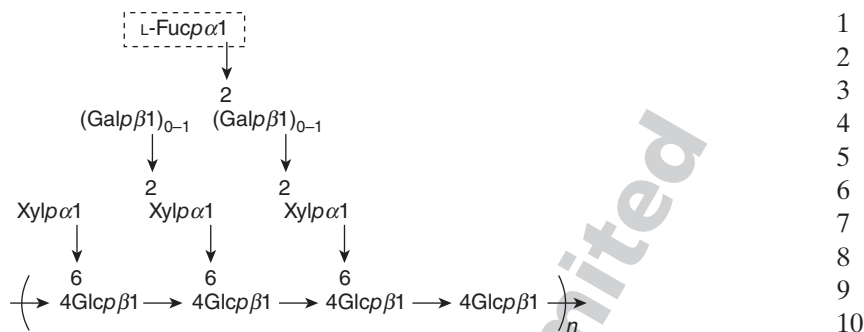
11.2.1 Xyloglucan in the vegetative cell wall

The XGs comprise a plant-specific family of polysaccharides based on a highly xylose-substituted $\beta(1 \rightarrow 4)$ glucan (cellulose) backbone (Carpita and McCann, 2000; Hoffman *et al.*, 2005). Fuco-galacto-xyloglucans (Fig. 11.1) are widely distributed among land plants (excepting many grass species), in which they act as the primary crosslinking glycans of cellulose microfibrils in the primary cell wall (Carpita and Gibeau, 1993; Carpita and McCann, 2000; Popper, 2008). As such, XGs constitute up to one-quarter of the dry weight of dicot cell walls (Busato *et al.*, 2001 and references therein), and are intimately associated with cellulose by adsorption onto and entrapment within the paracrystalline structure (Pauly *et al.*, 1999a). Indeed, XGs have a demonstrably tight and specific binding to cellulosic substrates, which is unique among polysaccharides (Zykwinska *et al.*, 2005, 2008). Moreover, this binding is effectively irreversible over a broad pH range (de Lima and Buckeridge, 2001; Lima *et al.*, 2004); strongly basic solutions (*e.g.*, 2 M NaOH) are required to release cellulose-bound XGs, presumably through partial ionization of the polysaccharide chains (Edwards *et al.*, 1985, 1986).

This remarkable, inherent affinity of XGs for cellulose forms the basis for their use in biofibre modification. Although an exact structural explanation is still lacking, elucidation of the molecular details of the strong cellulose–XG interaction has been an area of continued interest since the mid-1970s (Hanus and Mazeau, 2006, and references therein; Valent and Albersheim, 1974). The seminal study of Vincken *et al.* (1995) is particularly illuminating in the context of the practical utilization of XG as a cellulose modification reagent, and the subsequent development of the XET/XG-based technology. This study was the first to demonstrate that quantitative binding of XG to microcrystalline cellulose occurs when the polysaccharide is comprised of four or more xyloglucan oligosaccharide (XGO) repeats, *i.e.* when the XG chain has a backbone of 16 or more Glc residues ($n > 3$, Fig. 11.1). Subsequent studies have confirmed and extended these results to indicate that the binding of XG to microcrystalline cellulose is largely independent of pH over the range 2–8 and temperature of 5–60 °C (de Lima and Buckeridge, 2001; Lima *et al.*, 2004).

11.2.2 Xyloglucan in seeds

In addition to their structural role in the vegetative cell wall, XGs have been recruited as the primary storage carbohydrates in the seeds of certain



11.1 A common structure of XGs based on a Glc4Xyl3 core structure. Variable regioselective addition of galactosyl residues on one or two xylosyl branches further extends the core. Extension of Gal with an α -L-fucosyl residue (boxed) distinguishes XGs found in dicot primary cell walls from those found in seeds, cf. the molecular structure shown in Fig. 11.4.

species, including the tamarind (*Tamarindus indica*) and jatoba (*Hymenaea coubaril*) trees, as well as the ornamental nasturtium plant (*Tropaeolum majus*) (Buckeridge *et al.*, 1997; Edwards *et al.*, 1985; York *et al.*, 1993). In general, the seed XGs have the same basic oligosaccharide repeat structures as those in the primary plant cell wall, but are distinguished by a lack of terminal $\alpha(1 \rightarrow 2)$ -linked L-fucosyl residues on the β -D-Galp-(1 \rightarrow 2)- α -D-Xylp(1 \rightarrow 6)-D-Glcp sidechains (Buckeridge *et al.*, 1997; Hoffman *et al.*, 2005; York *et al.*, 1993) (Fig. 11.1). The importance of fucosyl residues in the cellulose–XG interaction *in vitro* binding is unclear (Chambat *et al.*, 2005; Whitney *et al.*, 2006; Zykwinska *et al.*, 2005), although they appear to be of little biological importance *in vivo*: mutant *Arabidopsis* plants that lack XG fucosylation have normal growth and cell wall strength (Reiter, 2002). However, selective enzymatic hydrolysis has indicated that the pendant galactose residues are important in maintaining XG solubility (Shirakawa *et al.*, 1998; Whitney *et al.*, 2006). The interested reader is referred to the review by Zhou *et al.* (2007) for a more detailed overview of XG structure–function relationships, including the effects on rheology and interactions with cellulose.

