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One Step Closer to a Sweet Conclusion

OtsA is required for the biosynthesis of trehalose, a nonreducing disaccharide that is important for bacterial survival and stress responses. In this issue of *Chemistry & Biology*, the structure of OtsA is uncovered and reveals an unexpected relationship between the enzyme's structure and function.

The synthesis and degradation of glycosidic bonds occurs via enzyme-catalyzed glycosyltransfer reactions. Much is known about the structures and mechanisms of the degradative enzymes, glycosidases. Over 80 sequence-derived families have been identified to date, and structural representatives are available for approximately 50 of these; revealing a large number of different folds. Mechanistically, they are divided into two classes. The inverting glycosidases function via an acid/base-catalyzed direct displacement mechanism, while the majority of the retaining glycosidases use a double-displacement mechanism in which a covalent glycosyl-enzyme intermediate involving an active-site carboxylic acid is formed and hydrolyzed with acid/base catalytic assistance. In an interesting variation found so far in hexosaminidases from families 18, 20, and 56, the substrate's own amide moiety functions as the nucleophile, and reaction occurs via a bound oxazoline intermediate [1].

By contrast, much less is known about the enzymes involved in glycoside synthesis, the sugar nucleotide-dependent glycosyltransferases. Some 60 sequence-derived families have been defined, but structures for only ten of these have been determined, of which seven are of the mechanistically predictable inverting transferases. A key distinction from the glycosidases is that only two fundamental protein folds (termed GT-A and GT-B; Figure 1) have been uncovered for transferases, and sequence analysis has suggested that this situation will hold true for a large number of the as yet structurally uncharacterized families [2]. The GT-A fold family comprises a single Rossman fold domain and has been identified in the structures of both inverting and retaining transferases (Figure 1). Enzymes of the GT-B fold have a twin Rossman fold structure, and up to this point all those identified have been inverting transferases, possibly suggesting a causal relationship. However, the paper in this issue by Gibson and coworkers [3] on the retaining glycosyltransferase, trehalose-6-phosphate synthetase (OtsA), also reveals a twin Rossman fold for this enzyme, showing clearly again that the fold does not dictate the mechanism. This provides a cautionary note on the overinterpretation of functional data from a predicted protein fold (a major premise of structural genomics).

The OtsA structural analysis also revealed a fascinating mechanistic story. Despite the absence of any detectable sequence similarity, the active site residues of OtsA are essentially superimposable on those of a nonnucleotide sugar glycosyltransferase of the GT-A class, glycogen phosphorylase. This enzyme catalyzes the reversible phosphorolysis of glycogen with net retention of configuration to produce glucose-1-phosphate. One of the more enigmatic aspects of the glycogen phosphorylase story has been its absolute requirement for the coenzyme pyridoxal phosphate, which is covalently bound at the active site as a Schiff's base. A reason for this requirement has eluded explanation. Structural and

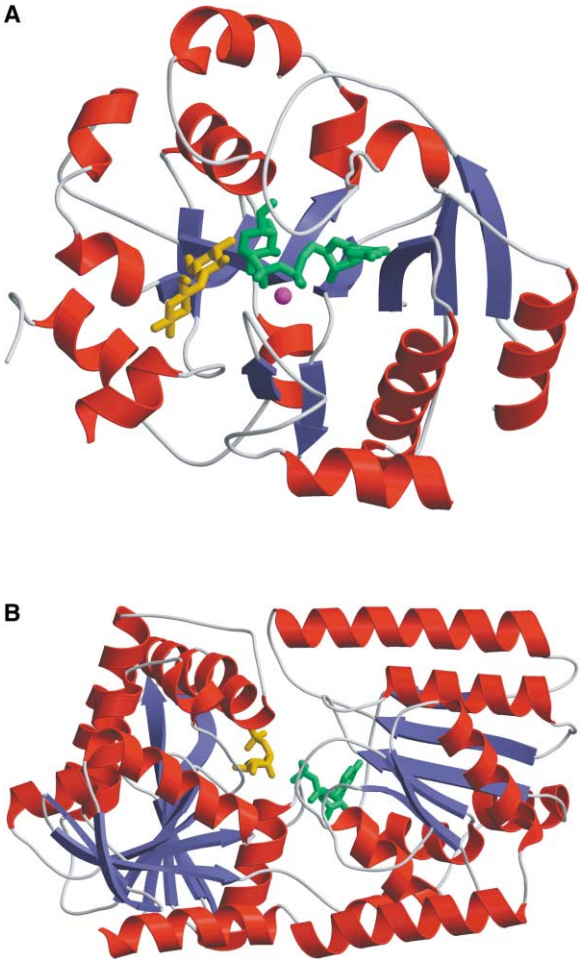

Figure 1. Transferase Folds

Ribbon diagrams [12] of the two known folds of retaining glycosyltransferases.

