Chemico-Biological Interactions 259 (2016) 23–30

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Sulfation pathways in plants

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ARTICLE INFO

Article history:
Received 23 March 2016
Received in revised form
2 May 2016
Accepted 16 May 2016
Available online 17 May 2016

Keywords:
Sulfation
Phosphoadenosine phosphosulfate
Sulfotransferase
Arabidopsis
Glucosinolates

ABSTRACT

Plants take up sulfur in the form of sulfate. Sulfate is activated to adenosine 5′-phosphosulfate (APS) and reduced to sulfite and then to sulfide when it is assimilated into amino acid cysteine. Alternatively, APS is phosphorylated to 3′-phosphoadenosine 5′-phosphosulfate (PAPS), and sulfate from PAPS is transferred onto diverse metabolites in its oxidized form. Traditionally, these pathways are referred to as primary and secondary sulfate metabolism, respectively. However, the synthesis of PAPS is essential for plants and even its reduced provision leads to dwarfism. Here the current knowledge of enzymes involved in sulfation pathways of plants will be summarized, the similarities and differences between different kingdoms will be highlighted, and major open questions in the research of plant sulfation will be formulated.

1. Introduction

Sulfur is an essential nutrient for all organisms, as a constituent of amino acids cysteine and methionine, many cofactors and prosthetic groups and other essential metabolites. In most of these compounds sulfur is in the reduced form of thiols or sulfides, however, the form of sulfur most available in nature is the oxidized oxyanion sulfate. Plants, yeast, fungi, and many bacteria are able to take the sulfate up, reduce it, and incorporate into bioorganic compounds in the pathway of sulfate assimilation (Fig. 1) [84]. Humans and other metazoans do not possess the capacity to reduce sulfate and are dependent on reduced sulfur compounds synthesized by other organisms. Both groups of organisms, however, can activate sulfate and incorporate it into diverse metabolites in its oxidized form (Fig. 1) [69]. The sulfate activation consists of two ATP dependent reactions, first sulfate is adenylated to form adenosine 5′phosphosulfate (APS) by ATP sulfurylase. This step is sufficient to allow sulfate reduction by APS reductase by some, but not all organisms [1,6,40,41]. In the second activation step APS kinase phosphorylates APS to 3′phosphoadenosine 5′-phosphosulfate (PAPS), which is the most important sulfate donor for the sulfation reactions and substrate for reduction by PAPS reductases in fungi, yeast, and some bacteria [35,69,81]. The sulfate from PAPS is transferred onto various acceptor molecules by sulfotransferases, a large family of enzymes with a varied substrate specificity [31,50].

Plant research traditionally focused on the primary reductive sulfur metabolism, however, sulfated compounds and their metabolism have become more attention in the last two decades. This is partly because of identification of sulfated peptides as important growth hormones [2,58], partly because PAPS was shown to limit glucosinolates synthesis [60,64], and partly because of the link of sulfation to phosphoadenosine phosphate (PAP), an important plant signal molecule [17]. Here the enzymes and genes playing part in plant sulfation pathways will be described, with special attention to similarities and differences between kingdoms, and the main open questions about this interesting part of plant sulfur metabolism will be formulated. Firstly, however, the major sulfated compounds in plants have to be introduced.

2. Plant sulfated compounds

2.1. Glucosinolates

The best described group of sulfated secondary metabolites are the glucosinolates, even though they are limited to the order Capparales, including the Brassicaceae family. These amino acid derived compounds play important roles in defense against herbivores, fungi, and bacteria [4,18,19,65,83]. They are also responsible for taste and smell of cruciferous vegetables and have positive impact on human health [87]. On the other hand, glucosinolates are antinutrients for animals and, therefore, modern varieties of oil-

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http://dx.doi.org/10.1016/j.cbi.2016.05.021

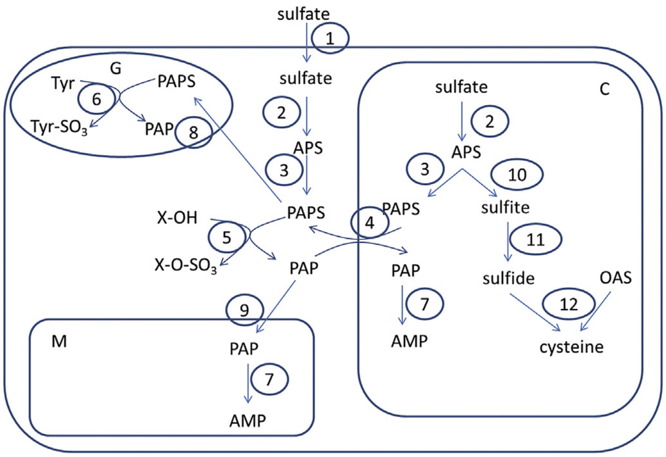

Fig. 1. Scheme of plant sulfation pathways. Shown are reactions connected with sulfation pathways in the three organelles chloroplast (C), mitochondria (M), and Golgi (G). The enzymatic and transport steps are indicated by numbers: 1, sulfate transporter; 2, ATP sulfurylase; 3, APS kinase; 4 PAPS transporter PAPST1; 5, sulfotransferase; 6, tyrosylprotein sulfotransferase; 7, 3′(2′),5′-bisphosphate nucleotidase (FRY1/SAL1); 8, Golgi PAPS transporter (unknown); 9 mitochondrial PAPS transporter (unknown); 10, APS reductase; 11, sulfite reductase; 12, O-acetylserine thiollyase. 10, 11, and 12 are part of primary sulfate assimilation.

seed rape possess only low amount of glucosinolates in the seeds, so that the press cakes can be used as source of animal feed [5]. Glucosinolates themselves, however, are only precursors of the active molecules, which are formed after enzymatic reaction with myrosinase. This enzyme hydrolyzes the thioglucose bond releasing glucose and an unstable product that undergoes chemical rearrangements into isothiocyanate or nitrile, depending on pH and on presence of modifying proteins. In plants therefore, these two components are spatially separated, the glucosinolates are stored in the vacuole and only upon tissue damage become available to the cytosolic myrosinase [9].