Owing to their availability in large quantities, tree seeds, and especially tamarind seed kernels, comprise the most relevant sources of XG for fundamental studies and industrial applications. ‘Tamarind kernel powder (TKP)’ is a large-scale (ca. 100 000 tonne y^{-1}) agricultural co-product from the production of tamarind fruit pulp for the food industry (Rao and Srivastava, 1973; Shankaracharya, 1998). De-oiled TKP contains ca. 60% XG by mass, which can be readily extracted by aqueous solutions to produce various crude preparations known as ‘tamarind gum’ (Gerard, 1980; Kumar

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1 and Bhattacharya, 2008; Rao and Srivastava, 1973). Repeated alcohol pre-
2 cipitation and dissolution, sometimes employing chemical or enzymatic
3 treatment steps (Rao and Srivastava, 1973), can be used to prepare high-
4 purity fractions of XG polysaccharide for diverse applications, including
5 thickeners, stabilizers, or gelling agents in food and pharmaceutical prod-
6 ucts (Coviello *et al.*, 2007; Gerard, 1980; Kumar and Bhattacharya, 2008;
7 Yamatoya and Shirakawa, 2003).

11.3 Technical uses of native xyloglucans in cellulose modification

11.3.1 Applications in textiles and papermaking

14 XG, extracted from tamarind kernel powder (TKP) in various purities, has
15 long been used to modify cellulosic fibres from cotton, jute, and wood,
16 especially in India, where the tree is indigenous (Gerard, 1980; Kumar and
17 Bhattacharya, 2008; Rao and Srivastava, 1973; Shankaracharya, 1998).
18 Sizing cotton, jute, and spun viscose with TKP improves yarn strength
19 during weaving and imparts smoothness and stiffness to fabrics (Gerard,
20 1980; Kumar and Bhattacharya, 2008; Rao and Srivastava, 1973). Here, XGs
21 are competitive with, or superior to, starches, and the strengthening effects
22 are likely, at least in part, to be the result of fibre–fibre crosslinking medi-
23 ated by the specific interaction of XG with cellulose. XG can also act as a
24 functional barrier coating for cellulose fibres: adsorption of XG on to yarns
25 or fabrics before dyeing, followed by treatment with a xyloglucanase (XG-
26 hydrolyzing enzyme) enhances partial dye removal, creating a desirable
27 abraded look (Kalum, 1998).

28 Regarding wood fibres, tamarind XG has been used in a crude form as a
29 binder for sawdust briquette and plywood manufacture, whereas, in combi-
30 nation with cross-linking borate salts, the polysaccharide gum has been used
31 as a paper adhesive (Gerard, 1980; Kumar and Bhattacharya, 2008; Rao
32 and Srivastava, 1973; Shankaracharya, 1998). The use of TKP to replace
33 starches and galactomannans for the improvement of paper strength prop-
34 erties has been long known (Gerard, 1980; Rao and Srivastava, 1973; Shan-
35 karacharya, 1998). More recently, the ability of XGs to improve paper
36 properties has been re-examined using contemporary approaches. Isolated
37 tamarind XG binds to chemical wood pulp fibres in aqueous suspension
38 with high affinity, improving both strength properties as well as fibre distri-
39 bution (formation) in paper sheets (Christiernin *et al.*, 2003; Lima *et al.*,
40 2003). In addition to wet-end application, XG has also been applied as a
41 spray coating, where the polysaccharide gives considerably higher improve-
42 ments in sheet strength properties for similar loading amounts (Ahrenstedt
43 *et al.*, 2008).

Using a flow loop system, Yan *et al.* (2006) demonstrated that the effect of improved formation was the result of reduced fibre flocculation in the wet-end of the papermaking process. Rutland and co-workers, working in collaboration with our group, have used cellulose colloidal probe atomic force microscopy (AFM) to perform fundamental surface force measurements of the cellulose–XG interaction in the context of papermaking (Nordgren *et al.*, 2008; Stiernstedt *et al.*, 2006a, 2006b). These results suggest that XG forms a boundary lubricating layer, which reduces friction and allows fibres to more easily slip past one another in the wet state, thus rationalizing the observed improvements in sheet formation. Paradoxically, cellulose adhesion is also enhanced by XG (decreased friction is almost always associated with decreased adhesion behaviour). Wet cellulose surfaces in contact display almost no adhesion, but when coated with an adsorbed XG layer, a significant time-dependent adhesion is manifested. This implies that the XG, although essentially irreversibly adsorbed, is nonetheless able to reorient and bind to an opposing surface on time scales of the order of a few seconds to minutes. This probably contributes to the increased paper strength observed with XG-treated pulps, because fibre bonds achieve significant adhesion levels before drying owing to bridging effects (Stiernstedt *et al.*, 2006a). Interestingly, the crosslinking of macroscopic cellulosic fibres by XG in paper and textiles may be analogous to the crosslinking of cellulose microfibrils by XG within the cell wall (Carpita and McCann, 2000). The capacity of wood pulp fibres to bind XG is closely correlated with the chemical composition of the fibre surface. Mechanical pulps, which have a high amount of residual surface lignin and extractives, bind less XG than chemical pulps with low lignin and hemicellulose content (Zhou *et al.*, 2006a).

