Research Advances on the S-Alk(en)yl Cysteine Sulfoxide Metabolic Pathway in Allium Plants (Postprint)

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Abstract

Allium is one of the largest genera of angiosperms, comprising numerous vegetable crops with characteristic pungent flavors such as garlic, onion, scallion, and leek. S-alk(en)yl cysteine sulfoxides are characteristic secondary metabolites unique to Allium plants, serving as precursors for various volatile sulfur compounds that confer the distinctive pungent flavor and medicinal value to these plants. Therefore, investigating the metabolic pathways of S-alk(en)yl cysteine sulfoxides in Allium plants is of great significance. Seven types of S-alk(en)yl cysteine sulfoxides have been identified in Allium plants, which are primarily synthesized in leaves via the glutathione pathway and subsequently transported to the cytoplasm of storage organs such as bulbs for accumulation. Currently, research on the degradation of S-alk(en)yl cysteine sulfoxides in Allium plants is relatively extensive, whereas studies on their biosynthesis remain limited. S-alk(en)yl cysteine sulfoxides represent downstream products of plant sulfur metabolism, whose upstream processes involve the uptake and transport of sulfur-containing compounds, as well as the metabolism of cysteine and glutathione; alterations in these metabolic processes may also influence the biosynthesis of S-alk(en)yl cysteine sulfoxides. Future research should focus on two aspects: first, continuing to clone and identify key enzyme genes in the biosynthetic pathway of S-alk(en)yl cysteine sulfoxides and investigating their functions; second, enhancing research on sulfur metabolism in Allium plants to establish a foundation for studying the regulation of S-alk(en)yl cysteine sulfoxide biosynthesis. These studies will provide valuable insights for deeply elucidating the metabolic pathways of S-alk(en)yl cysteine sulfoxides in Allium plants and for regulating the flavor of Allium crops through molecular breeding techniques.

Full Text

Research Progress on the Metabolic Pathway of S-Alk(en)ylcysteine Sulfoxides in Allium Plants

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Abstract

Allium is one of the largest genera of angiosperms, comprising many vegetable crops with distinctive pungent flavors, such as garlic, onion, Welsh onion, and Chinese chive. S-alk(en)ylcysteine sulfoxides are unique secondary metabolites in Allium plants that serve as precursors for various volatile sulfur compounds, imparting the characteristic pungent flavor and medicinal value to these species. Therefore, investigating the metabolic pathways of S-alk(en)ylcysteine sulfoxides in Allium plants holds significant importance. To date, seven S-alk(en)ylcysteine sulfoxides have been identified in Allium, primarily synthesized in leaves via the glutathione pathway and subsequently transported to the cytoplasm of storage organs like bulbs for accumulation. Current research has focused extensively on the catabolism of S-alk(en)ylcysteine sulfoxides, while studies on their biosynthesis remain limited. As downstream products of plant sulfur metabolism, S-alk(en)ylcysteine sulfoxide biosynthesis is influenced by upstream processes including sulfur compound uptake, transport, and the metabolism of cysteine and glutathione. Future research should prioritize two aspects: first, continued cloning and functional characterization of key enzyme genes in the S-alk(en)ylcysteine sulfoxide biosynthetic pathway; and second, strengthened investigation of sulfur metabolism in Allium to establish a foundation for understanding the regulation of S-alk(en)ylcysteine sulfoxide biosynthesis. These efforts will provide valuable insights for elucidating the metabolic pathways of S-alk(en)ylcysteine sulfoxides and for regulating Allium flavor through molecular breeding technologies.

