Phytochemistry 147 (2018) 89–124

Contents lists available at ScienceDirect

Phytochemistry

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

Review

Purine salvage in plants

Hiroshi Ashihara [a, *], Claudio Stasolla [b], Tatsuhito Fujimura [c], Alan Crozier [d]

[a] Department of Biology, Ochanomizu University, Bunkyo-ku, Tokyo, 112-8610, Japan
[b] Department of Plant Science, University of Manitoba, Winnipeg, R3T 2N2, Canada
[c] Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, 305-8572, Japan
[d] Department of Nutrition, University of California, Davis, CA, 95616-5270, USA

ARTICLE INFO

Article history:
Received 26 June 2017
Received in revised form
10 December 2017
Accepted 14 December 2017

This article dedicated to our friend and colleague at the University of Calgary, Professor Trevor A. Thorpe.

Keywords:
Review
Purine metabolism
Purine nucleotides
Salvage enzymes
Phosphoribosyltransferases
Purine nucleoside kinases
Nucleosidases
Nucleotidases
Caffeine biosynthesis
Cytokinin interconversion

ABSTRACT

Purine bases and nucleosides are produced by turnover of nucleotides and nucleic acids as well as from some cellular metabolic pathways. Adenosine released from the *S*-adenosyl-L-methionine cycle is linked to many methyltransferase reactions, such as the biosynthesis of caffeine and glycine betaine. Adenine is produced by the methionine cycles, which is related to other biosynthesis pathways, such those for the production of ethylene, nicotianamine and polyamines. These purine compounds are recycled for nucleotide biosynthesis by so-called "salvage pathways". However, the salvage pathways are not merely supplementary routes for nucleotide biosynthesis, but have essential functions in many plant processes. In plants, the major salvage enzymes are adenine phosphoribosyltransferase (EC 2.4.2.7) and adenosine kinase (EC 2.7.1.20). AMP produced by these enzymes is converted to ATP and utilised as an energy source as well as for nucleic acid synthesis. Hypoxanthine, guanine, inosine and guanosine are salvaged to IMP and GMP by hypoxanthine/guanine phosphoribosyltransferase (EC 2.4.2.8) and inosine/guanosine kinase (EC 2.7.1.73). In contrast to *de novo* purine nucleotide biosynthesis, synthesis by the salvage pathways is extremely favourable, energetically, for cells. In addition, operation of the salvage pathway reduces the intracellular levels of purine bases and nucleosides which inhibit other metabolic reactions. The purine salvage enzymes also catalyse the respective formation of cytokinin ribotides, from cytokinin bases, and cytokinin ribosides. Since cytokinin bases are the active form of cytokinin hormones, these enzymes act to maintain homeostasis of cellular cytokinin bioactivity. This article summarises current knowledge of purine salvage pathways and their possible function in plants and purine salvage activities associated with various physiological phenomena are reviewed.

1. Introduction

Purine nucleotides, such as ATP and GTP, are important metabolites as the energy source and building blocks of nucleic acids (Moffatt and Ashihara, 2002; Stasolla et al., 2003; Zrenner and Ashihara, 2011; Zrenner et al., 2006). In plants, purine nucleotides also act as substrates for the biosynthesis of nicotinamide adenine dinucleotides (NAD and NADP) (Ashihara et al., 2015; Zrenner and Ashihara, 2011), purine alkaloids (Ashihara et al., 2008b, 2017) and cytokinins (Ashihara et al., 2013b; Hirose et al., 2008). In most organisms, with the exception of parasites, purine nucleotides are synthesized *de novo* (Berens et al., 1995). In addition, purine nucleotides are synthesized by salvage pathways, recycling preformed purine bases and nucleosides for nucleotide synthesis (Moffatt and Ashihara, 2002). The salvage pathways are not supplementary routes leading to the biosynthesis of purine nucleotides. Although net formation of purine nucleotides is performed by the *de novo* pathway, rapid turnover of nucleic acids, especially RNA, is required for nucleotide production by the salvage pathways. Reduced salvage activity inhibits the normal growth of plants and other organisms (see Section 8.1.2).