11.3.2 Composites from xyloglucan and cellulose fibrils

In addition to whole plant fibres, complexes of pure cellulose micro/nanofibrils with XG have received continued attention owing to their relevance in understanding the molecular basis of plant cell wall morphology, see Whitney *et al.* (2006) and references therein. For bacterial cellulose composites, further processing with XG-active enzymes has been used to alter mechanical properties (Chanliaud *et al.*, 2004). Several studies have underscored the remarkable physical properties of cellulose networks based on bacterial (Yano *et al.*, 2005) and plant nanofibres (Iwamoto *et al.*, 2005), including microfibrillated cellulose from wood pulp (Henriksson *et al.*, 2008; Nakagaito and Yano, 2005). The admixture of cellulose nanofibres with XG thus represents an interesting new area of biofibre composite design that has, as yet, been little explored.

11.4 Chemo-enzymatic modification of xyloglucans to functionalize cellulose surfaces

The rich history of studies on the *in vivo* and *in vitro* association of XG with cellulose, including industrially relevant applications, forms a solid basis for the use of XGs as molecular anchors to attach chemical functionality to cellulosic surfaces. Indeed, the realisation that the selective modification of XG and subsequent aqueous adsorption on cellulosic fibres could circumvent some of the aforementioned difficulties associated with direct chemical derivatization has opened new possibilities to expand fibre properties.

As a polymer, XG possesses a number of potentially reactive sites for functionalization (Fig. 11.1, cf. Fig. 11.4). The most obvious are the numerous primary and secondary hydroxyl groups of the polysaccharide, which can be derivatized using well-known organic chemical methods with varying selectivity. Indeed, alkylated, carboxymethylated, sulfated, and oxidized derivatives of tamarind seed XG have been synthesized (Gerard, 1980; Lang *et al.*, 1992; Rao and Srivastava, 1973; Takeda *et al.*, 2008), and some of these have been applied in papermaking (Gerard, 1980; Rao and Srivastava, 1973). In contrast to traditional, 'chemical' methods of polysaccharide modification, enzymes are well-known to offer certain advantages in terms of specificity and gentle reaction conditions. Although a multitude of enzymes capable of degrading polysaccharides is known, the number of enzymes suitable for adding chemical groups onto polysaccharides is currently limited; however, two alternative, chemo-enzymatic approaches have been devised to functionalize XG for cellulosic fibre modification.

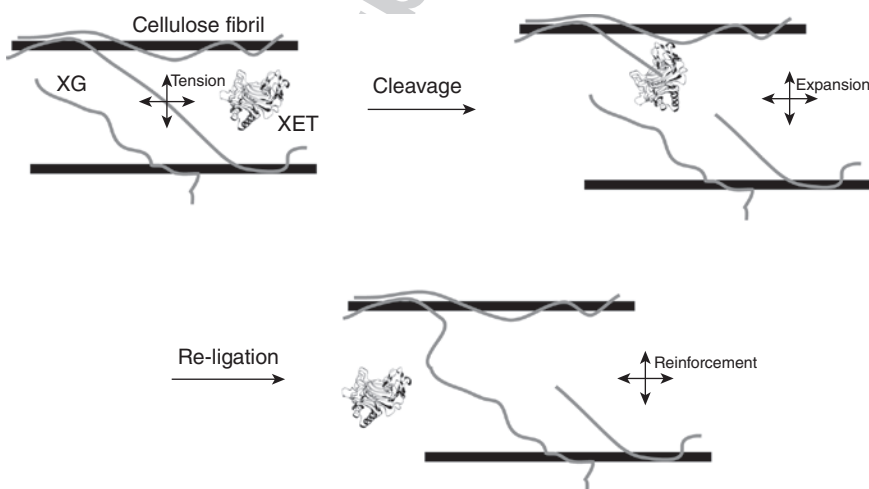
11.4.1 Activation of xyloglucans with galactose oxidase

Galactose oxidase catalyzes the regiospecific oxidation of the C-6 hydroxymethyl group (a primary alcohol) of galactose and galactosides to the corresponding aldehyde (Whittaker, 2003), which can be used as a reactive chemical handle for further derivatization. XGs are of course replete with subtending galactosyl residues along their backbones (Fig. 11.1). The seminal work by Gidley and colleagues on the oxidation of XG in solution using galactose oxidase formed the basis for the use of this enzyme to make polysaccharide conjugates (Lang *et al.*, 1992). Subsequently, the catalytic ability of galactose oxidase was harnessed to activate the galactose branches of both locust bean galactomannan and tamarind XG for conjugation with proteins (Berry *et al.*, 2001). In one example, the enzyme glucose oxidase and a monoclonal antibody were each covalently attached to the polysaccharides by direct reductive amination. In a complimentary approach, a thiolated recombinant single-chain antibody fragment was coupled with the

polysaccharides, which had been first activated by galactose oxidase and reacted with the thiophilic 4-(4-*N*-maleimidophenyl)butyric acid hydrazide. These protein- and enzyme-polysaccharide conjugates were readily adsorbed onto cotton cellulose powder in aqueous buffer. Most importantly, these gentle conditions retained the biochemical activities of the antibodies and enzyme, which were functional on the cellulosic substrate.

