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SOURCE ANALYSIS OF SULFUR DURING THIOSIDE BIOSYNTHESIS

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Abstract: Glucosinolates are a type of secondary metabolite rich in nitrogen and sulfur in Cruciferae plants. A lot of progress has been made in the research on the synthesis pathway of glucosinolates, especially the relationship between sulfur and glucosinolates synthesis. Sources of reduced sulfur donors, sources of activated sulfate, as well as cysteine (Cys), glutathione (GSH) and the high-energy sulfur donor 3′adenosine phosphate 5′phosphoryl sulfate (This paper reviews the research progress of sulfur sources in the process of glucosinolate synthesis, including the relationship between primary sulfur metabolites such as PAPS and glucosinolate synthesis, and proposes the balance between primary sulfur metabolism regulatory factors such as GSH, nitrogen and sulfur and other nutritional elements, and The regulatory mechanism of glucose and other signaling molecules on glucosinolate biosynthesis will become a new research hotspot, in order to provide a theoretical basis for research on the regulation of glucosinolate biosynthesis.
Keywords: Botany; Glucosinolates; Biosynthesis; Glutathione (GSH); Primary sulfur metabolism

1 REDUCED SULFUR DONORS DURING GLUCOSINOLATE SYNTHESIS

Glucosinolates (GS), referred to as glucosinolates, also known as glucosinolates, are a type of anionic substituents rich in nitrogen and sulfur in plants. Biometabolic substances mainly exist in Cruciferae, especially in Brassica plants, such as Brassica rapa ssp. pekinensis, Brassica oleracea, Brassica napus, Brassica juncea, Turnip Brassica rapa, Arabidopsis Arabidopsis thaliana et al[1]. Since BUSSY [2] first discovered glucosinolates from mustard seeds in 1839, the types and degradation products of glucosinolates have gradually been recognized. Currently, more than 132 glucosinolates have been identified [3]. All glucosinolates have a common chemical structure: generally composed of β-D-thioglucosyl, sulfoxime groups and side chain R groups derived from amino acids. According to the different R groups of amino acid side chains, glucosinolates can be divided into 3 categories: aliphatic glucosinolates, indole glucosinolates (side chains mainly derived from tryptophan) and aromatic glucosinolates (side chains mainly derived from phenylalanine or tyrosine) [1,4]. Glucosinolates themselves are relatively stable and have no biological activity. They mainly exist in the vacuoles of plant cells, while glucosinolates (also known as myrosinase) are located in specific protein bodies and only occur when plant tissues are broken, the two come into contact,
Glucosinolates are hydrolyzed under the action of myrosinase to produce isothiocyanates, thiocyanate esters, nitriles and other biologically active substances [4]. These hydrolysates have important biological functions. They are not only the main source of the unique flavor substances of cruciferous vegetables, but also play a role in resisting insect feeding [5-7], pathogenic bacteria infection [8] and various abiotic stresses [9] It also plays an important role in plant defense responses such as moisture, temperature, light, and salt stress. More importantly, it can prevent the occurrence of cancers such as colon cancer, breast cancer, and lung cancer in the human body [10-11]. After decades of research, the biosynthetic pathway of glucosinolates and its regulatory genes have been basically elucidated in the model plant Arabidopsis [12-14]. The biosynthetic process of glucosinolates mainly includes the following three stages: extension of amino acid side chains, formation of core structure and secondary modification of side chains [12]. During the formation of the glucosinolate core structure, nitro compounds or oxidized nitriles react with sulfur donors under the action of glutathione-S-transferase (GST). Combined to form S-alkylthiohydroxime; and under the catalysis of sulfotransferase (SOT), desulfothioside reacts with the high-energy sulfur donor 3′-adenosine phosphate 5′-phosphoryl sulfate (3′-phospho -adenosine- 5′-phosphosullfate (PAPS) binds to generate an SO42- at the N terminus, thus forming the basic glucosinolate structure. Both of these two-step reactions require sulfur donors, and the final glucosinolates contain a large amount of sulfur and are transported to seeds for storage to cope with sulfur deficiency stress and ensure sulfur balance in plants [15]. The author summarized the research progress on sulfur sources in the process of glucosinolate biosynthesis in recent years, and on this basis analyzed the relationship between primary sulfur metabolism and glucosinolate synthesis, hoping to further improve the metabolic network of glucosinolates and provide a basis for future research on sulfur primary and glucosinolate synthesis. Interactions between secondary metabolic pathways provide theoretical guidance.

