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Previews

Expanding the Repertoire of Glycosynthases

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As reported in this issue, Moracci and coworkers have now expanded the glycosynthase concept to two retaining α-L-fucosidases. The newly generated α-fucosynthases can use β-L-fucosyl azide as donors for transglycosylation, enabling the synthesis of fucose-containing oligosaccharides.

Structurally defined oligosaccharides are indispensable tools for deciphering the functional roles of glycans in biological systems. Yet chemical synthesis of complex oligosaccharides is still a daunting task that usually requires tedious protection and deprotection steps in order to control the regio- and stereo-selectivity in glycosidic bond formation. In contrast, enzymatic glycosylation usually gives high regio-selectivity and perfect control of the anomeric (α or β) stereo-chemistry without the need of protecting groups. Enzymatic glycosylation can be catalyzed by two families of enzymes, glycosyltransferases and glycosidases, when functioning in a transglycosylation mode. Glycosidase-catalyzed synthesis has attracted much attention in recent years, because the widespread glycosidases are usually easy to obtain, are capable of taking readily available donor substrates, and, in many cases, have relaxed substrate specificity.

To address the issue of product hydrolysis, a bottleneck associated with glycosidase-catalyzed synthesis, a conceptual breakthrough has been achieved in recent years with the invention of glycosynthases, a class of glycosidase mutants that promote glycosidic bond formation when a suitable activated glycosyl donor is used but are devoid of product hydrolysis activity because of the point mutation at the critical nucleophilic residue (Hancock et al., 2006; Perugino et al., 2004; Wang and Huang, 2009). Since the first introduction of the glycosynthase concept in 1998, a number of new glycosynthases have been discovered that belong to more than a dozen glycoside hydrolase families. Nevertheless, creating glycosynthases is not a trivial task. The fact that only a small fraction of thousands of known glycosidases has been converted to glycosynthases suggests that we have only begun to unveil the mystery of the enzymatic catalysis. It should be emphasized that most glycosynthases reported so far are derived from β-glycosidases. The fact that only two α-glycosidases (a retaining α-glucosidase and an inverting α-fucosidase) have been converted into glycosynthases (Okuyama et al., 2002; Wada et al., 2008) indicates the challenge of creating α-glycosynthases. In addition, almost all currently available glycosynthases exploit the active glycosyl fluoride with an opposite anomeric configuration as the donor substrate (Hancock, et al., 2006; Perugino, et al., 2004). As reported in this issue (Cobucci-Ponzano et al., 2009), Moracci and co-workers have now expanded the glycosynthase approach to two retaining α-L-fucosidases of *Sulfolobus solfataricus* and *Thermotoga maritima*. More impressively, they have successfully generated new α-fucosynthases that are able to use β-L-fucosyl azide as donors for transglycosylation, which enables the synthesis of diverse fucose-containing oligosaccharides.

First, Moracci and co-workers created point mutations at the nucleophilic residue D242 of the *S. solfataricus* α-L-fucosidase that are devoid of hydrolytic activity. Subsequent studies indicated that the activity of D242S and D242G mutants toward 2-chloro-4-nitrophenyl α-L-fucopyranoside (2C4NP-α-L-Fuc) could be rescued by sodium azide, leading to the formation of β-fucosyl azide. Interestingly, a disaccharide product, Fucα1-3Fucβ1-N_3, was also isolated from the reaction, implicating a glycosynthase activity of the mutants. A mechanism was proposed (Figure 1) in which the β-glycosyl azide was first generated by azide rescuing, and then served as substrate for the subsequent transglycosylation to give the disaccharide. The glycosynthase activity was confirmed by incubating synthetic β-L-fucosyl azide with D242S, which led to the formation of the disaccharide Fucα1-3Fucβ1-N_3. Interestingly, the wild-type α-L-fucosidase could not act on the β-fucosyl azide. These results suggest that the D/S point mutation at the nucleophilic Asp created a sufficient space at the catalytic site that can accommodate the β-L-fucosyl azide (with an opposite configuration versus normal substrate), which is significantly larger than the conventional potential substrate, β-L-fucosyl fluoride. The glycosynthase approach was also applied to the *T. maritima* α-fucosidase, and a Tm224G mutant was identified as another α-fucosynthase that can also use β-L-fucosyl azide for transglycosylation.