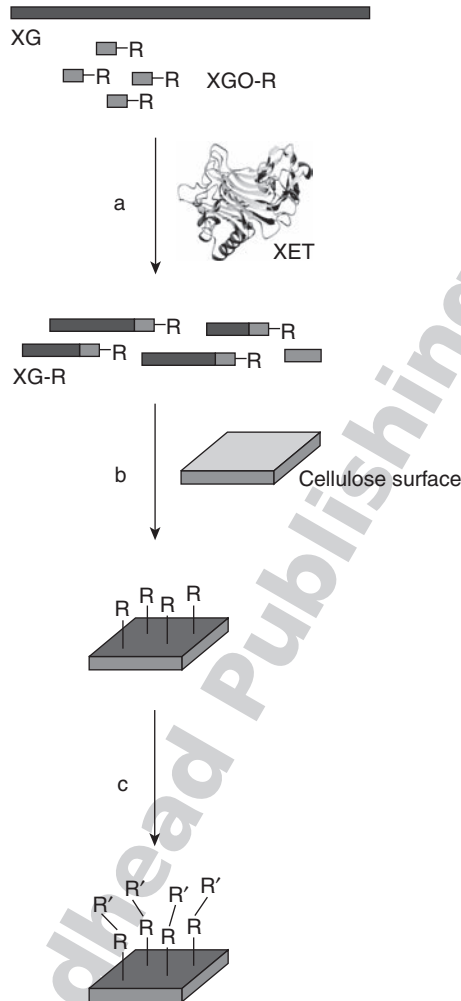
11.4.2 Xyloglucan endo-transglycosylase and xyloglucan as a toolkit for cellulose modification

Xyloglucan *endo*-transglycosylases (XET, EC 2.4.1.207) are plant enzymes that catalyze the endolytic cleavage and re-ligation of XG chains in the cell wall (Gilbert *et al.*, 2008; Rose *et al.*, 2002). This reaction, which proceeds without hydrolytic degradation of the XG polysaccharide, see Gilbert *et al.* (2008) for a full discussion, is implicated in *transient* wall loosening during cell growth, followed by reinforcement of the cellulose-XG network (Fig. 11.2) (Rose *et al.*, 2002). In contrast, cell wall polysaccharide hydrolases and lyases irreversibly cleave cellulose cross-linking glycans leading to irreversible wall softening (e.g., fruit ripening, rotting). Notably, early work on the biochemistry of XETs indicated that the catalytic reaction is somewhat promiscuous; these enzymes can utilize XG oligosaccharide derivatives as alternative glycosyl acceptor substrates, thereby incorporating them into



11.2 A biological role of xyloglucan *endo*-transglycosylases (XET) in plant cell wall growth and restructuring. XET cleaves and re-ligates high molar mass XG chains, which bind and crosslinked cellulose fibrils, without hydrolytic degradation. Transient wall loosening is believed to facilitate wall expansion, driven by turgor pressure.

1 the XG chain by transglycosylation (Fig. 11.3) (Fry, 1997; Fry *et al.*, 1992;
 2 Nishitani, 1992). Inspired by these observations, our laboratory has further
 3 developed this system as a biomimetic method to alter cellulosic fibre
 4 surface chemistry (Teeri *et al.*, 2007; Zhou *et al.*, 2007).

