

Advances in the Study of Glycosyltransferases in Plant Secondary Metabolite Biosynthesis

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Abstract

Glycosyltransferases are enzymes that can catalyze the transfer of glycosyl groups from an activated donor to a specific receptor molecule, which is ubiquitous in organisms and forms a super gene family. Glycosyltransferases are involved in various biological processes of plant life activities, and play an important role in plant secondary metabolism. This paper summarizes the progress of glycosyltransferases in plant secondary metabolic pathway, describes the gene family and its relationship with the evolution of plant secondary metabolic pathway, and summarizes the current methods of cloning glycosyltransferases Strategy.

Keywords

Glycosyltransferase; Plant Secondary Metabolic Pathway.

1. Introduction

Plants through the secondary metabolism of non-growth and development of large number of small molecules necessary for the development of organic compounds, a wide range of chemical structure is very different. There are now known about 100,000 secondary metabolites, including glycosides, terpenes, phenols, flavonoids, coumarins, lignans, alkaloids, steroids, saponins, polyynes, organic acids, etc., many Secondary metabolites have important pharmacological activity and economic value [1]. Secondary metabolites are formed in the basic framework of the structure, and then after hydroxylation, methylation, acylation, or combined with small molecules such as modification, and ultimately produce a variety of end products. Glycosylation is a widespread modification of the compound, is also an important transformation in vivo reaction -- the synthesis of many metabolites of the final step is a very important step, often occurs glycosylation reaction [2]. Catalytic glycosylation reaction enzyme is glycosyltransferase (Glycosyltransferase, GT, EC 2.4.x.y) [3]. Glycosyltransferases are enzymes that link glycosyl groups to specific receptors by the synthesis of glycosidic bonds. These enzymes are widely found in a variety of prokaryotes, eukaryotes, paleontology and viruses, can identify different receptors, donors and the formation of a wide variety of products. UDP - rhamnose, UDP - xylose and UDP - glucuronic acid, among which, UDP - glucose, UDP - galactose, UDP - rhamnose and UDP - glucuronic acid are the nucleoside diphosphorylated glycosyl - (Glucose, glycoprotein) and glycoside compounds (such as anthocyanin glycosides, flavonoids (such as glycosides), glycosides (such as glycosides), and so on. Glycosides), can catalyze the production of two different types of α and β -glycosidic bond [4-5]. Genome-wide data revealed that about 1% of genes in the eukaryotic genome encode glycosyltransferases [6]

2. The Role of Glycosylation in Plant Secondary Metabolites Synthesis

The role of glycosylation in the synthesis of secondary metabolites in plants From a chemical point of view, glycosyltransferases can alter the chemical stability and water solubility of the modified substances. Anthocyanins are able to determine the flower and fruit color of the plant,

but anthocyanins automatically become colorless anthocyanin cations at the cytoplasm or vacuolar pH, which can be inhibited by glycosylation of 3-OH, increase the aromatic ring stability. Multiple chemical modification of pigments can produce some unique traits, which has great potential in the fruit and color transformation projects [7~10]. Glycosylation can affect the residence area of metabolites, the accumulation of some small molecules in a fixed position (such as vacuole), but also can make some hydrophobic compounds glycosylated and reside in the hydrophilic environment [11]. Sweet Stevia (*Stevia rebaudiana*) leaves rich in a large number of steviol glycosides, the sweetness is about 300 times that of sucrose. Steviol glycosides in plastids are then glycosylated at the C-4 carboxyl position of steviol to form steviol glycosides, which are then transported to the vacuole. Because steviol glycosides begin to accumulate only after glycosylation at this step, it is generally believed that this step is critical for steviol glycoside transport to the vacuole. Using functional genomics to clone 3 in stevia leaves (UGT74G1, UGT76G1 and UGT85C2), in vitro experiments show that they are selective for steviol (Steviol) at different sites of glycosylation [12]. The chemical action of glycosylation also involves altering the chemical activity of the small molecule substance, thereby producing a detoxifying function. Fusarium is a common fungus in the growth and development of cereal crops. *Fusarium oxysporum* can produce the toxin deoxynivalenol (DON), which has serious harm to plant cell growth and human health. A glycosyltransferase UGT73C5 in *Arabidopsis thaliana* can glycosylate this toxic enzyme, resulting in loss of toxicity and increased resistance to UGT73C5-overexpressing transgenic plants [13]. Some plant antitoxins, such as cyanoguanosine CNGs, are found in vacuoles in large amounts in inactive precursors. When they are attacked by herbivores or pathogens, plant tissues are disrupted and immediately react with β -glucosidase and β -alcohol nitrile enzyme reaction, the release of toxic hydrogen cyanide, to achieve the purpose of defense [14].