(A) The GT-A family fold retaining glycosyltransferase LgtC [9] (1GA8.pdb). The donor (UDP-2-fluoro-galactose) and acceptor (4-deoxy-lactose) sugar analogs as well as the essential Mn^{2+} ion observed in the LgtC structure [11] are shown in green, gold, and magenta, respectively.

(B) The GT-B family fold retaining glycosyltransferase OtsA as described by Gibson et al. in this issue [3] (1GZ5.pdb). The UDP and acceptor sugar (glucose-6-phosphate) are in green and gold, respectively.

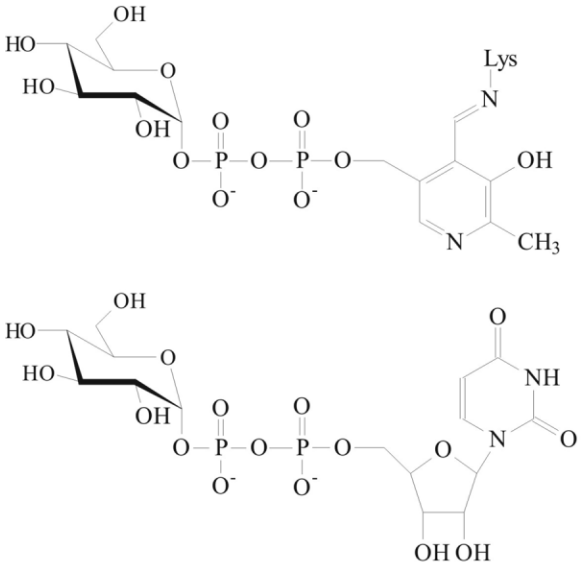

Figure 2. Substrate Similarities on OtsA and Phosphorylase

A comparative schematic highlighting the similarities of pyridoxal diphosphoglucose (top), a competent substrate analog for glycogen phosphorylase [6], and UDP-glucose (bottom), the substrate for OtsA.

mechanistic studies had shown that the coenzyme and the substrate/product glucose-1-phosphate bind in close proximity, with their phosphate moieties interacting [4, 5]. In addition, a synthetic analog in which the two are covalently linked, pyridoxal diphosphoglucose (Figure 2), underwent a regio- and stereospecific glucosyltransfer reaction onto oligosaccharide acceptors when incorporated into the active site [6]. The similarity of this analog to UDP-glucose was pointed out at that time and mechanistic similarity was suggested.

In strong support of this notion, Gibson et al. provide an overlay of the two structures, which reveals that the UDP and PLP groups bind in equivalent locations, with the distal phosphate of UDP overlapping the position of phosphate in glycogen phosphorylase [3]. Thus, the pyridoxal phosphate plus phosphate (from glucose-1-phosphate) in glycogen phosphorylase is behaving much like the uridine diphosphate of UDP-glucose in OtsA (Figures 2 and 3). Their analysis also shows the conservation of basic residues that stabilize the diphosphate moiety. These observations suggest a possible evolutionary explanation for the pyridoxal phosphate requirement of glycogen phosphorylase.

Bioinformatic analysis of genomes by Bernard Henrissat (P.M. Coutinho and B. Henrissat, Carbohydrate-Active Enzymes server at: http://afmb.cnrs-mrs.fr/~cazy/CAZY/index.html) has revealed that while nucleotide phosphosugar-dependent transferases are found throughout all organisms back to the Archea, α-glucan phosphorylases are not. It therefore seems possible that α-glucan phosphorylases evolved from NDP-sugar-dependent glycosyltransferases via the acquisition of a Schiff-base-bound PLP moiety, which took over the role of the UDP in stabilizing the active site. Therefore, the PLP requirement of glycogen phosphorylase may well be a reflection of the evolutionary origins of this enzyme. Indeed, it is hard to think of a better way in which an enzyme could acquire an internal phospho-monoester than to use PLP, with its electrophilic aldehyde as anchor point.

The other intriguing but incomplete conclusion of this comparison of glycogen phosphorylase and OtsA concerns the mechanisms of retaining glycosyltransferases. Although the obvious analogy with retaining glycosidases suggests a double-displacement mechanism via a covalent glycosylenzyme intermediate (Figure 4), many years of study have failed to trap such an intermediate on either α-glucan phosphorylases or retaining transferases [7, 8]. The current study reveals that the functional group best positioned for a role of nucleophile in OtsA is the main chain amide of His154, which per-