Glucosinolates are diverse in form, with more than 200 different metabolites belonging to this group, with 5–20 individual ones in a single species [16,18]. However, large variation exists within ecotypes of a single species, which has played a substantial role in understanding the control of glucosinolate synthesis [13,38]. This richness is enabled by a combinatorial effect of amino acid elongation in the first step and processing of the side chains after core synthesis [18,83]. The most prominent glucosinolates are derived from methionine and tryptophan and represent aliphatic and indolic glucosinolates, respectively. Glucosinolate synthesis requires action of at least 40 genes, most of them being known, at least for the glucosinolates found in Arabidopsis [83]. The synthesis of indolic glucosinolates from tryptophan is tightly connected with auxin synthesis, therefore mutants in this pathway have often auxin related morphological phenotypes [59,73]. Glucosinolates are sulfur rich compounds, because besides the methionine-derived side chains, two sulfur atoms are part of the core structure. One sulfur forms the thioglucoside bond and originates from glutathione, the other is oxidized as organic sulfate and is transferred onto desulfo-glucosinolate precursors from PAPS [20,64]. This last step in glucosinolates core synthesis links these metabolites to the sulfation pathways [64].

In Arabidopsis the sulfation of glucosinolates is catalyzed by three members of the sulfotransferase family, AtSOT16, AtSOT17, and AtSOT18 [29]. Sulfation is essential for glucosinolates function, as it is required for the formation of volatile isothiocyanates and other products after reaction with myrosinase, and therefore often a target of detoxifying mechanisms by specialists insects [71]. When provision of PAPS is limited, such as in mutants for two major APS kinase isoforms *apk1 apk2*, glucosinolate content is greatly reduced [64]. This triggers large alterations in sulfur metabolism, coordinated upregulation of the genes of glucosinolates synthesis and increased partitioning of sulfur towards the reduced compounds leading to accumulation of glutathione [62,64]. Similarly, the loss of ATPS1 isoform of ATP sulfurylase leads to reduced rate of glucosinolate synthesis [43]. The connection of the enzymes of primary sulfation pathway and glucosinolates synthesis is corroborated by the corresponding genes being parts of the same transcriptional network of two groups of MYB factors [97]. Given their high accumulation in Arabidopsis, changes in sulfation pathways are well manifested in alterations in glucosinolates accumulation.

2.2. Sulfated peptides

Among other sulfated compounds the sulfated peptides have particularly important functions. The first such peptides identified were the phytosulfokines, shown initially to be needed for proliferation of cultured plant cells [58]. Phytosulfokines are small peptides of five amino acids, containing two tyrosines which are sulfated. In plants, phytosulfokines control cell growth and somatic embryogenesis, and are also involved in pathogen defense [56,82]. Another sulfated peptide, the PSY1 peptide, has been found to contribute to the control of growth [2]. The sulfated peptides act through binding onto receptors, which have been identified in Arabidopsis, two LRR kinases for phytosulfokines and one for PSY1 [2,57]. Mutants of these three receptors show a dwarf phenotype, corroborating the importance of these peptides for plant growth. Due to increasing evidence of phytosulfokine acting in plant microbe interaction, it has been suggested that the peptides integrate growth and defense [76,80,82]. The exact mechanisms of action of phytosulfokines and PSY1 downstream the receptors are not known, yet.

Sulfated peptides, however, are not a plant specific invention, e.g., the gastric hormone cholecystokinin requires sulfation for its function as stimulus of pancreatic enzymes secretion and bile acid expulsion [12].

2.3. Other sulfated compounds

Plants synthesize a large number of secondary metabolites, with sulfation being one of possible modifications. Apart from glucosinolates, sulfated flavonoids are the best described group of sulfated secondary metabolites. They had been first characterized in plants of the genus *Flaveria* and were later found in many other families [85,90]. Sulfated flavonoids show anticoagulant properties, particularly those with multiple sulfate groups [25]. Other sulfated compounds in plants include turgorin, an inducer of seismonastic response in *Mimosa* [89] and various phenolic or terpenoid-derived compounds, including sulfated derivatives of phytohormones jasmonate, salicylate, and brassinosteroids [3,21,55]. Special class are sulfated polysaccharides found in many marine algae, such as carrageenans in red algae, fucoidans in brown algae or ulvans in green algae, which have many potentially beneficial biological activities [66]. These metabolites have been shown or are presumed to use the PAPS/sulfotransferase biosynthetic pathway. The number of sulfated metabolites is surely not final, partly because the substrate specificity of most sulfotransferases is not known (see below), partly because a large number of unknown sulfur containing compounds have been identified in Arabidopsis [24].

Other group of sulfated compounds important in plants and algae are the sulfolipids. They are found in chloroplast membranes and their synthesis is increased during phosphate limitation as a