In mammals, genetic deficiency of a purine salvage enzyme, hypoxanthine/guanine phosphoribosyltransferase (EC 2.4.2.8), causes the Lesch-Nyhan syndrome (Nyhan, 1997). In plants, adenine phosphoribosyltransferase (EC 2.4.2.7) appears to be a more important enzyme in purine salvage than hypoxanthine/guanine phosphoribosyltransferase. In addition to producing adenine nucleotides for energy metabolism and nucleic acid biosynthesis (Stasolla et al., 2003; Zrenner and Ashihara, 2011; Zrenner et al., 2006), adenine phosphoribosyltransferase is

* Corresponding author.
E-mail address: ashihara.hiroshi@ocha.ac.jp (H. Ashihara).

https://doi.org/10.1016/j.phytochem.2017.12.008
0031-9422/

involved in the removal of adenine and adenosine, which act as inhibitors of several reactions in cells (Moffatt and Ashihara, 2002), as well as being utilised for caffeine biosynthesis (Ashihara and Crozier, 2001), and activation and inactivation of cytokinin functions (Auer, 2002; Mok and Mok, 2001). Furthermore, deficiency of this enzyme causes male-sterility (Gaillard et al., 1998). Thus, purine salvage is not only the energy-saving route of nucleotide biosynthesis, but is an essential pathway in plants.

Since purine salvage is a topic of interest in medical science, including parasitology (Freitas-Mesquita and Meyer-Fernandes, 2017) and neurochemistry (Balestri et al., 2007), many reviews have been published. However, there is currently no review on purine salvage in plants. This is the first comprehensive article on this topic in plants which covers purine salvage literature published up to September, 2017.

This review will first describe the metabolic fate of purine bases and nucleosides (Section 2.1) and enzyme activity profiles in plant cells and tissues (Section 2.2), and then summarize the purine salvage enzymes (Section 3). After a brief summary of pathways for the supply of substrates for the salvage enzymes (Section 4), enzymes involved in substrate production for purine salvage enzymes (Section 5) and cellular concentration of substrates and products of purine salvage enzymes are summarised (Section 6). The transport mechanism of purine bases and nucleosides is then described (Section 7).

The second section of this review covers physiological aspects of purine salvage in plants (Section 8). This includes information on changes in purine salvage activity accompanied by physiological events (Section 8.1), the involvement of purine salvage enzymes in other aspects of metabolism, such as caffeine biosynthesis (Section 8.2), interconversion of cytokinins (Section 8.3) and various phenomena including male sterility (Section 8.4), pathogen responses (Section 8.5) and gravitropism (Section 8.6).

Finally, recent omics studies which may reveal new functions of purine salvage in plants are discussed (Section 8.7).

This article aims at describing the current knowledge of purine salvage studies at the biochemical, molecular biological and physiological levels, together with the historical survey of this topic. There are some reviews on general plant nucleotide metabolism including biosynthesis and degradation pathways of purine nucleotides, (Moffatt and Ashihara, 2002; Ross, 1981; Stasolla et al., 2003; Wagner and Backer, 1992; Wasternack, 1982; Zrenner and Ashihara, 2011; Zrenner et al., 2006). For related pathways, recent reviews are available on caffeine biosynthesis (Ashihara et al., 2013a, b; 2017), cytokinin metabolism (Hluska et al., 2016; Sakakibara, 2006, 2010), ethylene biosynthesis (Lin et al., 2009) and *S*-adenosyl-L-methionine (SAM) metabolism (Roje, 2006). Except for recombinant enzymes derived from special genes, enzyme names recommended by the IUBMB are used throughout the article.

