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Review article

β-Glucosidases: Multitasking, moonlighting or simply misunderstood?

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ABSTRACT

β-Glucosidases have a wide range of functions in plants, including roles in recycling of cell-wall oligosaccharides, defense, phytohormone signaling, secondary metabolism, and scent release, among others. It is not always clear which one is responsible for a specific function, as plants contain a large set of β-glucosidases. However, progress has been made in recent years in elucidating these functions. To help understand what is known and what remains ambiguous, we review the general approaches to investigating plant β-glucosidase functions. We consider information that has been gained regarding glycoside hydrolase family 1 enzyme functions utilizing these approaches in the past decade. In several cases, one enzyme has been assigned different biological functions by different research groups. We suggest that, at least in some cases, the ambiguity of an enzyme's function may come from having multiple functions that may help coordinate the response to injury or other stresses.

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1. Introduction

β-Glucosidases (3.2.1.21) remove the nonreducing terminal β-D-glucosyl residue from glucoconjugates, including glucosides, 1-*O*-glucosyl esters, and oligosaccharides. β-Glucosidases have been suggested to have many functions, since there are many compounds containing nonreducing terminal glucosides in plants [1].

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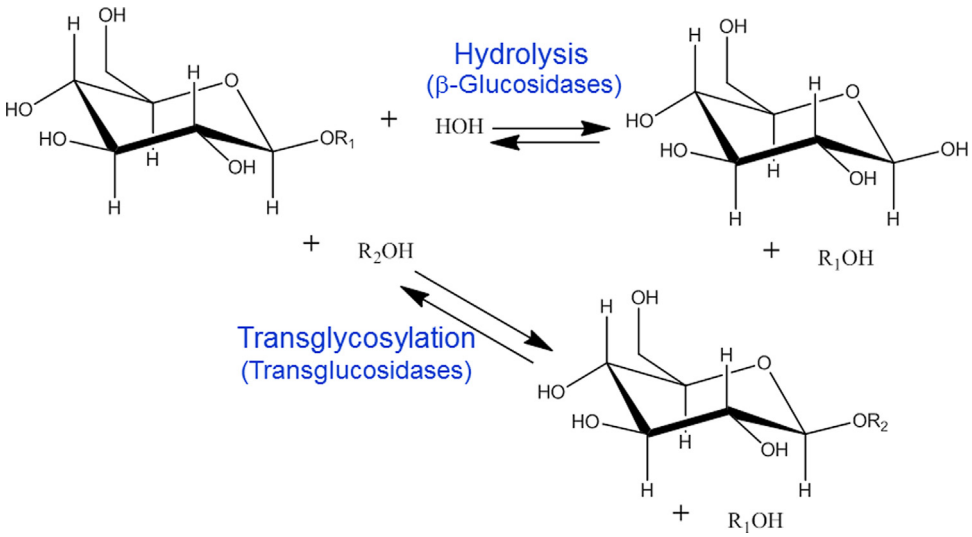

Fig. 1. Hydrolysis and transglycosylation reactions of β-glucosidases and transglucosidases. β-Glucosidases often have significant transglucosidase activity in addition to hydrolase activities, while transglucosidases generally have very little hydrolase activity, even at low acceptor substrate (R_2OH in the figure) concentrations.

These functions include recycling of cell-wall-derived oligosaccharides during cell wall remodeling, release of defense compounds from their innocuous glycoside storage forms, activation of phytohormones and metabolic intermediates by removal of glucosyl blocking groups, release of aroma components from involatile glycosides, and release of monolignols from their glycoside storage forms. Many β-glucosidases have transglucosidase activities in addition to their hydrolase activity (Fig. 1) [2]. This has led to the suggestion they may function in synthesis of glucoconjugates in the plant [3]. Indeed, acyl-glucose-dependent transglucosidases that function in anthocyanin synthesis are closely related to β-glucosidases [4,5].

Before the availability of large sets of genomic sequences around the year 2000, the literature gave the impression that each plant may have one to a few β-glucosidases. Once the genomic sequences started to come out, it became apparent that each plant has many putative β-glucosidase isoenzymes. β-Glucosidases have been categorized into the protein sequence-based glycoside hydrolase families GH1, GH2, GH3, GH5, GH9, GH30 and GH116, with those from plants falling in GH1, GH3, GH5 and GH116 (http://www.cazy.org) [6]. GH1 is the largest family, from which most plant β-glucosidases have been characterized. Arabidopsis (*Arabidopsis thaliana*) has 48 GH1 genes, including 8 apparent pseudogenes [7], rice (*Oryza sativa*) has 38, including 2 putative pseudogenes and 2 gene fragments [8], and maize (*Zea mays*) has 26, including 2 encoding protein fragments too short to be functional β-glucosidases, but excluding the galactolipid galactolipid galactosyl transferase homologue that was included in the Arabidopsis and rice gene counts [9]. However, the number of glucoconjugates in plants is likely larger than the number of β-glucosidases and the enzymes tend to have overlapping specificities, making determination of their exact functions complex.

Here, we review the information necessary to assign the functions of putative β-glucosidases, and the genetic and biochemical approaches that have been used, including their strengths and weaknesses. We then consider the recent investigations into the functions of plant β-glucosidase and related transglucosidases, including the apparent substrates and their functions. In doing so, we will highlight some of the ambiguities in functional assignments and instances where one enzyme has been assigned multiple substrates/activities. These cases of multiple functions may be due to "multitasking," where the enzyme carries out multiple functions at the same time, "moonlighting," where the enzyme has two different functions that occur in separate situations, or simply misunderstanding of the data. Much of this discussion will focus on Arabidopsis and rice, from which the most β-glucosidases have been characterized, although enzymes from other plants will be mentioned where appropriate. We concentrate on family GH1 enzymes, but one must be aware that the enzymes from GH3, GH5 and GH116 (and perhaps as yet unidentified β-glucosidase families) may also contribute to β-glucosidase functions. Many of the issues that are considered for β-glucosidases are similarly relevant for the study of other enzymes with multiple isoenzymes in plants, such as glycosyltransferases.

2. Assignment of functions of β-glucosidases

One must know the biochemical activity, in terms of the substrate and reaction specificity, what substrates the enzyme has access to in the plant, and under what conditions in what tissue, type of cell and cellular compartment the enzyme and substrate are in contact in order to assess the biological role of a putative β-glucosidase. Furthermore, this information is of limited value without understanding the roles of the substrates and products in the plant, and what other factors may regulate the activity of the enzyme and its substrate or product. Biochemical, genetic and analytical chemical approaches have been developed to tease out this information. None of these can define the function of an enzyme on its own, leading to many ambiguities and contradictions in the literature.

2.1. Traditional biochemical approaches

The traditional approach to enzyme function is to identify a putative function, as defined by a specific substrate, and purify a protein with the ability to hydrolyze or transglucosylate that substrate. For instance, three scopolin β-glucosidase isoenzymes were purified and identified from Arabidopsis roots based on this strategy [10]. However, many β-glucosidases are rather promiscuous, leading to uncertainty in assigning the most relevant biological function. This is compounded by the fact that most laboratories have access to a limited number of glucosides and focus on the substrate of interest, and perhaps a few related compounds, and thus do not check for all possible substrate specificities.

In fact, many of the substrates of interest are in relatively short supply or present challenges to assay, so investigators often