Keywords: Allium, S-alk(en)ylcysteine sulfoxide, alliinase, flavor compounds, metabolic pathway

1. S-Alk(en)ylcysteine Sulfoxides and Their Biosynthetic Intermediates in Allium Plants

1.1 S-Alk(en)ylcysteine Sulfoxides in Allium Seven S-alk(en)ylcysteine sulfoxides have been isolated and identified from Allium plants (Figure 1 [Figure 1: see original paper]). Stoll and Seebeck (1948) first isolated S-allylcysteine sulfoxide (commonly known as alliin) from garlic. Subsequently, Virtanen and Matikkala (1959) identified S-1-propenylcysteine sulfoxide (isoalliin), S-methylcysteine sulfoxide (methiin), and S-propylcysteine sulfoxide (propiin) in onion. These four S-alk(en)ylcysteine sulfoxides constitute the primary source of Allium' s distinctive flavor and medicinal properties. In addition to these four, three minor S-alk(en)ylcysteine sulfoxides have been discovered: S-ethylcysteine sulfoxide (ethiin), S-butylcysteine sulfoxide (butiin), and cycloalliin (Ueda et al., 1994; Kubec et al., 2000; Kubec et al., 2002). Among the seven compounds, alliin, isoalliin, and cycloalliin are structural isomers.

Different Allium species produce distinct flavor profiles due to variations in their S-alk(en)ylcysteine sulfoxide composition. Alliin reaches its highest concentration in garlic bulbs, while being nearly absent in onion and Welsh onion. Isoalliin is the predominant S-alk(en)ylcysteine sulfoxide in onion bulbs and Welsh onion, whereas methiin is most abundant in Chinese chive (Edwards et al., 1994b; Kubec et al., 2000; Fritsch & Keusgen, 2006; Yamazaki et al., 2011). Methiin is ubiquitous across Allium species, while propiin has only been detected in some species at low concentrations (Fritsch & Keusgen, 2006).

1.2 Biosynthetic Intermediates of S-Alk(en)ylcysteine Sulfoxides In addition to S-alk(en)ylcysteine sulfoxides, Allium plants contain numerous biosynthetic intermediates, including γ-glutamyl-S-alk(en)ylcysteine, γ-glutamyl-S-alk(en)ylcysteine sulfoxide, S-alk(en)ylglutathione, and S-alk(en)ylcysteine (Whitaker, 1976). These intermediates not only serve as precursors for S-alk(en)ylcysteine sulfoxides but also function in nitrogen and sulfur storage (Jones et al., 2004; Rose et al., 2005).

Research has demonstrated that these intermediates in the S-alk(en)ylcysteine sulfoxide biosynthetic pathway possess medicinal and health-promoting properties. S-allylcysteine from garlic exhibits anticancer and cholesterol-lowering effects, and shows promise in preventing and treating Alzheimer' s disease (Ray et al., 2011; Ng et al., 2012; Colín-González et al., 2015). Additionally, the stereoisomer S-1-propenylcysteine demonstrates efficacy in preventing and alleviating cardiovascular diseases, particularly hypertension (Kodera et al., 2017; Matsutomo et al., 2017).

2. Biosynthetic Pathway of S-Alk(en)ylcysteine Sulfoxides in Allium Plants

2.1 The Biosynthetic Pathway Radioisotope tracing studies have revealed that the cysteine moiety of S-alk(en)ylcysteine sulfoxides primarily originates from cysteine residues in glutathione, while the alk(en)yl groups (allyl, propenyl, and propyl) are derived from methacrylic acid produced through valine metabolism. The origins of methyl, ethyl, and butyl groups remain unclear (Lancaster & Shaw, 1989; Edwards et al., 1994a). Cycloalliin and isoalliin are isomers that exist in minimal amounts in onion at room temperature, but isoalliin converts substantially to cycloalliin upon heating (Ueda et al., 1994).