2. Metabolism of purine bases and nucleosides in plants

2.1. Metabolic fate of purine bases and nucleosides

Purine bases and nucleosides are metabolised by three different routes (i) salvage pathways, (ii) catabolic (degradation) pathways, and (iii) in some plant species, purine alkaloid biosynthesis. The pathways that have been established in plants are summarised in Fig. 1 (Ashihara and Crozier, 1999; Moffatt and Ashihara, 2002; Stasolla et al., 2003; Zrenner and Ashihara, 2011). The metabolic fate of purine bases and nucleosides has been investigated using radiolabelled purine precursors. ^{14}C-Labelled purine bases and nucleosides are taken up rapidly and immediately metabolised in plant cells and tissues. Purine salvage activity is usually estimated from a summation of the radioactivity incorporated into nucleotides and nucleic acids. A portion of purines is catabolised by the conventional purine catabolic pathway and radioactivity from ^{14}C-labelled purine bases and nucleosides is incorporated into the purine catabolites, allantoin and allantoic acid as well as CO_2. In some plant species such as *Camellia sinensis* (tea) and *Coffea arabica* (coffee), purine bases and nucleosides are utilised for the biosynthesis of purine alkaloids, (Ashihara and Crozier, 1999; Ashihara et al., 2008a, b, 2017). Profiles of the metabolic fate of a series of purine bases and nucleosides have been comprehensively investigated in *A. thaliana* cells (Yin et al., 2014), potato tubers (Katahira and Ashihara, 2006a), leaves and roots of tea seedlings (Deng and Ashihara, 2010) and cacao leaves (Koyama et al., 2003).

The metabolic fate of [8-^{14}C]adenine, [8-^{14}C]hypoxanthine, [8-^{14}C]guanine and [8-^{14}C]xanthine in the exponential growing phase of *A. thaliana* cells (Yin et al., 2014), disks of young developing potato tubers (Katahira and Ashihara, 2006a), young leaves of tea (Deng and Ashihara, 2010) and cacao (Koyama et al., 2003) are shown in Fig. 2A–D. Of the four purine bases investigated, adenine is the favoured precursor for purine salvage being converted to AMP (step 3 in Fig. 1) which is converted to ATP by subsequent phosphorylation reaction steps and then incorporated into RNA by RNA polymerase. The second most efficient salvaged purine base precursor is guanine. Guanine is converted to GMP (step 7) and then used for GTP and RNA synthesis. The conversion of AMP to GMP occurs in plants, but conversion of GMP to AMP is limited. In several species radioactivity from [8-^{14}C]adenine is distributed in adenine residues (~70%), and the remainder (~30%) is incorporated in guanine residues, whereas radioactivity from [8-^{14}C]guanine is incorporated principally into guanine residues (>95%) (Deng and Ashihara, 2010; Katahira and Ashihara, 2006a; Yin et al., 2014). This suggests that some AMP produced by adenine salvage is converted to IMP by AMP deaminase (EC 3.5.4.6) and then converted to GMP via XMP (steps 11–13 in Fig. 1). Conversion of GMP to AMP is negligible, because GMP reductase (EC 1.7.1.7, step 14 in Fig. 1) is absent in plants (Stasolla et al., 2003). Small amounts of radioactivity from [8-^{14}C]hypoxanthine is recovered in the guanine residues of RNA. This implies that IMP derived from hypoxanthine is utilised preferentially in GMP synthesis, and conversion to AMP is restricted.

In potato tubers, adenine and guanine are salvaged predominantly for nucleotide synthesis, but <2% of these purine bases is catabolised (Katahira and Ashihara, 2006a) (Fig. 2B). Since deaminases for adenine and guanine are absent in plants (Stasolla et al., 2003; Katahira and Ashihara, 2006a), adenine appears to be converted to AMP (step 3), which is then deaminated to IMP (step 11) and converted to xanthine via inosine and hypoxanthine (steps 10, 8 and 17a). Xanthine is catabolised via the conventional purine catabolic pathway (step 17–22). In the case of guanine, it is first converted to GMP (step 7) and then catabolised via a GMP → guanosine → xanthosine → xanthine pathway (steps 10, 15 and 9). The salvage activity of hypoxanthine in plants is usually lower than that of adenine and guanine, although relatively high hypoxanthine salvage is observed in species such as cacao (Fig. 2D). Since hypoxanthine is a substrate for both purine salvage (hypoxanthine/guanine phosphoribosyltransferase, step 7) and purine catabolism enzymes (xanthine dehydrogenase, step 17), the relative activity of purine salvage is reduced in plant materials where the purine degradation is pronounced. In purine alkaloid-forming species, xanthosine is a key substrate for the biosynthesis of theobromine and caffeine (steps 23–25). In contrast to other purine bases, xanthine is degraded extensively (steps 17–22) and only limited amounts are utilised for purine alkaloid synthesis in tea and cacao leaves (Fig. 2C and D).

The metabolic fate of [8-^{14}C]adenosine, [8-^{14}C]inosine, [8-^{14}C]