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Research review paper

Glycoside phosphorylases: Structure, catalytic properties and biotechnological potential ☆

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ABSTRACT

Glycoside phosphorylases (GPs) are the enzymes that reversibly phosphorolytically process glycosidic bond in sucrose (6′-phosphate), α-1,4-glucan and maltodextrins, α-glucobioses, α-1,3-oligoglucan, β-glucobioses and β-glucodextrins, chitobiose, β-galactosides and β-mannosides, and transfer non-reducing end terminal glycosyl residue to inorganic phosphate. They are modular enzymes that form biologically active homooligomers. From a mechanistic as well as structural point of view, they are similar to glycoside hydrolases or glycosyltransferases. Regardless the stereochemical outcome of the phosphorylase-catalyzed reaction (inversion or retention) the phosphorolytic cleavage of glycosidic bond is reversible, therefore glycosyl phosphates may efficiently be used for oligosaccharide synthesis. Although majority of GPs show very high substrate and positional selectivity, they might be employed for a green, inexpensive and often one-pot conversion of one sugar (cheap) to another one (expensive). This fascinating capability is due to the fact that pathways of several GPs share the same glycosyl phosphate, i.e. a product of one phosphorylase is simultaneously consumed as a substrate by another one, or even the same enzyme in a second step if the phosphorylase possesses a relaxed acceptor specificity. In some cases glycosyl phosphates may be interconverted using other auxiliary carbohydrate-active enzymes, achieving for example galactoside synthesis from *gluco*-configured sugar donors, thus widening synthetic potential of these biocatalysts. In comparison with common hydrolysis, the energy of glycosidic bond is not annihilated during phosphorolysis. This energetic aspect of the reactions catalyzed by GPs and their physiological role is discussed in relation to often concurrently occurring glycoside hydrolases.

Contents

Abbreviations: GH, glycoside hydrolase; GP, glycoside phosphorylase; GT, glycosyltransferase.

☆ This review provides an overview of the enzymes phosphorolytically processing glycosidic bond, their catalytic properties, substrate specificity and the structural and mechanistic relation to glycoside hydrolases and glycosyltransferases, with the emphasis on their physiological role and potential for oligosaccharide synthesis.

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1. Introduction

Carbohydrates are ubiquitous molecules that play many functions (Snider, 1981). For most living heterotrophic organisms they are a principle source of energy. Due to a low reactivity they are used as energy storage as well, predominantly in the form of various polysaccharides. Polysaccharides also have a structural function, being crucial components of the cell walls. Oligosaccharides generated from these polysaccharides by various carbohydrate catabolic enzymes (glycoside hydrolases, polysaccharide lyases, glycoside phosphorylases and esterases), or synthesized by anabolic enzymes (glycosyltransferases, transglycosidases and glycoside phosphorylases) often play a role of information and signaling molecules. They mediate cell-environment as well as intracellular compartment communication in order to maintain homeostasis.

Although the number of the most common sugars hardly exceeds that of natural amino acids, the spectrum of their combinations is much more diverse. No doubt that this is due to the fact that each sugar residue carries several hydroxyl groups, each of which may be independently used for an attachment of another carbohydrate or non-carbohydrate moiety. This fact results in a plethora of oligosaccharides identified so far. Despite this complexity in composition and structure, in all living organisms both oligosaccharide synthesis and degradation are accurately controlled at various levels.

Oligosaccharide synthesis usually proceeds from an activated precursor and is catalyzed by glycosyltransferases (GTs). The form of precursor activation varies and may be exemplified by other oligosaccharides, lipid-linked phosphates, sugar pyrophosphates and mainly nucleotide phosphates (Lee et al., 2013). Polysaccharides and oligosaccharides are degraded by several mechanisms. Some of them are subjected to oxidoreductase or lyase cleavage but the most frequent is hydrolysis mediated by batteries of endo- and exo-acting glycoside hydrolases (GHs). Much lesser attention has been paid to still another modification of glycosidic bonds, phosphorolysis, that is analogous to hydrolysis but in this case, the role of water is played by inorganic phosphate. The phosphorolytic processing of glycosides is catalyzed by so-called glycoside phosphorylases (GPs). These enzymes are a topic of this review that is focused on their catalytic properties, structure–function–activity relationship and the potential of their biotechnological exploitation.

