COMMENTARY

Glycosynthase-catalysed formation of modified polysaccharide microstructures

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Glycosynthases are mutant glycosidases, genetically engineered to catalyse the synthesis of oligosaccharides. A study by Faijes et al. in this issue of the *Biochemical Journal* has expanded the ability of glycosynthases to catalyse the polymerization of carbohydrates to form unnatural oligosaccharide products that can attain unique crystalline forms. These findings reinforce the poten-

tial of glycosynthases as interesting and important tools for the enzymic synthesis of novel carbohydrates with specific and controlled structures.

Key words: carbohydrate, (1 → 3,1 → 4)- β -D-glucan, glycosynthase, polysaccharide structure, polysaccharide synthesis.

INTRODUCTION

Nature has long appreciated the importance and usefulness of oligosaccharide polymers. β -(1 → 4)-, β -(1 → 3)- and β -(1 → 3, 1 → 4)-linked glucans are naturally abundant polysaccharides synthesized by plants, yeasts, fungi, bacteria and some algae, primarily as structural and energy storage materials. The unique physical properties exhibited by these polymers, such as their high mechanical strength, hydrophilicity, thermoplasticity and biodegradability, as well as their complex microcrystalline architecture, has led to the development of polysaccharides for use as advanced biomaterials and in various biomedical applications [1,2]. For example, bacterially synthesized cellulose has found several applications in human and veterinary medicine as artificial blood vessels for microsurgery and as protective covers for micro-nerve sutures [1]. Natural cellulose forms highly ordered microscopic fibrils with distinct crystalline morphologies, the two most common allomorphs being cellulose I and cellulose II [3]. Cellulose I has neighbouring glucan chains packed in a parallel orientation, while cellulose II, which can be formed by recrystallization of natural cellulose, is a thermodynamically more stable form with antiparallel packing of glucan chains. It is these highly ordered molecular and supramolecular structures that make cellulose useful for a variety of advanced materials applications.

The synthesis of polysaccharides, such as cellulose, is carried out naturally by a variety of enzymes including glycosyltransferases, transglycosidases and phosphorylases. Unfortunately, the polysaccharides obtained from natural sources are often quite heterogeneous, requiring (sometimes extensive) purification before they are suitable for advanced materials applications [1]. As such, there is great interest in developing improved methods for synthesizing complex polysaccharides with specific and well-defined structures. Recent work by Faijes et al. [4] in this issue of the *Biochemical Journal* reports another use of glycosynthases to catalyse the polymerization of carbohydrates for the production of complex polysaccharides. The products from this glycosynthase-catalysed polymerization reaction are found to be highly ordered, adopting a novel crystalline structure. Of considerable interest is the possibility that this method may be further applied to the production of chemically functionalized polysaccharide structures, and could prove to be an important step forward in the development of advanced carbohydrate-based biomaterials.

Alternative methods of polysaccharide synthesis

Complex polysaccharides are difficult to synthesize through conventional synthetic methods due to the vast number of possibilities for the combination of monomeric units (due to the presence of several hydroxy groups of similar reactivity) as well as the need for glycosidic linkages to be formed stereospecifically (α - or β -anomers). For this reason, the use of purified enzymes to synthesize polysaccharides *in vitro* has received considerable attention [5,6], as enzyme-catalysed reactions are generally specific with regards to substrates, stereo- and regio-chemistry.

Glycoside hydrolases normally catalyse the hydrolysis of glycosidic linkages, but under conditions that favour a reversal of their hydrolytic activity can be used successfully to promote the synthesis of polysaccharides. This may be achieved either by shifting the equilibrium using high substrate concentrations (thermodynamically controlled) or by using activated glycosyl donors (kinetically controlled) [7]. The kinetically controlled method takes advantage of sugar donors with excellent leaving groups, such as glycosyl fluorides or aryl glycosides, to achieve a high concentration of the covalent glycosyl-enzyme intermediate, which can then be 'turned over' by reacting with an acceptor other than water [7].

Synthetic cellulose was first synthesized *in vitro* using a cellulase to catalyse the polymerization of β -cellobiosyl fluoride. However, glycosylation was only found to be substantially favoured over hydrolysis when the reaction was carried out in a mixed solvent system (acetonitrile/acetate buffer, pH 5), indicating that the polymerization reaction proceeded under kinetic control as well as thermodynamic control. Interestingly, when crude cellulase was used for the polymerization the product formed was the cellulose II allomorph [7]. However, when a purified cellulase was used, the cellulose microfibrils formed apparently gave an electron-diffraction pattern characteristic of metastable cellulose I, previously believed to be impossible to form *in vitro* [8]. Therefore glycosidase-catalysed polymerization may provide a method for the preparation of new higher-order molecular assemblies.