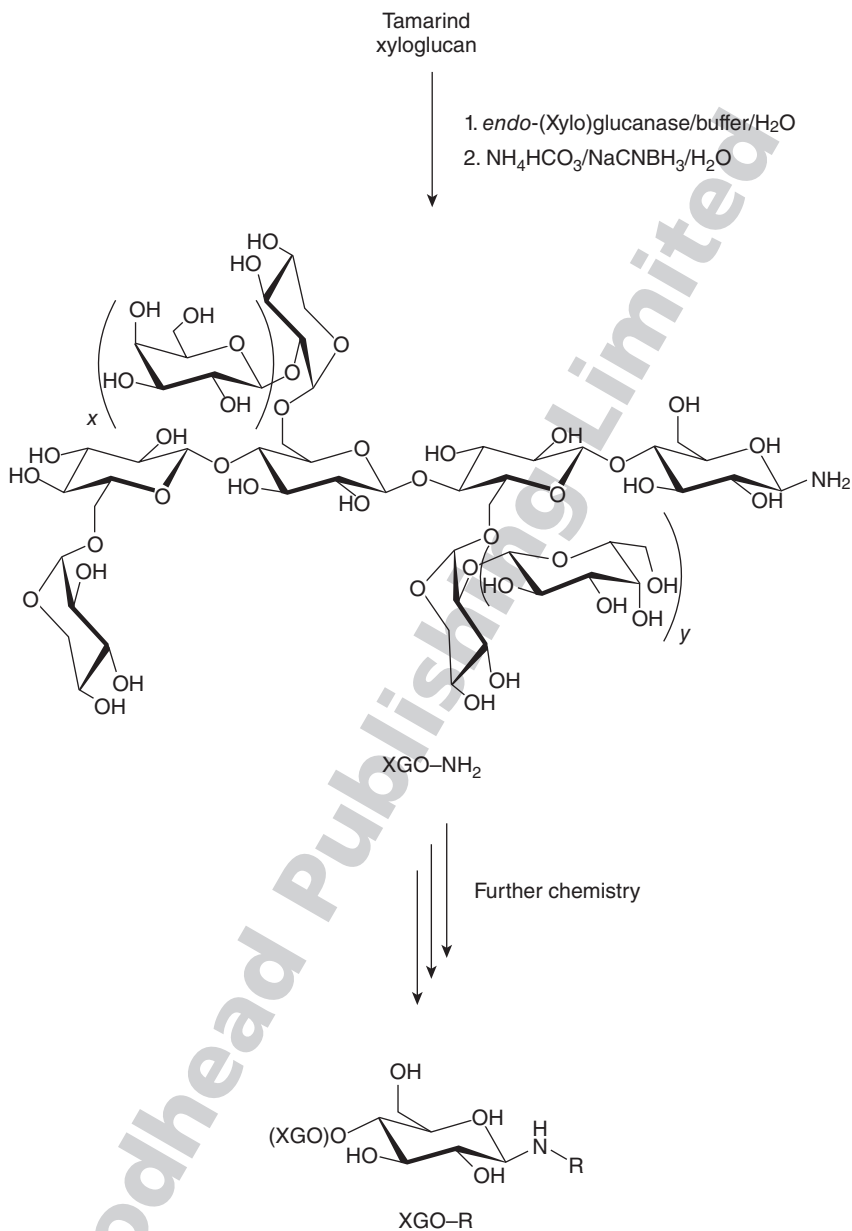


11.3 The use of XET and XG for cellulose modification: (a) XET produces modified XG (XG-R) by transglycosylation of XG onto XGO-R; (b) subsequent adsorption of XG-R from aqueous solution, driven by the strong cellulose–XG interaction, brings the functional group onto the cellulose surface; and (c) further reactions of surface-bound R groups are possible with appropriate reagents, including photoactivation, electrophilic/nucleophilic reactions, grafting from polymerisation, and biomolecule capture.

This technology requires three essential components (Fig. 11.3): (1) high molar mass XG polysaccharide, (2) chemically modified xyloglucan oligosaccharides (XGO-R), and (3) a xyloglucan *endo*-transglycosylase (XET).

1. Xyloglucan polysaccharide (XG). For reasons of convenience and large-scale availability (*vide supra*), tamarind seed XG is the base material of choice. The oligosaccharide composition of this polysaccharide is well-established, and the native high molar mass is sufficiently high ($M_w > 500\,000$).
2. Chemically modified xylogluco-oligosaccharides (XGO-R). These enzyme substrates are readily produced by the digestion of tamarind XG with microbial xyloglucanases (Gilbert *et al.*, 2008; Pauly *et al.*, 1999b; York *et al.*, 1993), followed by reductive amination to install an amino group on the first Glc residue of the XGO (Fig. 11.4). This amino group provides a uniquely reactive chemical handle, which may be further functionalized, depending on the application. Reductive amination is practically convenient because the reaction occurs in aqueous solution without the need for hydroxyl protecting groups. Chemical handles may also be installed by anomeric glycosylation (Ibatullin *et al.*, 2008), lipase-catalyzed esterification (Gustavsson *et al.*, 2005), or via oxidation with galactose oxidase (Priem *et al.*, 1997). Xyloglucanase digestion of tamarind XG produces a mixture of the four constituent oligosaccharides (Fig. 11.1 and 11.4) and, although this may be simplified by β -galactosidase digestion and/or chromatographic separation (Greffé *et al.*, 2005), it is practically more convenient to use the mixture directly on a large scale.
3. Xyloglucan *endo*-transglycosylase (XET). This enzyme is responsible for the recombination of the XGO-R substrates into the XG polysaccharide. Work in our laboratory has routinely employed a XET, *PttXET16-34* from the hybrid aspen (*Populus tremula* \times *tremuloides*), produced recombinantly in the yeast *Pichia pastoris* (Bollok *et al.*, 2005; Kallas *et al.*, 2005). Recombinant expression has the distinct advantage over extraction from native sources that it is highly scalable and industrially widespread (Bollok *et al.*, 2005). *PttXET16-34* is biochemically well-characterized (Kallas *et al.*, 2005), including three-dimensional enzyme structural analysis (Johansson *et al.*, 2004). Notably, these structural studies, which include XET-XGO ligand complexes, indicate that this enzyme has an active site cleft with the potential to tolerate a diversity of sterically bulky XGO-R functional groups.