Glycosylation also plays an important role in modulating the activity of hormones in plants. In addition to ethylene, glycosylated compounds of other classical hormones have been found in plants. However, the mechanism of glycosylation regulating hormone balance is controversial. It was found that glycosyltransferases decrease or even eliminate phytohormones through catabolism in the process of regulation of IAA, ABA, cytokinin (CKs) and brassinosteroids (BRs) biological activity; its mechanism may be the receptor on the hormone identification, it may be changed in some aspects of the physical and chemical properties of hormones. Glycosylated hormone molecules can be metabolized either as inactivated products or stored as transition states, and can be rejuvenated by the hydrolysis of β -glucosidase if desired. The biosynthetic precursor of lignin is a glycosylated form that can transport lignin precursors from intracellular to extracellular, and is essential for lignin biosynthesis [15]. It is generally believed that the combination of small molecules is a hormone inactivation mechanism. However, a recent study of jasmonic acid signal activity found that the binding of isoleucine molecules to jasmonates increased their bioactivity, suggesting that the binding of small molecules could also enhance the biological activity of hormones [16]. Glycosyl is also an indispensable component of some active molecules. An oligosaccharide chain is present at the C-3 position of the saponin which acts in the transmembrane transport and has significant antifungal activity, and the removal of the oligosaccharide chain results in the inactivation of such compounds [17]. In addition, some glucose esters have the role of coenzyme, the conjugates and biosynthetic intermediates in the metabolic pathway with transesterification. Sinapoyl-glucose is an important auxiliary compound in the synthesis of sinapoylmalate in *Arabidopsis thaliana* leaf, and is an important auxiliary compound in the synthesis of sinapoylcholine, an antinutritional compound in *Arabidopsis thaliana* seeds [18-19].

3. The Glycosyltransferase Gene Family and its Relationship with the Evolution of Metabolic Pathways

According to substrate identification, sequence similarity and phylogenetic analysis, glycosylation can be divided into 91 gene families. Most of the genes related to plant secondary metabolism belong to Family I. Glycosylation occurs in its -OH, -COOH, -NH₂, -SH or C-C groups [20]. The glycosylation reaction of the glycosyltransferases in Family I is a small molecule lipophilic compound.

The primary structure of the glycosyltransferases catalyzing secondary metabolism has a common feature - a 44-amino acid conserved region at the C-terminus, the PSPG box (Plant Secondary Product Glycosyltransferase box) [21]. In Family I, about 50% of the glycosyltransferase gene contains a PSPG box, suggesting that this conserved sequence may be associated with glycosyl donor-bound uridine diphosphate-Sugar (UDP-sugar) related. N-terminal sequence variation is large, may be associated with the recognition and binding of different receptors. In the protein structure level, through the trunculus alfalfa (*Medicago truncatula*) in the glycosyltransferase The analysis of the structure of UGT71G1 and donor UDP - glucose indicates that the glycosyltransferase contains two Rossmann coils ($\beta \alpha \beta$ structure), the C - terminal is the activated donor binding domain, the N - terminal is the receptor binding domain, And the role of the C-terminal conserved region-specific amino acid residues in binding sugar donors was found [22]. Through Arabidopsis genome analysis, 119 genes were found in Arabidopsis to encode possible glycosyltransferases by sequence alignment, which were divided into 14 genes. Homology analysis showed that the glycosyltransferase gene in Arabidopsis thaliana had high similarity. Comparison of the glycosyltransferase gene in Arabidopsis with other species showed that although the activity of many enzymes and substrate similar, but the sequence difference. Phylogenetic tree analysis revealed that the glycosylated cytokinase gene in maize and legumes was located on a different branch from the five glycosylated cytokinase genes in Arabidopsis [23]. In Arabidopsis, more than 1/2 of the glycosyltransferase does not contain introns, the remaining genes contain 1-2 introns. By conservative analysis and intron position analysis, it was deduced that there were at least 10 independent intron insertions and 1 or 2 intron loss during the evolution of Arabidopsis thaliana glycosyltransferase gene.

More and more genome data show that, as a large family of genes containing hundreds of members, the evolution of glycosyltransferase gene is not independent, and its evolution needs to consider three factors: one is a gene family, collateral Homologous genes are more likely to be selected and mutated than single-copy genes [24]. Second, there is a large number of co-regulatory and protein-protein interactions in a single metabolic pathway, which may be the co-evolutionary junction of enzymes in the same metabolic pathway [25,26]; and third, chemical analysis indicates that some of the compounds are mutually exclusive between metabolic pathways, such as terpenoid alkaloids and steroid alkaloids in *So lanaceae* [27]. This mutual exclusion indicates that a compound is not required to accumulate other similar compounds and avoid unnecessary energy consumption as long as the accumulation in the plant body has been achieved to protect the plant from injury. The evolution of glycosyltransferase also depends on gene duplication. As a large family of genes, glycosyltransferases perform tandem repeats rather than fragment repeats [28]. UGT genes in Arabidopsis have a common origin [29] suggesting that all of the UGTs in other species genomes may also be a large gene family originating from the same ancestor. This also means that there may be some UGTs gene family is independent of origin. Therefore, the substrate specificity and function of the glycosyltransferase gene can not be deduced by sequence analysis. Glycosyltransferase is the first line of defense of plant itself, which can continuously buffer

environmental pressure and adapt to changes in the environment. They have a high plasticity in plant secondary metabolism, and can be turned at high speed.