1.1 Cysteine (Cys) as a Reduced Sulfur Donor

Cysteine (Cys) is the hub of sulfur nutritional metabolism. The inorganic sulfur absorbed by plants enters the organic skeleton through a series of reduction and assimilation reactions to form Cys. Plants use Cys as a precursor to synthesize many substances with important biological functions. Sulfur-containing compounds; therefore, the accumulation of Cys in cells is very low, but the flux is very high [16]. Cys has long been considered as a reducing

sulfur donor in the synthesis of glucosinolates, and is combined with nitro compounds or nitrile oxides to form S-alkylthiohydroximes under the catalysis of GST or GST-like functional enzymes. In vivo experiments with isotope labeling have shown that cysteine is more likely to participate in the synthesis of glucosinolates than methionine and other thiol substances; however, in vitro experiments have shown that thiol substances are easily combined with nitro compounds [17]. Recent studies have shown that glutathione can also provide reducing sulfur for the synthesis of glucosinolates.

1.2 Glutathione (GSH) as a Reduced Sulfur Donor

Glutathione (GSH) is a tripeptide compound composed of glutamic acid, cysteine and glycine. It is a biologically active substance widely present in plants. Its active site is the sulfhydryl group of cysteine [18-19]. The presence of sulfhydryl groups makes it have strong reducing ability; in addition, there is a rare γ-peptide bond between glutamic acid and cysteine, which can protect GSH from being hydrolyzed by many peptidases [18]. The special chemical structure of GSH enables it to have important biological functions. It plays an important role in the storage and transport of reduced sulfur, the regulation of enzyme activity, the removal of reactive oxygen species, and resistance to various adversity stresses (heavy metals, drought stress, salt stress, and bacterial infection) [20-26]. Recent studies have shown that GSH can also serve as a reduced sulfur donor combined with nitro compounds or oxidized nitriles to directly participate in the biosynthesis process of glucosinolates.

The first experiment that pointed out that GSH is involved in glucosinolate synthesis came from the analysis of pad2 mutants. This mutant lacks γ-glutamylcysteine synthetase (γ-Glu-Cys synthetase, GSH1) [27], that is, GSH synthesis The key enzyme of the mutant, the GSH content of the mutant was only 20% of the wild type, while the Cys content increased 5 times, but the glucosinolate content did not change significantly compared with the wild type; while after 24 h of insect (Noctuidae)) induction, mutant indole-3- Methyl glucosinolate and 4- The content of methylsulfinylbutyl glucosinolate is only 50% of the wild type [17,28], which shows that there is a certain relationship between the synthesis of GSH and glucosinolates. However, due to the complexity of GSH function, the specific relationship is was not elucidated in this study.

GEUFLORES et al. [29] used genetic engineering methods to demonstrate for the first time that GSH can provide reducing sulfur for glucosinolate synthesis in tobacco Nicotiana tabacum. When benzylglucosinolate (BGLS) synthesis genes (CYP79A2, CYP83B1, SUR1, UGT74B1, SOT16) were co-expressed in tobacco leaves, low levels of BGLS were produced, but at the same time GSH and nitro compounds were accumulated. Yoke (S-[(Z)-phenylacetohydroximoyl]-L-glutathione, GS-B)[29]. This is because CS lyase (CS lyase, SUR1) cannot catalyze the hydrolysis of the γ-glutamyl group between glutamate and cysteine. This means that there is an enzyme that hydrolyzes the γ-glutamyl group. However, the γ-glutamyl transpeptidase family (GGT family), which has similar functional enzymes, is located in aplastids or vacuoles, while the synthesis of glucosinolates is located in the cytoplasm, which excludes the role of GGT enzymes in glucosinolate synthesis. function[30]. In addition, an enzyme with similar functions was also found in Escherichia coli. This enzyme contains a γ-glutamyltransferase domain [30]. When the γ-glutamyl peptidase (GGP) recombinant containing the homotypic domain was co-expressed in the above-mentioned tobacco leaves, the accumulation of GS-B decreased and the content of BGLS increased (about 5 times). This is Proved the existence of non-GGT family γ-glutamyl hydrolase [29]. In addition, in vitro experiments also demonstrated that GGP1 can catalyze the hydrolysis of γ-glutamyl.