Subsequent studies have shown remarkably relaxed acceptor substrate

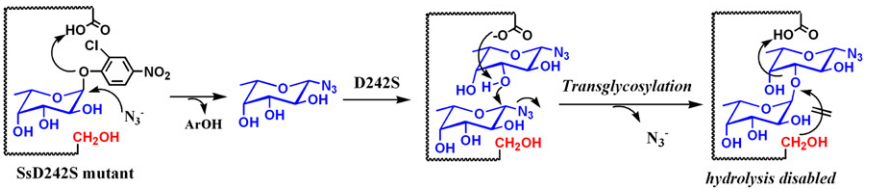

Figure 1. Putative Mechanisms for the Azide-Rescuing Reaction at the Fucα-1,3-Fucα1-N_3 Disaccharide Formation

specificity for the α-fucosynthases. Using β-L-fucosyl azide as the donor, the SsD242S mutant was able to take 16 mono- and disaccharides (so far tested) as acceptors for transglycosylation. All the reactions proceeded in an absolute stereo-specific control, leading to the formation of α-linked fucoside derivatives. The regio-selectivity seemed to be dictated by the structures of the acceptor substrates. Thus, various fucose-containing oligosaccharides with α-1,2-, α-1,3-, α-1,4- and/or α-1,6-fucosylated linkages could be formed, depending on the acceptors (Figure 2). Another interesting feature of the fucosynthase-catalyzed transglycosylation is the formation of branched trisaccharides. Notably, nonlinear trisaccharides were observed in the fucosynthase-catalyzed reactions, implicating a novel acceptor recognition mode for the new α-fucosynthases. It should be pointed out that the ability of the new α-fucosynthases to use the stable β-fucosyl azide for transglycosylation is of particular importance for practical synthesis. A recently reported α-1,2-fucosynthase that was evolved to use the labile β-fucosyl fluoride could only give a very low (6%) yield, because of the spontaneous hydrolysis of the β-fucosyl fluoride during transglycosylation (Wada et al., 2008). The discovery of the new α-fucosynthases thus opens a new avenue to the practical synthesis of biologically interesting fucose-containing oligosaccharides.

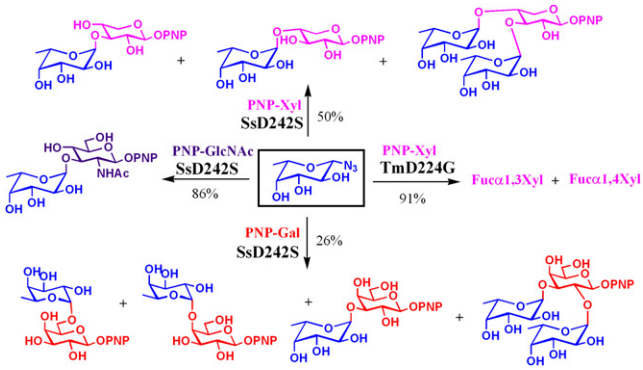

Figure 2. Synthesis of Fucose-Containing Oligosaccharides by Fucosynthase-Catalyzed Transglycosylation

In summary, the new α-fucosynthases described in this study constitute the first α-fucosynthases derived from retaining α-fucosidases. The remarkable acceptor substrate promiscuity of these new α-fucosynthases provides a unique opportunity to discover various new α-fucosynthases with tailored specificity and/or enhanced activity by directed evolution, as exemplified by a recent report for evolving endo-glycoceramidase (Hancock et al., 2009). Most importantly, the new α-fucosynthases represent the first α-glycosynthases that can use β-glycosyl azide as donor substrate for transglycosylation. It is likely that other glycosynthases can be evolved to take corresponding glycosyl azides for transglycosylation, which clearly indicates an exciting direction to expand the repertoire of glycosynthases.

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Tools to Tackle Protein Acetylation

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In the recent issue of *Molecular Cell*, Neumann et al. dissect the effect of H3K56 acetylation on chromatin structure using a novel method for generation of acetylated proteins. This is a valuable addition to the toolkit for those interested in unraveling how posttranslational modifications regulate protein function.

In the nucleus of eukaryotic cells, DNA is tightly associated with proteins forming a structure known as chromatin. These protein/DNA associations allow high compaction of the DNA, and e.g., at the same time, ensure its accessibility for the transcription and replication machinery. The building blocks of chromatin are nucleosomes, which consist of a protein component, built as an octamer of four core histones (H2A, H2B, H3 and H4), around which ~147 base pairs of DNA are