Admixture of these three components in buffered aqueous solution results in the XET-catalyzed incorporation of XGO-R into high mass XG, to yield functionalized XG, XG-R, with a concomitant reduction in polysaccharide chain length (Fig. 11.3, step a). Subsequent adsorption of XG-R



11.4 Chemo-enzymatic synthesis of amino-modified xyloglucan oligosaccharides (XGO-NH₂) and further derivatives (XGO-R) from tamarind XG. Digestion of the polysaccharide produces a mixture of oligosaccharides in the ratio 2:4:3 (Glc₄Xyl₃/Glc₄Xyl₃Gal/Glc₄Xyl₃Gal₂) (York *et al.*, 1993). XGO-NH₂ and diverse XGO-R compounds are substrates for XET, which are incorporated into XG polysaccharide (see Fig. 11.3).

onto cellulosic fibres in water results in gentle, surface-specific chemical functionalization (Fig. 11.3, Step b). The incorporation of XGO-R into XG by the enzyme is essential since, as discussed above, short XG oligosaccharides do not have a significant affinity for cellulose. Thus, the extended polysaccharide chain of the XG-R product functions as a molecular anchor, the length of which can be adjusted by the molar ratio of XG to XGO-R. The functional group density on the cellulosic surface can therefore be controlled either by adjusting the length of the XG-R anchoring tail, or varying the loading amount of XG-R with respect to the cellulose. Depending upon the nature of the R group, further elaboration of the surface chemistry is possible (Fig. 11.3, step c).

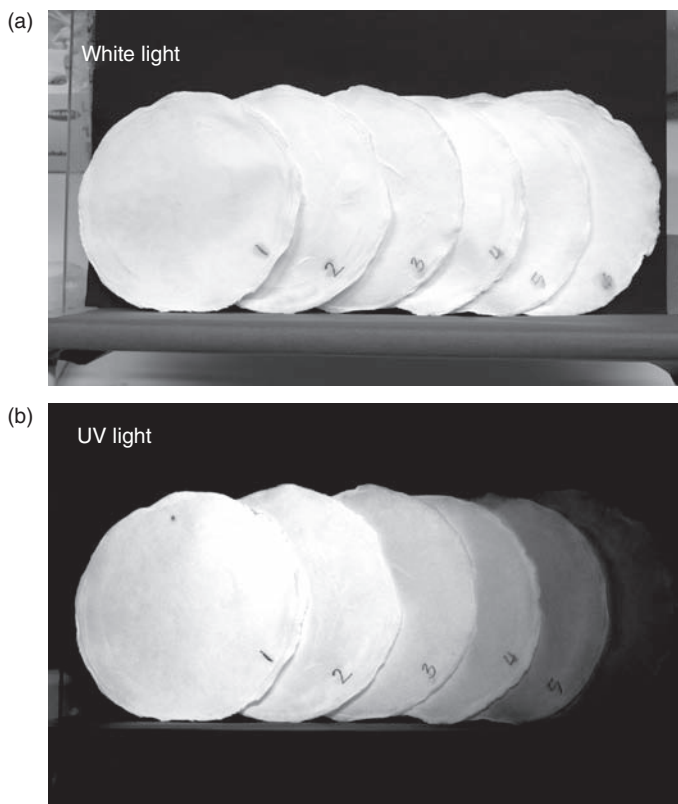
Our initial proof-of-concept work (Brumer *et al.*, 2004) focused on the well-known fluorophore fluorescein, which was conjugated to aminated XGOs (XGO-NH₂) by reaction with fluorescein isothiocyanate (FITC) to yield XGO-FITC. XET-catalyzed incorporation of XGO-FITC into XG, following the scheme shown in Fig. 11.3, produced XG-FITC. Both the enzyme-catalyzed production and binding of XG-FITC to the model cotton cellulose substrate Whatman No. 1 filter paper were extensively characterized. To demonstrate the substrate versatility of the method, XG-FITC was adsorbed onto regenerated cellulose films and a XG-sulforhodamine conjugate (XG-SR) was bound to regenerated cellulose fibres (Brumer *et al.*, 2004).

Functionalized xyloglucan (XG-R) for the activation of cellulosic surfaces

Although the production of yellow paper and pink fibres is somewhat trivial, the established methodology has a certain industrial relevance. The attachment of dyes and fluorophoric optical brightening agents (OBA) is widespread in both the paper and textile industries, whereas retention of these small organic molecules on cellulosic fibres is sometimes poor. Indeed, our laboratory collaborated with a large industrial wood pulp producer to test the potential of the XET/XG system to increase the efficiency of OBA retention. To this end, we generated a sulfated aminostilbene derivative of XG (XG-OBA) using XET, and demonstrated that it was quantitatively bound to pulp fibres, producing a dosage-dependent UV brightening effect (Fig. 11.5). Notably, the XG-OBA was retained on the pulp even after harsh mechanical refining (Zhou *et al.*, 2006b and Brumer *et al.*, unpublished results).

Although inclusion of the final functionality on XG before cellulose binding may be preferred in some applications, in many cases it is desirable to produce activated (or activatable) cellulose surfaces that are capable of further reactions. As mentioned above, the amino group provides such a chemical handle for further elaboration, and cellulosic fibres activated with

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11.5 An example of the use of the XET/XG method to retain an optical brightening agent on chemical wood pulp handsheets. Pulps were treated with six different amounts of XG-OBA and formed into sheets. Irradiation with ultraviolet light (a) shows a clear dose-dependent UV brightening effect, which is not observed under normal laboratory fluorescent tube lighting (b).