The above proves that GSH provides sulfur for the synthesis of glucosinolates in plants that do not synthesize glucosinolates (tobacco), and proves that GGP1 hydrolyzes γ- glutamyl groups, but it still needs to be proven that this also exists in plants that contain glucosinolates. mechanism. GEUFLORES et al. [31] also used the GGP1 and GGP3 double mutant of the model plant Arabidopsis thaliana as the research object. The study showed that the glucosinolate content of this mutant significantly decreased and accumulated 10 This is a conjugate of GSH and nitro compounds; at the same time, subcellular localization shows that GGP1 and GGP3 are located in the cytoplasm, which is consistent with the enzyme system related to glucosinolate synthesis, which means that GSH can serve as a reducing sulfur in the process of glucosinolate synthesis. The donor provided more clear and direct evidence, and also further proved the hydrolysis of γ-glutamyl group by GGP. However, the double mutant of GGP1 and GGP3 still contains a large amount of glucosinolates, which indicates that there may be other γ-glutamyl hydrolases, which await further study and confirmation [30].

2 THE HIGH-ENERGY SULFUR DONOR 3′ ADENOSINE PHOSPHATE 5′ PHOSPHORYL SULFATE (PAPS) PROVIDES THE ACTIVATED SULFATE FOR GLUCOSINOLATE SYNTHESIS.

The high-energy sulfur donor 3′ adenosine phosphate 5′ phosphoryl sulfate (PAPS) is the accumulated form of activated sulfate in cells and is also the substrate for sulfotransferase (SOT) [32]. In the last step of the synthesis of the glucosinolate core structure, PAPS transfers the sulfate radical (SO42-) to the hydroxyl group of desulfothioside under the action of SOT to form the basic glucosinolate structure [33]. Currently, a total of 18 members of the sulfotransferase family have been found in Arabidopsis, which can be divided into 7 subfamilies based on the homology of their coding sequences. Their main function is to catalyze secondary metabolites such as glucosinolates, flavonoids, and phytosulfopeptins. sulfation reaction [34]. Among them, SOT16, SOT17 and SOT18 are mainly responsible for the sulfation reaction of glucosinolates. Their expression levels are affected by tissues and organs, growth stages and light

conditions, and have different substrate specificities. SOT16 mainly catalyzes indole and aromatic compounds. Sulfation reaction of glucosinolates, while SOT17 and SOT18 have higher affinity for long-chain aliphatic desulfothioglucosinolates [34-35].

PAPS is SO42 absorbed by plants-It is synthesized catalyzed by ATP sulfurylase (ATPS) and APS kinase (APS kinase) with the participation of adenosine triphosphate (ATP). Currently, APK family members have been found in Arabidopsis thaliana There are 4 members, but only the apk1apk2 double mutant has a significantly reduced glucosinolate content and accumulates a large amount of desulfothiosides [34,36-37]. And subcellular localization shows that: the synthesis of glucosinolates is located in the cytoplasm (SOT16, SOT17 and SOT18 are all located in the cytoplasm), while only APK3 in the APK gene family is located in the cytoplasm, and APK1, APK2 and APK4 are all located in the chloroplast [34,37]. This indicates that there is a transport mechanism that transports PAPS Transported from chloroplasts to the cytosol for synthesis of glucosinolates. Recent studies have confirmed that PAPS transporter (PAPST1) can transport PAPS across the membrane to the cytoplasm along the concentration gradient [37].