The currently accepted biosynthetic pathway for S-alk(en)ylcysteine sulfoxides in Allium is the glutathione pathway (Figure 2 [Figure 2: see original paper]). In this pathway, glutathione first conjugates with methacrylic acid from valine metabolism to form S-(2-carboxypropyl)glutathione. Subsequent removal of the glycyl group yields γ-glutamyl-S-(2-carboxypropyl)cysteine, which undergoes decarboxylation and oxidation of the S-2-carboxypropyl group to form γ-glutamyl-S-allylcysteine or γ-glutamyl-S-propenylcysteine (Chhabria & Desai, 2014; Yoshimoto & Saito, 2019; Sun et al., 2020). γ-Glutamyl-S-allylcysteine and γ-glutamyl-S-propenylcysteine are interconvertible isomers, and their S-allyl and S-propenyl groups can be reduced to form γ-glutamyl-S-propylcysteine (Lancaster & Shaw, 1989). These three γ-glutamyl compounds are then converted to alliin, isoalliin, and propiin through S-oxygenation and deglutamylation reactions. The order of these reactions varies among Allium species and S-alk(en)ylcysteine sulfoxide types. In onion isoalliin biosynthesis, S-oxygenation likely precedes deglutamylation (Lancaster & Shaw, 1989), whereas in garlic alliin biosynthesis, the opposite occurs—S-oxygenation follows the removal of the γ-glutamyl group (Yoshimoto et al., 2015a; Yoshimoto et al., 2015b). Methiin biosynthesis follows a similar pattern (Lancaster & Shaw, 1989). In addition to the glutathione pathway, an alternative route may exist in Allium that bypasses glutathione, involving direct alkylation of cysteine or thioalkylation of O-acetylserine to form S-alk(en)ylcysteine, which then undergoes S-oxygenation to produce various S-alk(en)ylcysteine sulfoxides (Ikegami & Murakoshi, 1994).

2.2 Enzymes Involved in S-Alk(en)ylcysteine Sulfoxide Biosynthesis Each step in plant secondary metabolite biosynthesis typically requires specific enzyme catalysis, and S-alk(en)ylcysteine sulfoxide biosynthesis in Allium is no exception. However, research on the enzymes involved remains limited. To date, only γ-glutamyl transpeptidase (GGT; EC 2.3.2.2) catalyzing deglutamylation and flavin-containing monooxygenase (FMO; EC 1.14.13.8) catalyzing S-oxygenation have been molecularly characterized in garlic (Yoshimoto et al., 2015a; Yoshimoto et al., 2015b). In Arabidopsis thaliana, γ-glutamylcysteine synthetase (GSH1; EC 6.3.2.2) and glutathione synthetase (GSH2; EC 6.3.2.2) are key enzymes in glutathione biosynthesis, while phytochelatin synthase (PCS;

EC 2.3.2.15) catalyzes the removal of glycyl groups from glutathione conjugates (May & Leaver, 1994; Rawlins et al., 1995; Blum et al., 2007). Sun et al. (2020) identified four Arabidopsis GSH1 homologs, one GSH2 homolog, and one PCS homolog in the garlic genome as candidate genes for alliin biosynthesis, and examined their spatiotemporal expression patterns, though functional studies were not pursued further.

2.2.1 The De-γ-glutamyl Reaction in S-Alk(en)ylcysteine Sulfoxide Biosynthesis Two classes of enzymes possess de-γ-glutamyl activity in plants: the GGT family (four members in Arabidopsis) that primarily catalyzes the removal of γ-glutamyl groups from glutathione and its conjugates in extracellular or vacuolar compartments (Martin et al., 2007), and the γ-glutamyl peptidases (GGPs) family (five members in Arabidopsis) that catalyzes this reaction for glutathione conjugates in the cytoplasm (Geu-Flores et al., 2011). In the Allium S-alk(en)ylcysteine sulfoxide biosynthetic pathway, the intermediate γ-glutamyl-S-alk(en)ylcysteine must undergo deglutamylation to generate the final S-alk(en)ylcysteine sulfoxide, suggesting that de-γ-glutamyl enzymes are essential components of this pathway.