2. Overview of glycoside phosphorylases

In addition to oxido-reductive (lytic polysaccharide monooxygenases) and lyase-mediated (polysaccharide lyases) cleavage, three major enzymatic routes are known for the construction or breakdown of a glycosidic linkage (Fig. 1). The most common mechanism for its breakdown is hydrolysis that is mediated by exo- and endo-acting GHs. In contrast, glycosidic linkage is built up by other enzymes called glycosyltransferases. The reactions of both GHs and GTs are essentially irreversible, i.e. the equilibrium is shifted towards the glycosidic bond hydrolysis and synthesis, respectively.

GPs are the enzymes that transfer a glycosyl moiety from the non-reducing end of a polysaccharide or oligosaccharide sugar donor to inorganic phosphate to generate a glycosyl phosphate. However, the phosphorolytic reaction is reversible and its equilibrium constant is of 10^0 order of magnitude (between 0.1 and 10; Doudoroff, 1943; Fitting and Doudoroff, 1952; Kitaoka et al., 1992b; Zhang and Lynd, 2004) since the free energy of glycosidic linkage between carbohydrates is approximately the same as that of ester linkage in glycosyl phosphate. Hence, the enzymes are able to synthesize new glycosidic bond from appropriate glycosyl phosphate and an acceptor. Such a synthetic reaction is usually called reverse reaction and the synthetic potential of these enzymes will be discussed later.

GPs that are currently known (November 2014) and their specificities are summarized in Table 1. Their number has increased since the last reviews on this subject (Kitaoka and Hayashi, 2002; Nakai et al., 2013). According to the third digit of their EC numbers all entries in the table with one exception are hexosyltransferases transferring single hexopyranosyl (aldohexose) residue to inorganic phosphate. The last entry is maltosyltransferase that transfers aldobiose moiety and generates α-maltosyl phosphate (Elbein et al., 2010).

The EC numbers were being given to the phosphorylases roughly chronologically. Therefore, it is not surprising that the first enzyme having the lowest EC number 2.4.1.x, i.e. glycogen/starch phosphorylase (EC 2.4.1.1) is known for almost eight decades (Cori and Cori, 1936). Further GPs, sucrose phosphorylase (EC 2.4.1.7) and maltose phosphorylase (EC 2.4.1.8), were discovered in 1942 (Kagan et al., 1942) and 1952 (Fitting and Scherp, 1952), respectively. On the other hand, it is worth to note that several GPs reported in 2012 and 2013 having the highest EC numbers 2.4.1.3xx (Kawahara et al., 2012; Nihira et al., 2012a,b, 2013b,c; Ladevèze et al., 2013; Verhaeghe et al., 2014; Nihira et al., 2014a,b; Nakajima et al., 2014) were not included in EC classification a half a year ago. The newest enzymes are not included in the EC classification at present (Chiku et al., 2014).

3. Determination of glycoside phosphorylase activity

Classical determinations of GP activity include thin layer chromatography and high-performance liquid chromatography. While the TLC is used as a qualitative assay, HPLC is routinely used for a quantitative analysis. Its drawback is a requirement of a relatively expensive equipment including appropriate chromatography columns. Recently a simplification of the quantification of GP activity has been introduced that is not negatively influenced by a contaminating acid phosphatase. This method relies on a spectrophotometric detection of the phosphate anion based on the formation of blue-color complex with ammonium molybdate (de Groeve et al., 2010b). Since the phosphate anion is consumed during phosphorolysis and generated from glycosyl phosphate during reverse reaction, both processes, i.e. GP-catalyzed phosphorolysis and synthesis, can be monitored and quantified using this methodology. It should be noted that glycosyl phosphates may be also quantified by mass spectrometry (Mizanur et al., 2007), however, it seems not to have found widespread application, presumably due to an expensive equipment.

4. Stereochemical outcome of the reaction catalyzed by glycoside phosphorylases, their classification and substrate specificity

Theoretically, there could be two types of GPs in regard to anomeric configuration of the glycosyl donor and the resulting glycosyl phosphate.