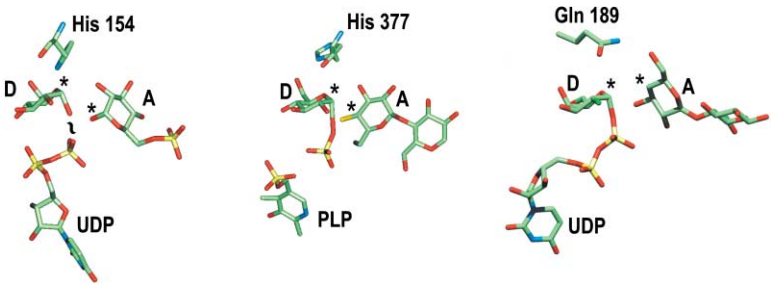

Figure 3. Comparison of Key Active-Site Elements

Comparison of the similarity in disposition of key elements (donor sugar, D; acceptor sugar, A; and potential nucleophile) in the active sites of OtsA [3], maltodextrin phosphorylase [13], and LgtC [10]. The reactive center of the donor sugar and reactive hydroxyl of the acceptor sugar are shown by an asterisk in each case. On the left is shown the abortive complex of OtsA [3] highlighting the UDP and the acceptor sugar glucose-6-phosphate. The donor sugar glucose is not observed in the structure but has been modeled as in [3]. The disposition of a main chain amide oxygen of His154 relative to the anomeric center is shown. In the middle is an active-site representation of maltodextrin phosphorylase. For the sake of this discussion, we have modeled the position of the donor sugar (glucose 1-phosphate) based on the −1 sugar and free phosphage position of a previously published product complex with the thio-oligosaccharide analog, 4-S-α-D-glyco-pyranosyl-4-thiomaltotetraose [13]. The positions of the PLP and acceptor sugar (+1) as well as that of the +2 sugar position are those observed in the published structure (the +3 sugar has been removed for clarity). On the right is shown the active site of LgtC [11] highlighting the donor sugar analog, UDP-2-fluoro-galactose, and acceptor sugar analog, 4-deoxy-lactose (i.e., the reactive hydroxyl at C4 is absent). A potential nucleophile, the side chain amide oxygen of Gln189, is shown in a similar position to the main chain amide oxygen atoms of His154 in OtsA and His377 of phosphorylase.

fectly overlays with the main chain amide of His377 in glycogen phosphorylase. A role as nucleophile for the His377 main chain amide had been suggested previously [9]. Adding to the intrigue is the recent demonstration that the best candidate for this role in the retaining α-galactosyltransferase LgtC from *Neisseria meningitidis* (a GT-A rather than GT-B family fold) is the side chain amide of Gln189 [10]. While an amide might seem an unlikely candidate for such a role, good precedent exists with the hexosaminidases alluded to earlier. However, kinetic analysis of LgtC mutants modified at this position cast doubt on the existence of a true covalent intermediate and have led to the rekindling of an earlier suggestion of an S_Ni-like mechanism [11] involving a late oxocarbenium ion-like transition state wherein the departure of the leaving group and attack of the nucleophile occur in a concerted but asynchronous fashion on the same (α) face of the glycoside. The work of Gibson et al. [3] extends this idea further to include the GT-B fold family retaining transferases, now exemplified by their structure OtsA, and, by analogy, the structurally related glycogen phosphorylases. This controversial mechanism will undoubtedly be the focus of considerable analysis in the coming years.

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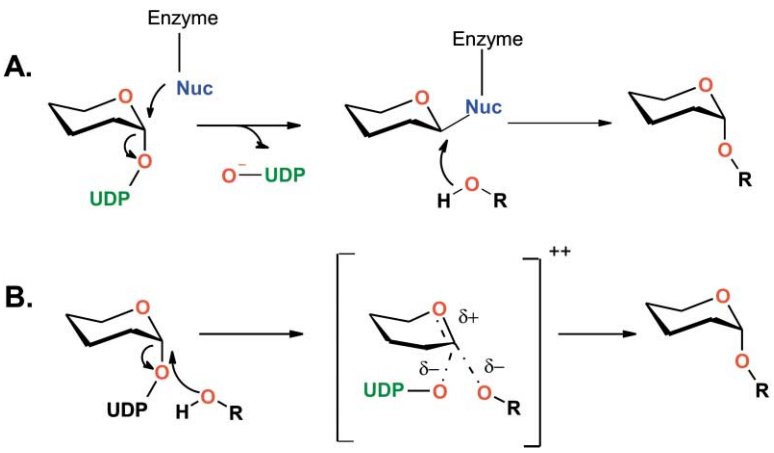

Figure 4. Two Possible Reaction Schemes for Retaining Glycosyltransferases

(A) A base-catalyzed double-displacement mechanism via a covalent intermediate with the enzyme.

(B) An S_Ni-like mechanism [11] involving a late oxocarbenium ion-like transition state wherein the departure of the leaving group and attack of the nucleophile occur in a concerted but asynchronous fashion on the same (α) face of the glycoside.

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