Cho et al. (2012) cloned a GGT sequence fragment from garlic whose expression increased significantly during low-temperature storage, suggesting its involvement in alliin synthesis during the greening process. Yoshimoto et al. (2015a) subsequently cloned three GGT genes from garlic, designated AsGGT1, AsGGT2, and AsGGT3, with AsGGT3 being identical to the sequence reported by Cho et al. (2012). Phylogenetic analysis revealed that AsGGT1 and AsGGT2 are most closely related to Arabidopsis AtGGT4, while AsGGT3 clusters with AtGGT1 and AtGGT2. In vitro enzyme assays demonstrated that all three GGTs catalyze the removal of γ-glutamyl groups from γ-glutamyl-S-allylcysteine to produce S-allylcysteine, but show negligible activity toward γ-glutamyl-S-allylcysteine sulfoxide, indicating that deglutamylation precedes S-oxygenation in garlic. Subcellular localization analysis revealed that AsGGT2 primarily localizes to the vacuole, whereas AsGGT1 and AsGGT3 lack clear signal peptides and may reside in the cytoplasm. In Arabidopsis, AtGGT1 and AtGGT2 localize to the outer side of the plasma membrane, while AtGGT3 and AtGGT4 localize to the vacuole; no cytoplasmic GGTs have been reported (Grzam et al., 2007; Ohkama-Ohtsu et al., 2007a; Ohkama-Ohtsu et al., 2007b). Therefore, AsGGT2 may share functional similarity with AtGGT4, while the cytoplasmic roles of AsGGT1 and AsGGT3 require further investigation. To date, two GGT genes have been identified in onion: one AcGGT purified from germinating onion bulbs exhibits high substrate specificity for intermediates in S-alk(en)ylcysteine sulfoxide synthesis (Lancaster & Shaw, 1994), while another shows high specificity for glutathione and its S-conjugates but no activity toward γ-glutamyl-S-propenylcysteine sulfoxide (Shaw et al., 2005).

No functional studies of GGP family genes have been reported in Allium.

In Arabidopsis, GGP1 and GGP3 play important roles in glucosinolate and phytoalexin biosynthesis, primarily catalyzing deglutamylation of glutathione S-conjugates in the cytoplasm (Geu-Flores et al., 2011). In onion cells, S-alk(en)ylcysteine sulfoxides and their γ-glutamyl intermediates accumulate mainly in the cytoplasm (Lancaster et al., 1989). Therefore, we hypothesize that the deglutamylation reaction in Allium S-alk(en)ylcysteine sulfoxide biosynthesis occurs in the cytoplasm. Whether Arabidopsis GGP1 and GGP3, which localize to the cytoplasm, participate in this process requires further investigation.

2.2.2 The S-Oxygenation Reaction in S-Alk(en)ylcysteine Sulfoxide Biosynthesis S-oxygenation represents one of the most crucial steps in Allium S-alk(en)ylcysteine sulfoxide biosynthesis, as alliinase is only active toward S-alk(en)ylcysteine sulfoxides and not other sulfur-containing intermediates. Flavin-containing monooxygenases (FMOs) are widely distributed in animals, plants, and microorganisms, catalyzing oxygenation reactions. In the presence of the FAD cofactor and NADPH, FMOs can transfer hydroxyl groups to small, nucleophilic, heteroatom-containing (e.g., nitrogen, sulfur, selenium, or iodine) substrates (Krueger & Williams, 2005; Schlaich, 2007). Plant FMOs are classified into three evolutionary clades based on amino acid sequence similarity and play important roles in natural product biosynthesis (Schlaich, 2007). For example, FMO1 from clade I participates in biosynthesis of N-hydroxypipecolic acid, a key regulator of systemic acquired resistance (Hartmann et al., 2018); clade II members are important for auxin synthesis (Yamamoto et al., 2007; Mashiguchi et al., 2011); and clade III FMOs catalyze S-oxygenation of methylthioalkyl glucosinolates, playing crucial roles in side-chain modification of aliphatic glucosinolates (Hansen et al., 2007; Li et al., 2008; Kong et al., 2016). Studies on mammalian FMOs have shown that some can catalyze the conversion of S-allylcysteine to alliin (Krause et al., 2002; Novick & Elfarra, 2008).