4. Glycosyltransferase Gene Cloning Methods and New Strategies

The first discovered glycosyltransferase gene in plants is the Bronze1 gene that controls melanin deposition in maize seeds, discovered by Nobel Prize winner McClintock, which encodes a UDP-glycosyltransferase that produces flavonol glycosides [30]. With the development of biotechnology, biochemistry, bioinformatics, molecular biology and genetics have been successfully applied to the gene cloning, identification and analysis of glycosyltransferase. The classical biochemical method is to obtain the protein with specific glycosyltransferase activity by isolating and purifying, and then cloning the corresponding gene sequence according to the protein sequence. The rhamnosyltransferase gene related to bitterness formation in Citrus which is cloned by this method [31]; bioinformatics is based on glycosyltransferase gene conserved sequence, the target plant EST, cDNA library or genomic sequence homologous alignment, from which found the possible glycosyltransferase gene. The gene of UGT74M1, which is involved in the biosynthesis of hexose ester biosynthesis, was identified and cloned by searching the EST database of *Saponaria vaccaria*. The molecular biology method is based on the conserved sequence of glycosyltransferase gene, designing degenerate primers and cloning the glycosyltransferase gene by RT-PCR method, or cloning the gene by using difference display library screening method. In the *Medicago truncatula*, we analyzed the gene chip of Affymetrix arrays to observe the changes of gene expression in the transcriptome of the cells induced by MJ, and predicted the nine glycosyltransferase genes [32]. The genetic method was mainly through the mutant screening separation purpose gene, by screening blue fluorescence mutants, from the south to cloning to a blue fluorescence formation related to the glycosyltransferase gene UGT74F2 [33]. However, the traditional gene sequence acquisition method can only obtain a single gene, can not glycosyltransferase this large family of genes to conduct a comprehensive systematic study. With the completion of plant genome sequencing of Arabidopsis and rice, the botanical research has entered the post-genome era, that is, functional genomics era. Functional genomics (Functional genomics) as a new research field, emphasizing the development and application of the whole (genomic level or system level) experimental method, the basic strategy is to study from a single gene from the system point of view of all genes, genome sequence analysis information, to clarify gene function [34~35]. However, the Sanger sequencing method used in the Arabidopsis thaliana genome sequencing has high sequencing cost and small amount of data, so it can not be used in large quantities of gene sequences. With the development of sequencing technology, the second generation sequencing platform, represented by the 454 sequencer (Roche GS FLX sequencer), Illumina / Solexa (Illumina Genome Analyzer) and Solid Sequencer (ABI SOLID sequencer), has been widely used. Genome sequencing into a new era of rapid development. All the three sequencing platforms use the principle of pyrosequencing, which has the characteristics of high sequencing throughput, long read length and low cost, and is increasingly applied to transcriptome research. Liet al [36] in 2010 using high-throughput 454 GS FLX 5-year-old licorice vegetative organ transcriptome sequencing group, through bioinformatics analysis of glycosyltransferase gene was 172 key genes. Sun et al. [37] sequenced the transcriptome of 4-year-old American ginseng roots by high-throughput 454 GS FLX in 2010, and sequenced the candidate gene sequences of 235 glycosyltransferases by methyl jasmonate induction and tissue-specific expression analysis. Four glycosyltransferase genes that may be involved in ginsenoside biosynthesis. Functional genomics and high-throughput analysis have contributed to the accuracy and efficiency of glycosyltransferase identification.

5. The Summary and Outlook

As a multi-gene family, which is widely involved in many metabolic pathways, glycosyltransferases are required to respond to various changes in plant growth, development and environment, and are directly involved in the biosynthesis and degradation of different secondary metabolites of plants, So the plant secondary metabolic pathway glycosyltransferase research has important scientific significance. Although the activity and substrate specificity of many plant glycosyltransferases have been tested and characterized in vitro, their function in vivo needs to be further validated. It is encouraging that the biochemical characteristics of most plant glycosyltransferases found so far are consistent with their physiological characteristics in plants. Therefore, the in vitro enzyme activity of glycosyltransferase provides a good basis for its functional analysis in plants. More and more plant glycosyltransferases have been identified for their biological functions, which will provide new insights into the molecular mechanism of plant cell homeostasis and plant growth and development. Plant glycosyltransferase has important application value in enzymatic synthesis of carbohydrate complexes, and also has broad application prospect in improving crop quality.

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