Further studies have shown that: 3′, 5′-phosphoadenosine (PAP), the product of PAPS after removing the sulfate group, is cytotoxic (inhibits the decomposition of malformed RNA by RNase), and PAPST1 can PAP is transported to chloroplasts along the concentration gradient. In the chloroplasts, PAP phosphatase (FRY1) exists to catalyze the degradation of PAP to form adenosine monophos- phate, AMP) [37]. Therefore, based on the transmembrane transport mechanism of PAPST1 along the concentration gradient, the synthesis and utilization of PAPS and the degradation of PAP interact to jointly regulate the biosynthetic pathway of glucosinolates. The Arabidopsis mutant lacking PAPST1 also had a significantly reduced glucosinolate content and accumulated desothioglucosinolates, but the degree of accumulation was not as good as that of the apk1apk2 double mutant, indicating that there may be other PAPS transporters, which need to be confirmed by further studies. At the same time, this also shows that the sulfation of desulfothioside catalyzed by SOT is not only inhibited by low concentrations of PAPS, but also when the PAPS/PAP transport mechanism is disordered, this reaction is also inhibited [37].

3 THE RELATIONSHIP BETWEEN PRIMARY SULFUR METABOLISM AND GLUCOSINOLATE SYNTHESIS

The sulfate absorbed by plant roots passes through the xylem and phloem under the synergistic action of multiple sulfate transporters, and finally enters the chloroplast or plastid and is activated by ATPS to form adenosine 5'-phosphosulfate (APS) [16,38-39]. APS is energetically unstable and can be reduced by APS reductase (APR) to form SO32-, enters the primary metabolic pathway of sulfur to form sulfur-containing compounds such as cysteine, glutathione, and methionine, and further provides raw materials for the synthesis of secondary metabolites such as glucosinolates; it can also be produced under the action of APK Phosphorylation forms PAPS, which enters the secondary metabolic pathway and provides activated sulfate for the synthesis of secondary metabolites such as glucosinolates [34,37]. Therefore, there is a close relationship between the synthesis of glucosinolates and sulfur metabolism.

3.1 Primary Sulfur Metabolism Provides Raw Materials for the Synthesis of Glucosinolates

Primary sulfur metabolism provides precursor amino acids (methionine, Met), reduced sulfur donors (Cys and GSH) and activated sulfate (PAPS) for the synthesis of glucosinolates. The synthesis of glucosinolates is affected by the sulfur nutrition level in the plant. control. Studies have shown that increasing the application of sulfur fertilizer can significantly increase the accumulation of glucosinolates in plants [40]; under sulfur deficiency conditions, the expression of glucosinolate synthesis genes in Arabidopsis is down-regulated, and the expression of myrosinase-encoding genes is up-regulated, indicating that On the one hand, plants reduce the utilization of sulfur by secondary metabolism by reducing the synthesis of glucosinolates; on the other hand, they increase its degradation to improve the conversion of sulfur from secondary metabolism to primary metabolism, thereby alleviating sulfur deficiency stress and ensuring normal growth and development of plants. [41]. HUSEBY et al. [42] showed that under light conditions, the expression of primary sulfur metabolism genes such as sulfate transporter and APR is up-regulated. At the same time, the expression of glucosinolate synthesis genes is also up-regulated, and the glucosinolate content increases. In addition, Met is the precursor amino acid for the synthesis of aliphatic glucosinolates, and compared with indole and aromatic glucosinolates, aliphatic glucosinolates are more responsive to environmental sulfur supply levels [41].

3.2 Regulation Mechanism of Primary Sulfur Metabolism and Glucosinolate Synthesis

The regulatory mechanisms of primary sulfur metabolism and glucosinolate synthesis are intricate and are affected by key sulfur metabolism genes such as APK and APR, glucosinolate synthesis transcription factors such as MYB, and intracellular redox levels.

In the sulfur metabolism pathway, APS can react with ATP under the action of APK to form PAPS, which provides activated sulfate for the synthesis of glucosinolates; or it can enter the sulfur reduction and assimilation pathway under the action of APR [16,39], Therefore, APS becomes the branch point of the primary and secondary metabolism pathways of sulfur, and APK and APR become important regulatory factors. MUGFORD et al. [36] showed that the glucosinolate content of the apk1apk2 Arabidopsis mutant was only 15% of that of the wild type. Microarray analysis showed that the transcription levels of glucosinolate synthesis genes (UGT74B1, CYP83B1, SUR1, SOT16, SOT18)