Based on these findings, we hypothesize that FMO proteins also catalyze S-oxygenation in Allium. Before cloning of the Allium S-oxygenase, some of its properties were already known. S-oxygenases from different Allium species show no substrate selectivity among various S-alk(en)ylcysteine types—for example, onion S-oxygenase can catalyze formation of isoalliin from S-propenylcysteine, as well as methiin, ethiin, and alliin from S-methylcysteine, S-ethylcysteine, and S-allylcysteine, respectively. Moreover, S-oxygenases from various Allium species can all catalyze alliin formation from S-allylcysteine (Ohsumi et al., 1993). Using homology-based cloning, Yoshimoto et al. (2015b) identified a 1,371 bp gene from garlic encoding a 457-amino acid protein belonging to the plant FMO family clade III, localized to the cytoplasm, and designated it AsFMO1. In vitro protein activity assays revealed that recombinant AsFMO1 exhibits strong stereoselectivity, producing only (+)-alliin (RCSS-S-allylcysteine sulfoxide), and strong substrate selectivity, showing high activity toward S-allylcysteine but weak activity toward γ-glutamyl-S-allylcysteine. Whether other FMO family members

in Allium participate in S-alk(en)ylcysteine sulfoxide biosynthesis requires further investigation.

2.3 Sites of Biosynthesis and Accumulation S-alk(en)ylcysteine sulfoxide content varies dramatically among Allium tissues. Garlic and onion bulbs are the primary storage organs, while Chinese chive flowers contain the highest concentrations (Yoshimoto & Saito, 2019; Liu et al., 2021). These compounds are primarily synthesized in green vegetative leaves, with chloroplasts being essential for biosynthesis (Lancaster et al., 1988; Yoshimoto & Saito, 2019). The synthesized S-alk(en)ylcysteine sulfoxides are transported via vascular systems to developing bulbs (Yamazaki et al., 2002). In mature garlic bulbs, substantial amounts of alliin accumulate along with some γ-glutamyl-S-allylcysteine in storage leaves. During garlic bulb germination, γ-glutamyl-S-allylcysteine is converted to alliin through deglutamylation and S-oxygenation, potentially protecting young shoots from pathogens and herbivores (Ichikawa et al., 2006; Yoshimoto et al., 2015a; Yoshimoto & Saito, 2019). At the subcellular level, S-alk(en)ylcysteine sulfoxides accumulate mainly in the cytoplasm, though the precise synthetic compartment remains unclear and requires further investigation. This is because glutathione, a key participant in biosynthesis, is synthesized in both cytoplasm and chloroplasts, while methacrylic acid, another participant, is produced through valine metabolism occurring in mitochondria or peroxisomes (Lancaster et al., 1989; Binder et al., 2007).

2.4 Effects of Sulfur and Selenium Fertilizers S-alk(en)ylcysteine sulfoxide biosynthesis in Allium is influenced by soil sulfur content. Increased sulfur fertilization significantly enhances S-alk(en)ylcysteine sulfoxide accumulation in garlic and onion bulbs (Randle et al., 1995; Bloem et al., 2004; Lundegårdh et al., 2008). Sulfur levels also affect the proportion of S-alk(en)ylcysteine sulfoxides among total sulfur compounds and the ratio between isoalliin and methiin. Under sulfur deficiency, the proportion of S-alk(en)ylcysteine sulfoxides among total sulfur compounds increases. Additionally, while isoalliin is the dominant S-alk(en)ylcysteine sulfoxide in onion bulbs under sufficient sulfur conditions, methiin becomes predominant under sulfur deficiency (Randle et al., 1995).

Selenium is an essential trace element for human health. The ability of Allium to accumulate sulfur compounds makes it a potential crop for selenium biofortification. Due to similar chemical properties, many enzymes involved in sulfur metabolism also function in selenium metabolism. Allium plants can incorporate selenium instead of sulfur into their metabolic pathways, forming Se-alk(en)ylselenocysteine sulfoxides (Trippe & Pilon-Smits, 2021; González-Morales et al., 2017). Selenium-enriched Allium primarily accumulates selenium as organic selenium compounds such as Se-methylselenocysteine and γ-glutamyl-Se-methylselenocysteine (Dong et al., 2001; Shah et al., 2004; Arnault & Auger, 2006), which exhibit excellent antitumor effects. Therefore, appropriate selenium fertilization during Allium cultivation can significantly increase organic selenium content, enhancing both health benefits and economic value.