XG-NH₂ have been reacted with a number of electrophiles, including isocyanates, isothiocyanates, thioesters, and activated carboxylic acids (Brumer *et al.*, 2004; Teeri and Brumer, 2003). For example, XG-NH₂-modified Whatman No. 1 filter paper can be reacted with FITC to yield paper effectively identical to that from direct XG-FITC modification (Brumer *et al.*, 2004). Thiol groups on cellulosic fibres, from the adsorption of a thiolated XG derivative (XG-SH), can be specifically reacted with methanethiosulfonate reagents or disulfides in water to form derivatives of the general structure XG-S—S—R. Subsequently, reduction of the disulfide bond (represented by the em dash) under gentle, aqueous conditions, can be used to remove the functional groups with concomitant regeneration of the XG-SH thiol, which can be re-derivatized (Brumer *et al.*, 2004; Gustavsson

et al., 2005). As such, this chemical cycling represents a proof-of-concept for dynamically altering cellulose surface chemistry.

Biomolecule capture

The capture of biological molecules represents a special kind of surface activation, with numerous applications in separations, diagnostics, and biomedicine. Cellulose surfaces, including bacterial cellulose hydrogels, display low non-specific protein adsorption and high biocompatibility (Brumer *et al.*, 2004; Helenius *et al.*, 2006; Klemm *et al.*, 2001; Miyamoto *et al.*, 1989; Wan *et al.*, 2006). Although desirable in certain applications, this biological ‘non-stickiness’ is a limitation in the use of cellulose to capture biological molecules or as a scaffold for tissue engineering. Here also the XET/XG system provides a solution. As a first example, adsorption of a XG bearing the small organic ligand biotin (XG–biotin) to Whatman No. 1 has yielded filters that are able to bind the protein streptavidin conjugated to the reporter enzyme alkaline phosphatase. Sensitive detection via conversion of a phosphatase substrate indicated that the XG–biotin-activated filters bound significant amounts of the protein conjugate, whereas control filters showed no detectable background binding (Brumer *et al.*, 2004).

We have recently extended this methodology to improve the adhesion of endothelial cells to artificial blood vessels composed of bacterial cellulose (Bodin *et al.*, 2007a). Here, the adsorption of a XG–pentapeptide conjugate, XG–GRGDS, improves the ability of bacterial cellulose to function as a tissue scaffold and facilitates cell proliferation by interaction with integrin receptor proteins on endothelial cell surfaces. Importantly, adsorption of XG–FITC to the bacterial cellulose hydrogel, which is 95–99% water, indicates that the gentle aqueous binding conditions employed do not alter the morphology and, thus, the material properties of the substrate (Bodin *et al.*, 2007a).

Although the use of XG conjugates to anchor or capture biological molecules on cellulosic fibres is only in its infancy, one can readily envision a range of biomolecular probe–target systems of practical importance (Table 11.1).

Extension to polymers: multivalent effects

A potential limitation of the XET/XG-based method of cellulose modification is that in all of the examples described thus far, a single functional group is appended per XG chain. Given that a minimum XG–R chain length is required for binding, this ultimately limits the functional group density on the cellulosic material. Consequently, we have developed XG–initiator conjugates for the grafting of polymers from cellulose surfaces. This

1 **Table 11.1** Examples of molecular probes and their targets of importance in
 2 biological applications

3	4	5
6	Probe	Target
7	Small molecule (e.g. biotin*)	Protein (e.g. streptavidin*)
8	Carbohydrate	Protein
9	Protein (e.g. RGD peptides*)	Protein (e.g. cell surface integrins*)
10	Enzyme (e.g. alkaline phosphatase*)	Substrate (e.g. indolyl phosphates)
11	Substrate	Enzyme
12	Oligonucleotide (DNA or RNA)	Oligonucleotide (DNA or RNA)
13	Chelator	Inorganics

14 * See text.

15 approach is conceptually identical to the surface activation described above,
 16 although here activation allows for the elaboration of complex polyvalent
 17 molecular architectures.

18 In a first demonstration of the potential of XG-immobilized polymeriza-
 19 tion initiators, we successfully attached an atom-transfer radical polymeriza-
 20 tion (ATRP) initiator, the 2-bromopropionyl group, onto cellulose via
 21 the XG conjugate XG- INI_{ATRP} . Subsequently, methylmethacrylate and
 22 styrene were independently polymerized from filter paper sheets to create
 23 highly hydrophobic surfaces (Zhou *et al.*, 2005). A particular advantage of
 24 controlled polymerization techniques, such as ATRP, is that polymer chains
 25 grow at the same rate, which results in low polydispersity and well-defined
 26 molecular properties (Matyjaszewski and Xia, 2001). Furthermore, the
 27 'living' nature of ATRP facilitates the production of complex polymer block
 28 structures (Carlmark and Malmström, 2003), whereas the combination of
 29 graft polymerization with subsequent chemical modification opens a multi-
 30 tude of possibilities to further tailor cellulosic material properties (Golas
 31 and Matyjaszewski, 2007; Nyström *et al.*, 2006).

