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Enzymatic synthesis using glycoside phosphorylases

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ABSTRACT

Carbohydrate phosphorylases are readily accessible but under-explored catalysts for glycoside synthesis. Their use of accessible and relatively stable sugar phosphates as donor substrates underlies their potential. A wide range of these enzymes has been reported of late, displaying a range of preferences for sugar donors, acceptors and glycosidic linkages. This has allowed this class of enzymes to be used in the synthesis of diverse carbohydrate structures, including at the industrial scale. As more phosphorylase enzymes are discovered, access to further difficult to synthesise glycosides will be enabled. Herein we review reported phosphorylase enzymes and the glycoside products that they have been used to synthesise.

1. Introduction

Enzymatic synthesis of glycans represents an attractive alternative to chemical synthesis, avoiding the need for tedious and extensive protecting group chemistry. Much effort has focused on the exploitation of naturally catabolic glycoside hydrolases, and mutants thereof, in synthesis; glycosyltransferases have also received much attention, in spite of occasional issues with enzyme stability and accessibility of sugar nucleotide donor substrates.[1,2] In contrast, glycoside phosphorylases have received much less attention for the biotransformation of carbohydrates until recently. These latter enzymes utilise accessible and relatively stable sugar-1-phosphates in effecting stereo- and regio-selective synthesis of glycosidic linkages (Fig. 1).[3] This has allowed their use in the synthesis of diverse carbohydrate structures, including the commercial synthesis of 2-*O*-α-D-glucosyl glycerol (**31**)[4] and the kilogram scale synthesis of lacto-*N*-biose (**56**)[5] (vide infra).

The currently characterised glycoside phosphorylases are soluble enzymes that catalyse the reversible addition of phosphate across glycosidic linkages in a stereospecific manner (Fig. 2).[6] The phosphorylase reaction may be inverting or retaining with respect to the glycosidic linkage formed, which is determined by their reaction mechanism and is reflected in their classification in CAZy families.[7]

A recent article from Nakai et al. provides a useful overview of phosphorylase structure and mechanism.[3] Herein we review the utilisation of phosphorylases in glycoside synthesis, providing an update on earlier reviews of this topic.[3,6,8] In a number of instances, the kinetic characterisation of phosphorylase action has been reported but the products of such experiments have not been fully characterised. Nonetheless, such examples are included herein in order to project the potential of phosphorylases as synthesis tools.

Phosphorylases have been found for many types of glycosides, but most identified to date act on D-glucosyl residues (Table 1). In fact, there are characterised disaccharide phosphorylases for every conceivable D-Glc-D-Glc linkage except β-1,1 and α- or β-1,6 (Fig. 3).

In the following sections, current state of knowledge of the various classes of glycoside phosphorylases is outlined.

2. α,α-1,1-D-Glucan phosphorylases

Trehalose (D-Glc-α,α-1,1-D-Glc, **1**), which can accumulate to very high levels in a variety of bacteria, fungi, insects and plants,[9] is used in many different biological roles, including in energy storage,[10] in abiotic stress responses[11] and for osmoregulation.[12] Trehalose metabolism can be by hydrolysis, but it is often by phosphorolysis (Fig. 4).

2.1. Inverting trehalose phosphorylase

The protozoan alga *Euglena gracilis* produces an enzyme that shows phosphorolytic activity towards trehalose.[13] In the

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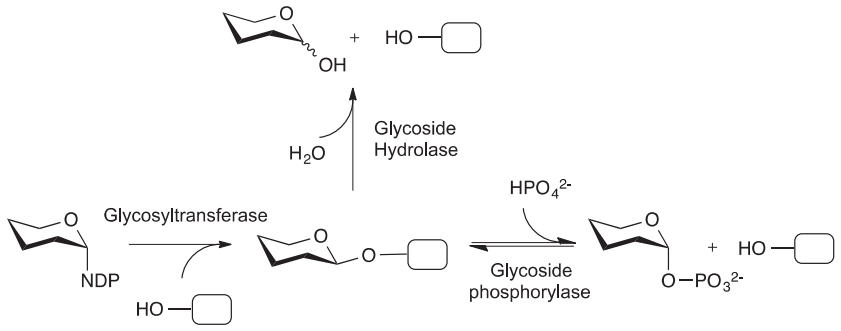

Figure 1. Reactions performed by glycosyltransferases, glycoside hydrolases and glycoside phosphorylases. NDP = nucleotide diphosphate.