3. Degradation Pathway of S-Alk(en)ylcysteine Sulfoxides in Allium Plants

3.1 The Degradation Pathway S-alk(en)ylcysteine sulfoxides serve as precursors for various flavor-active compounds in Allium. Subcellular fractionation and immunohistochemical studies have shown that these sulfoxides accumulate in the cytoplasm of mesophyll cells, while alliinase is sequestered in the vacuoles of bundle sheath cells (Lancaster & Collin, 1981; Ellmore & Feldberg, 1994; Yamazaki et al., 2002). Upon cellular disruption, alliinase contacts S-alk(en)ylcysteine sulfoxides, catalyzing their conversion to alk(en)ylsulfenic acids, pyruvate, and ammonia (Rose et al., 2005). With the exception of onion, which contains lachrymatory factor synthase (LFS) that catalyzes conversion of (Z)-1-propenylsulfenic acid to the lachrymatory factor ((E)-propanthial S-oxide), other Allium species lack enzymes that act on alk(en)ylsulfenic acids (Imai et al., 2002). Alk(en)ylsulfenic acids are highly unstable and spontaneously undergo a series of reactions to form over 50 sulfur-containing compounds without enzymatic catalysis (Nohara et al., 2017; Yoshimoto & Saito, 2019; Nohara et al., 2021).

Two molecules of alk(en)ylsulfenic acid can spontaneously condense, eliminating one H_2O molecule to form thiosulfinates. The first characterized thiosulfinate was diallyl thiosulfinate from garlic, commonly known as allicin (Cavallito & Bailey, 1944). Thiosulfinates are also unstable and can further react spontaneously to generate various sulfur compounds through [3,3]-σ rearrangements, intramolecular cycloadditions, and Diels-Alder reactions, producing compounds such as ajoene, diallyl trisulfide (DATS), diallyl disulfide (DADS), diallyl sulfide (DAS), 2-vinyl-4H-1,3-dithiin, and 3,4-dimethiolane (Nohara et al., 2017; Block et al., 2018; Kubec et al., 2018). These compounds not only confer Allium' s characteristic pungent flavor but also contribute to its high medicinal and health value.

The greening or reddening reactions that occur during Allium processing and storage also relate to S-alk(en)ylcysteine sulfoxide degradation. The pigments responsible for these color changes consist of multiple chromogenic substances. Although the specific pigments differ between species (e.g., garlic greening vs. onion reddening), their biosynthetic mechanisms are similar (Kubec et al., 2004; Imai et al., 2006; Kato et al., 2013; He et al., 2017; Kubec et al., 2017). Most studies have confirmed that isoalliin is the key compound for these discoloration reactions (Lukes, 1986; Kubec et al., 2004; Cho et al., 2009; Dong et al., 2010). Isoalliin-derived 1-propenylsulfenic acid, generated by alliinase cleavage, can spontaneously condense to form 1-propenyl thiosulfinate or intermolecularly condense with other sulfenic acids to generate 1-propenyl-containing thiosulfinates (Kubec & Velíšek, 2007). These thiosulfinates can react with virtually all amino acids in Allium to form pyrrolyl amino acids, precursors of pigment substances (Cho et al., 2009; Lee et al., 2012). As

pigment precursors, pyrrolyl amino acids then react with naturally occurring (thio)carbonyl compounds to produce the final pigments (Kato et al., 2013; Kubec et al., 2017).

3.2 Key Enzymes in the Degradation Pathway

3.2.1 Alliinase Alliinase, also known as alliin lyase or S-alk(en)ylcysteine sulfoxide lyase, is a type I pyridoxal 5' -phosphate (PLP)-dependent C–S bond lyase that hydrolyzes S-alk(en)ylcysteine sulfoxides to produce alk(en)ylsulfenic acids, pyruvate, and ammonia. This enzyme is crucial for forming Allium's flavor and bioactive compounds (Nock & Mazelis, 1987; Manabe et al., 1998). Notably, significantly reduced expression of alliinase genes in onion affects lachrymatory factor and flavor compound synthesis, producing non-pungent, tearless onions (Kato et al., 2016). Therefore, alliinase genes represent important targets for artificial regulation of Allium flavor profiles.