were up-regulated, and desulfurization Glucosinolate accumulation; obstruction of PAPS synthesis leads to the upregulation of primary sulfur metabolism pathway, the contents of Cys and GSH increase significantly, but the APS content decreases, and there is no obvious change in APR activity; and the expression of sulfate transport genes and ATPS (ATPS1 and ATPS3) is upregulated. It may be induced by insufficient PAPS [34]. In APK-overexpressed materials, the glucosinolate content did not change significantly (although the transcription levels of MAM3 and SOT17 were up-regulated), but APR activity was induced and the flux into primary sulfur metabolism increased [34,37]. APR is an important regulator of the sulfur reduction and assimilation pathway, and is feedback-inhibited by Cys and GSH. That is, sulfur deficiency will cause the activity of APR to significantly increase; and when the concentration of Cys or GSH is high, the activity of APR will be inhibited. This also reflects the demand-driven nature of the sulfur metabolism pathway [16,39].

MYB is a type of transcription factor that can regulate the synthesis of glucosinolates at the transcription level by regulating the expression of its related genes [12]. Among them, MYB28, MYB29 and MYB76 can regulate the synthesis of aliphatic glucosinolates, and MYB34, MYB51 and MYB122 can regulate the synthesis of indole glucosinolates [12]. Studies have shown that MYB can also directly regulate primary sulfur metabolism genes such as ATPS and APR [39]. Overexpression of MYB leads to up-regulation of the transcription levels of ATPS1 and ATPS3, induction of the primary sulfur metabolism pathway, and increase in GSH content; in mutants lacking MYB, the transcription level of APR is down-regulated [34].

In addition, primary sulfur metabolism and glucosinolate synthesis are also regulated by intracellular redox levels. Studies have shown that: APR is induced by oxidative stress, while APK is more active when the content of reduction products is high. When plants are in a state of stress, the production of reactive oxygen species leads to an increase in APR activity, while APK activity is inhibited and the primary sulfur metabolism pathway is upregulated [34,37]. Therefore, when cells are in an oxidative state, the flux of sulfur into primary metabolism increases, while when cells are in a reducing state, sulfur is more likely to enter secondary metabolic pathways such as glucosinolate synthesis.

4 SUMMARY AND OUTLOOK

Glucosinolates are secondary metabolites rich in nitrogen and sulfur in cruciferous plants. Glucosinolates have been known for a long time as the source of flavor substances in cruciferous vegetables. Glucosinolates and their degradation products are important in plant defense and reduction of It has received increasing attention due to its important biological effects on human cancer incidence. So far, the biosynthetic process of glucosinolates and its regulatory genes have been basically elucidated in the model plant Arabidopsis thaliana. Glucosinolates are an important storage form of organic sulfur in plants, and their synthesis process is also accompanied by the reduction and assimilation of sulfur. Cys and GSH, the products of the sulfur reduction and assimilation pathway, are the reduced sulfur donors in the synthesis process of glucosinolates, and PAPS is glucosinolates. The synthesis of glucosinolates provides activated sulfate, so there is a close connection between the synthesis of glucosinolates and the sulfur reduction and assimilation pathway (primary sulfur metabolism pathway). However, the primary metabolism of sulfur and the synthesis of glucosinolates are affected by key sulfur metabolism genes such as APK and APR, glucosinolate synthesis transcription factors such as MYB, and intracellular redox levels. Their regulatory mechanisms are intricate and there are still many unresolved issues. Issues such as sulfur nutritional signal transduction, coordination between sulfur and other nutrients such as nitrogen, and biological regulation of glucosinolate synthesis by signal exchange between glucose and other plant hormones need to be further studied to clarify the primary origin of sulfur. Interactions between metabolic and secondary metabolic pathways. In addition, when GSH is used as the reduced sulfur donor for glucosinolate synthesis, it is not clear whether the hydrolysis of carboxypeptidase still exists, and further exploration is needed; the specific mechanism of action of GGP is still in the inference stage and still needs further research; the GSH The regulatory effect of dynamic equilibrium on glucosinolate synthesis is also a matter of concern and may become a new research hotspot. With the continuous deepening of scientific research, genetics, proteomics, metabolomics, genetic engineering and other means can be used to artificially manipulate the biosynthesis of glucosinolates in the future, which will be helpful for improving crop resistance to pests and diseases and anti-cancer activity of crops. And the selection and improvement of new varieties are of great significance.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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