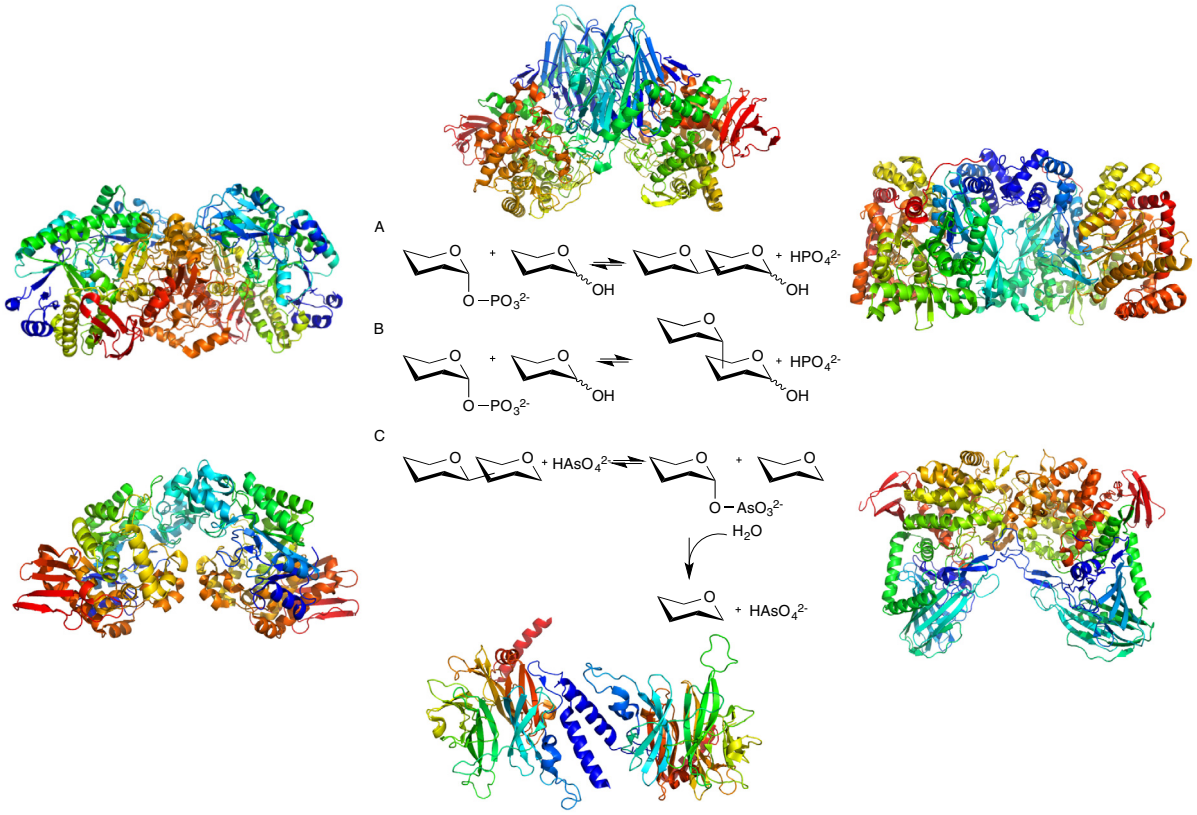

Figure 2. General scheme for phosphorylase actions and representatives of the six families for which structures have been solved. (A) The reaction of inverting phosphorylases. (B) The reaction of retaining phosphorylases. (C) Arsenolysis. GH112, GH94, GT35, GH65, GH130 and GH13, clockwise from top left. (2ZUS,[61] 3QDE,[141] 1GPA,[65] 1H54,[142] 4KMI[127] and 1R7A[137]). Replacement of inorganic phosphate with arsenate releases an unstable sugar-arsenate, which readily decomposes to the free monosaccharide, giving rise to net glycoside hydrolysis.[143]

glycoside synthesis sense, this enzyme activity shows promiscuity towards the 6 position of the acceptor, with both 6-deoxy-D-glucose and D-xylose serving as acceptor substrates, giving glucosides (**13**) and (**14**), respectively (Fig. 5).[14] A similar activity has also been identified in bacteria, including plant symbionts[15] and thermophiles.[16] In the latter case, this activity was shown to be promiscuous with respect to acceptor configuration, as highlighted in Figure 5.[16]

2.2. Trehalose-6-phosphate phosphorylase

As part of their normal trehalose metabolism, many acid bacteria phosphorylate the trehalose and then use trehalose-6-phosphate phosphorylase[17] to release β-D-Glc-1-P (**9**) and D-Glc-6-P (**12**) into the hexose phosphate pool (Fig. 4).[18]

2.3. Retaining trehalose phosphorylase

An alternative trehalose phosphorylase was identified in *Flammulina velutipes* which is retaining, and thus produces α-D-Glc-1-P (Fig. 4, **11**).[19] This enzyme has since been found in many other fungi,[20] but it has not yet been the subject of investigation for use in synthesis.

3. α-1,2-D-Glucan phosphorylases

3.1. Kojibiose phosphorylase

In nature kojibiose, (D-Glc-α-1,2-D-Glc, **2**), is found in honey[21] and as a component of *Leuconostoc* dextran,[22] for instance, whilst Kojidextran is produced by *Rhizobium* species.[23] It is also part of