Previous studies indicate that alliinase is encoded by multigene families in Allium (Ovesná et al., 2015; Sayadi et al., 2020). Alliinase genes have been cloned from multiple species including garlic, onion, Chinese chive, and shallot (Van Damme et al., 1992; Manabe et al., 1998; Lancaster et al., 2000; Do et al., 2004; Tang, 2013; Bai, 2017). Without genomic information, the exact number and chromosomal distribution of alliinase genes remained unclear until 2020, when Sun et al. sequenced the garlic genome and identified 60 alliinase genes, 38 of which are expressed across different tissues. Alliinases from bulbs and leaves differ from those in roots, showing high homology within the same tissue but low homology between different tissues (Rabinkov et al., 1994; Tang, 2013).

Most Allium alliinase genes encode approximately 480 amino acids, with an N-terminal signal peptide of 30–40 residues targeting the enzyme to the vacuole (Van Damme et al., 1992; Manabe et al., 1998). Alliinases typically exist as homo-oligomers: garlic alliinase is a dimer composed of two 51.5 kDa subunits, while onion alliinase forms trimers or tetramers of 50 kDa subunits (Nock & Mazelis, 1986; Nock & Mazelis, 1987; Rabinkov et al., 1994). Alliinase has broad substrate specificity—all six non-cyclic S-alk(en)ylcysteine sulfoxides can serve as substrates, though enzyme activity varies among different Allium species and substrates (Cheng & Guo, 2001). Activity is also affected by temperature, pH, and metal ions (Jansen et al., 1989; Krest & Keusgen, 1999).

Comparative analysis of cloned Allium alliinase sequences reveals four conserved domains: an epidermal growth factor-like (EGF-like) domain, a PLP-binding domain, an aspartate aminotransferase superfamily domain, and a catalytic domain (Tang, 2013). The PLP-binding domain, located in the central region, has been extensively studied. The PLP cofactor binds to a lysine residue within this domain, with the binding site well-characterized across species: Lys-251 in mature garlic alliinase, Lys-280 in Chinese chive alliinase, and Lys-285 in onion bulb alliinase (Kitamura et al., 1997; Manabe et al., 1998; Shimon et al., 2007). The EGF-like domain at the N-terminus contains six cysteine residues

arranged as C-x18-19-C-x-C-x2-C-x5-C-x6-C. Its exact function remains unclear but may be involved in vacuolar targeting (Kuettner et al., 2002). This domain is rare in plant proteins but highly conserved in alliinases, serving as a structural marker for identifying novel alliinases (Sayadi et al., 2020). Crystal structure analysis of garlic alliinase shows that eight of its ten cysteine residues form four disulfide bonds: Cys20-Cys39, Cys41-Cys50, Cys44-Cys57, and Cys368-Cys376. The first three are located in the EGF-like domain, while the fourth at the C-terminus is important for stabilizing the catalytic region and maintaining the relative orientation of substrate and cofactor. The remaining two cysteine residues, Cys220 and Cys350, are free thiols distant from the active site, and their chemical modification does not affect enzyme activity (Weiner et al., 2009). Alliinase is also a glycoprotein with multiple glycosylation sites per monomer. Sequence analysis predicts four potential glycosylation sites in garlic alliinase: Asn19, Asn146, Asn191, and Asn328 (Rabinkov et al., 1995). Crystal structure analysis reveals that only Asn146 and Asn328 are utilized: Asn146, located at the subunit interface, links two subunits via glycan chains to maintain dimer stability, while Asn328 resides on the dimer surface without contacting any enzyme atoms (Kuettner et al., 2002; Shimon et al., 2007).

3.2.2 Lachrymatory Factor Synthase When onion cells are damaged, they release a volatile substance that causes tearing, known as the lachrymatory factor. As early as 1971, Brodnitz and Pascale identified (E)-propanthial S-oxide as the lachrymatory factor in onion. For a long time, the conversion of (Z)-1-propenylsulfenic acid to the lachrymatory factor was considered a spontaneous, non-enzymatic reaction. However, in 2002, Imai et al. first identified the enzyme catalyzing this conversion in onion, naming it lachrymatory factor synthase (LFS), for which they received the 2013 Ig Nobel Prize in Chemistry. The onion LFS gene spans 737 bp and encodes a 169-amino acid protein. LFS shows no activity toward (E)-1-propenylsulfenic acid, yet its product is (E)-propanthial S-oxide, leading to its classification as a (Z)-1-propenylsulfenic acid isomerase (Masamura et al., 2012).