Glycosynthase-catalysed synthesis of polysaccharides

Despite the promising advances made in the use of native glycosidases to synthesize polysaccharides, yields rarely exceed 50 % because these enzymes prefer to catalyse the hydrolysis of

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glycosidic linkages. A new approach to the enzymic synthesis of oligosaccharides relies upon the use of specifically engineered retaining glycosidases, called glycosynthases [9]. These enzymes have their catalytic nucleophile removed, rendering them incapable of catalysing the hydrolysis of glycosidic linkages. However, glycosynthases are capable of processing glycosyl fluorides of the 'wrong' anomeric configuration, as this reactive compound mimics the covalent glycosyl-enzyme intermediate, and the mutant enzyme can catalyse its transfer to another sugar. The first glycosynthase reported was derived from the well-studied exoglucosidase Abg, from *Agrobacterium* sp. [9]. This glycosynthase transferred single sugar units in each catalytic step to produce di-, tri-, and small amounts of tetra-saccharide products. More recently, several examples of glycosynthases derived from endoglycosidases have been reported. These enzymes are capable of transferring disaccharides in a single catalytic step and have been used successfully to synthesize polysaccharides with β -(1 $\rightarrow$ 4)-, β -(1 $\rightarrow$ 3)- and mixed β -(1 $\rightarrow$ 3,1 $\rightarrow$ 4)-linkages. The first endoglycosidase engineered to act as a glycosynthase was a (1 $\rightarrow$ 3,1 $\rightarrow$ 4)- β -glucanase from *Bacillus licheniformis*. The mutant enzyme was found to catalyse glycosylation reactions using laminaribiosyl fluoride to form tri- and tetra-saccharides with mixed β -(1 $\rightarrow$ 3)- and β -(1 $\rightarrow$ 4)-linkages in excellent yields [10].

Later, an endocellulase Cel7B from *Humicola insolens* was transformed into a glycosynthase, capable of polymerizing cellobiosyl fluoride to produce crystalline β -(1 $\rightarrow$ 4)-linked polysaccharides, again in high yield [11]. The structure of the insoluble material produced by this glycosynthase was found to be consistent with low-molecular-mass cellulose II. Importantly, this enzyme was also capable of polymerizing cellobiosyl fluorides that possessed alternative chemical functionalities at the C-6 position, such as azide and bromide. Thus glycosynthases provide a unique and convenient method for introducing specific and controlled modifications into crystalline polysaccharide structures. The immobilization of proteins and enzymes on to the surface of polysaccharides has been investigated as a method of improving the biocompatibility of these polymers in medical applications [12]. Up to now, however, the covalent attachment of functional groups on to the surface of crystalline polysaccharides has relied heavily upon the use of toxic reagents such as cyanogen bromide and epichlorohydrin.

The synthesis of crystalline β -(1 $\rightarrow$ 3)-linked polysaccharides has also been reported using a glycosynthase derived from a barley β -(1 $\rightarrow$ 3)-glucan endohydrolase [13]. The solid products from the polymerization of laminaribiosyl fluoride by this glycosynthase were structurally similar in all respects to curdlan, which is an essentially linear β -(1 $\rightarrow$ 3)-linked glucan. Of interest here was the finding that 3-thiolaminaribiosyl fluoride [a β -(1 $\rightarrow$ 3)-S-linked disaccharide] was also polymerized by the glycosynthase, producing oligosaccharides with alternating S- and O-linkages between the sugar residues. These polymers were found to be much more resistant to enzymic hydrolysis than natural curdlans containing only O-linked sugar residues.

The work carried out to date suggests that glycosynthases are attractive tools for the synthesis of various polysaccharide structures. The recent work by Faijes et al. [4] takes the use of glycosynthases one step further, showing that these enzymes are also capable of generating unnatural crystalline polysaccharides with new morphologies. Expanding on their work with the original endoglycosynthase from *Bacillus licheniformis* [10], the Planas group has found that this enzyme also catalyses the polymerization of laminaribiosyl fluoride to produce crystalline polysac-

charides with alternating β -(1 $\rightarrow$ 3)- and β -(1 $\rightarrow$ 4)-linkages, with an average degree of polymerization of 12 [4]. In Nature, mixed β -(1 $\rightarrow$ 3,1 $\rightarrow$ 4)-glucans formed *in vivo* exist as linear polymers where the degree of polymerization can reach as high as 1200; however, the content of β -(1 $\rightarrow$ 3)-linkages does not usually exceed 25–30 % [14]. So these polymers essentially consist of β -(1 $\rightarrow$ 3)-linked cello-oligosaccharides. As the glycosynthase polymerization products contain alternating β -(1 $\rightarrow$ 3)- and β -(1 $\rightarrow$ 4)-linkages, the content of β -(1 $\rightarrow$ 3)-linkages is necessarily 50 %, and so these polymers represent structures not normally found in Nature. Light microscopy revealed the products of this glycosynthase to be low-molecular-mass polysaccharides forming spherulites composed of lamellar elements, while crystallographic analysis indicated that the molecular conformation of the products is likely to be similar to that of cellulose I crystals. This is the first example of a glycosynthase product adopting a novel crystalline morphology.

These results open up a range of possibilities for the synthesis of biomaterials using glycosynthases. Certainly, the ability to use chemically modified monomers to introduce functionalizable groups, such as azide, into novel polysaccharide structures would be highly desirable. Quite exciting, also, is the possibility that glycosynthase reactions may be carried out in the presence of other polymers to direct the formation of composite polymer materials. With regard to the range of new glycosynthase-produced polysaccharide structures that will be available, the physical properties that these polymers will possess and the importance of these structures as advanced biomaterials, only time will tell.

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