32 Attachment of the ring-opening polymerization (ROP) initiator
 33 2,2-bis(methylol)propionic acid (bis-MPA) onto cellulose via the XG-
 34 INI_{ROP} conjugate has been used to extend the grafting-from-XG concept to
 35 so-called biodegradable polymers. Both poly(ϵ -caprolactone) (PCL) and
 36 poly(L-lactic acid) (PLLA) polyesters have been successfully grafted from
 37 XG- INI_{ROP} -modified filter paper to yield hydrophobic surfaces (Lönnerberg
 38 *et al.*, 2006). Notably, initiation from XG- INI_{ROP} produced papers that con-
 39 tained less polymer and were slightly less hydrophobic, for all polymers and
 40 graft lengths studied, than those resulting from direct esterification of the
 41 initiator onto cellulose. However, whereas the esterified and grafted cellu-
 42 lose paper surfaces were completely resistant to cellulase enzyme attack,
 43 papers onto which PCL and PLLA were grafted from XG- INI_{ROP} could be

degraded (Lönnberg *et al.*, 2006). This suggests that the XG, which can be readily digested by *endo*-glucanases (*endo*-cellulases) (Gilbert *et al.*, 2008; Pauly *et al.*, 1999b), acts as a selectively cleavable linker that can facilitate recycling of grafted fibres in biocomposite materials. Indeed, existing technology involving the use of cellulases in paper and textile de-inking could potentially be adapted for such an application (Soni *et al.*, 2008; Zeyer *et al.*, 1994a; 1994b).

11.5 Conclusion and future trends

The use of XG as a molecular anchor to functionalize cellulose surfaces holds considerable promise for the development of new materials. Building upon a solid platform of knowledge of XG chemistry and enzymology, biomimetic adaptation of the unique XG–cellulose interaction is opening new possibilities for cellulosic substrates inherently derived from natural sources. The ability of XG conjugates to bind to all types of cellulose, from high-purity sources such as Whatman No. 1 filters (98% cotton cellulose, mainly I_α allomorph), cotton powder, bacterial and regenerated cellulose to complex industrial wood pulp fibres, highlights the broad potential of the system (Berry *et al.*, 2001; Bodin *et al.*, 2007a; Brumer *et al.*, 2004; Zhou *et al.*, 2006a). Moreover, there is currently an unprecedented focus on the use of cellulosic biofibres in diverse applications, from the nanoscale to the macroscale, as a consequence of their unique strength-to-density ratio, biodegradability, and renewability (Dufresne, 2008; John and Thomas, 2008; Mohanty *et al.*, 2000; Pandey *et al.*, 2005; Samir *et al.*, 2005).

Continued development of biological and chemical methods to control cellulose surface chemistry will be implicit in achieving the maximum performance of cellulosic materials in individual applications. Simultaneous with stepwise improvements in traditional commodities such as paper, packaging, and textiles, revolutionary smaller-volume, higher value-added products will be demonstrators of emerging technologies. In particular, the use of cellulosic fibres in nanocomposites, biomedical applications, and functional textiles has tremendous scope. Chemo-enzymatically modified XGs are thus a useful addition to the available toolkit necessary to realize this potential. The application of these molecules to bast fibres, or in combination with other types of enzymatic treatments (Mikolasch and Schauer, 2009), represent currently untapped areas that may bear fruit.

11.6 Sources of further information and advice

In the first instance, the reader is referred to the numerous articles and reviews cited in the text, which have been selected as a starting point for further exploration of the literature (the author regrets any omissions

owing to space limitations or simple oversight). The following sources are particularly recommended:

- Zhou *et al.*, 2007: a detailed review of the use of XG in cellulose modification, including an extensive bibliography.
- Carpita and McCann, 2000: a highly recommended textbook introduction to the fundamentals of polysaccharide composition and plant cell wall structure.
- Hoffman *et al.*, 2005: an unparalleled overview of the structural diversity of XGs across plant species.
- Gerard, 1980; Kumar and Bhattacharya, 2008; Rao and Srivastava, 1973; Shankaracharya, 1998: although each has its particular limitations, this quartet of reviews provides a holistic summary of traditional uses of XG.
- Gilbert *et al.*, 2008: a useful introductory primer on glycoside hydrolases and transglycosidases, including the XET enzyme.
- <http://www.biotech.kth.se/glycoscience/>: homepage of the Division of Glycoscience (previously Wood Biotechnology) at the Royal Institute of Technology (KTH) in Stockholm, where the XET/XG cellulose modification biotechnology was elaborated.
- <http://www.swetree.com/>: homepage of SweTree Technologies, a Swedish biotechnology company working toward biofibre improvement, including industrialization of the technology developed at KTH.

11.7 Acknowledgements

The author wishes to thank Professor Tuula Teeri and all of our co-authors who have contributed to the development of the XET/XG cellulose modification technology in Stockholm. Dr Peter Piispanen and Mr Kaj Kauko are especially thanked for acquiring the photographic images used to produce Fig. 11.5. Our work has been funded by the Knut & Alice Wallenberg Foundation, Swedish Foundation for Strategic Research, Formas, and the Swedish Research Council. The Swedish Research Council and the Swedish Foundation for Strategic Research (*via* Biomime, the Swedish Center for Biomimetic Fibre Engineering) are thanked for current funding during the writing of this chapter.

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