The release of lachrymatory factor during onion processing poses significant inconvenience to workers. The discovery of LFS enabled the possibility of creating tearless onions. RNAi-mediated silencing of LFS expression dramatically reduces lachrymatory factor synthesis, causing (Z)-1-propenylsulfenic acid to be converted primarily to di-1-propenyl thiosulfinate and subsequently to various thiolane-type compounds (Eady et al., 2008; Aoyagi et al., 2011). Such tearless onions significantly reduce human cyclooxygenase-1 and α-glucosidase activity while decreasing platelet aggregation (Aoyagi et al., 2011; Thomsom et al., 2013). Thus, genetically engineered tearless onion germplasm not only alleviates processing inconveniences but also increases beneficial bioactive compounds.

Since the mid-20th century discovery of S-alk(en)ylcysteine sulfoxides and al-

licin, research on their chemical structures, bioactivities, and pharmacokinetics has expanded due to their significant medicinal and health value. As understanding of their bioactivities deepens, the importance of elucidating their metabolic pathways has become increasingly apparent. However, while degradation studies are abundant—particularly on alliinase and lachrymatory factor synthase—biosynthesis research remains limited, with only a few reports in garlic and onion (Jones et al., 2004; Yoshimoto & Saito, 2019). S-alk(en)ylcysteine sulfoxide metabolism constitutes an important component of plant sulfur metabolism, involving cysteine, glutathione, and valine metabolism. To date, only garlic GGTs (AsGGT1, AsGGT2, and AsGGT3) and FMO (AsFMO1) have been identified as key biosynthetic enzymes (Yoshimoto et al., 2015a; Yoshimoto et al., 2015b). Both GGTs and FMOs belong to multigene families, and whether other family members participate in S-alk(en)ylcysteine sulfoxide biosynthesis requires further investigation. Studies on sulfur compound metabolism in model plants like Arabidopsis provide valuable references. Sun et al. (2020) examined spatiotemporal expression patterns of garlic GSH1, GSH2, and PCS homologs based on the Arabidopsis glutathione pathway, but functional studies are lacking. While valine-derived methacrylic acid has been confirmed to participate in biosynthesis, the specific metabolic pathway and key enzymes remain unclear. Therefore, future research should focus on identifying and functionally characterizing key biosynthetic enzymes.

Regarding metabolic regulation, only a few studies have reported on controlling S-alk(en)ylcysteine sulfoxide degradation (Aoyagi et al., 2011; Thomson et al., 2013; Kato et al., 2016). Additional research has examined effects of tissue culture and fertilization on content and composition (Prince et al., 1997; Xu et al., 2007), but molecular regulation of biosynthesis remains unreported, primarily due to incomplete understanding of the biosynthetic pathway. Moreover, as downstream products of sulfur metabolism, S-alk(en)ylcysteine sulfoxide biosynthesis is influenced by upstream processes including sulfur compound uptake, transport, and cysteine synthesis. However, sulfur metabolism in Allium is poorly understood and requires strengthened investigation to establish a foundation for regulatory studies.

With rapid advances in omics technologies, genomics, transcriptomics, and metabolomics have been applied to Allium research, particularly with the completion of garlic genome sequencing (Khandagale et al., 2020; Li et al., 2020; Liu et al., 2021; Sun et al., 2016; Sun et al., 2020), providing abundant reference sequences for metabolic pathway studies. Future priorities should include: (1) comprehensive identification of key intermediates, enzymes, and genes in the S-alk(en)ylcysteine sulfoxide biosynthetic pathway; (2) elucidation of cellular compartmentalization, subcellular localization, product transport, and interpathway relationships; and (3) construction of gene regulatory networks. These efforts will provide theoretical foundations for manipulating S-alk(en)ylcysteine sulfoxide metabolism in Allium through molecular breeding technologies.

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