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Chorismate derived C6C1 compounds in plants

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ing anthranilate, *p*-aminobenzoate, *p*-hydroxybenzoate, salicylate and 2,3-dihydroxybenzoate.

Introduction

The secondary metabolites are the products of interaction of the producing organism with its environment and have a restricted occurrence. Many have economical importance as, e.g. drugs, antioxidants, flavors, fragrances, dyes, insecticides and pheromones (Verpoorte et al. 2002). Secondary metabolites can be classified according to their biosynthetic building blocks or their carbon skeleton. The C6C1 compounds are compounds having an aromatic six-carbon ring with one carbon attached. They are generally derived from the shikimate pathway (Dewick 2002).

The shikimate pathway, restricted to microorganisms and plants, includes seven metabolic steps, starting with phosphoenolpyruvate and D-erythrose-4-phosphate, and ending with chorismate (an important metabolic branch-point) (Fig. 1). All enzymes involved have been purified and the cDNAs characterized from some prokaryotes and eukaryotes (Herrmann and Weaver 1999). In plants, the pathway is localized in plastids. Gallic acid and protocatechuic acid (3,4-dihydroxybenzoic acid) are C6C1 compounds that can derive from either shikimate pathway (by dehydration and dehydrogenation of 3-dehydroshikimic acid) or phenylalanine pathway (via 3,4,5-trihydroxycinnamic acid) (Torssell 1997; Ossipov et al. 2003). Gallic acid can also derive from orsellinic acid via the polyketide pathway by decarboxylation and oxidation (Torssell 1997). This review will focus on chorismate-derived C6C1 compounds in plants, includ-

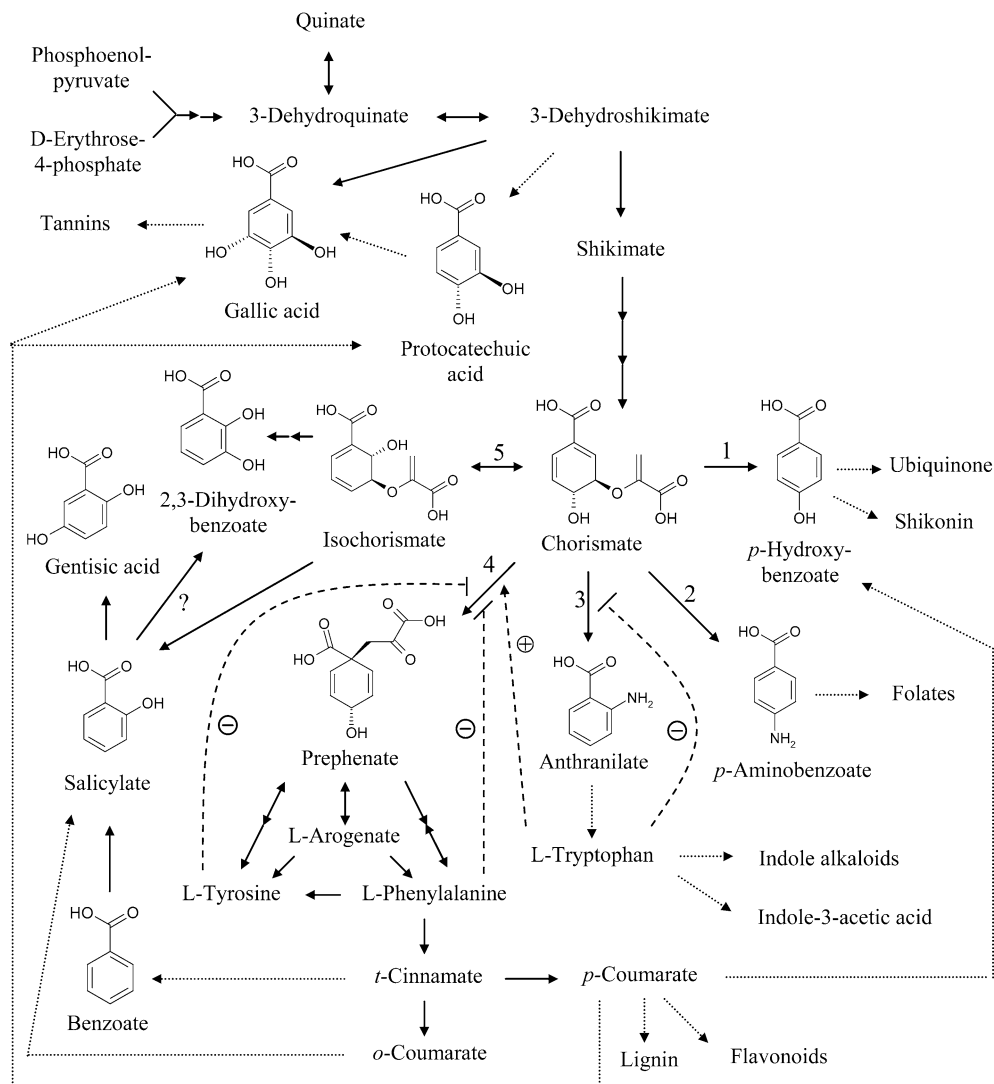
Anthranilate

Anthranilate is the product of anthranilate synthase (AS, EC 4.1.3.27), the first enzyme of the tryptophan biosynthesis. The flux through this pathway is controlled by feedback inhibition by tryptophan on AS (Li and Last 1996). AS is a key regulator for alkaloid accumulation induced by elicitors in *Ruta graveolens* (Bohlmann et al. 1995) and it may be a rate-limiting enzyme in the biosynthesis of avenanthramides, indole phytoalexins in oats (Matsukawa et al. 2002).

AS holoenzymes are characterized as tetramers consisting of two α - and two β -subunits, encoded by separate nuclear genes, synthesized in the cytosol and transported into the plastid to obtain the mature active form (Zhang et al. 2001). Two genes encoding AS α subunits (*ASA1* and *ASA2*) were isolated from *Arabidopsis thaliana* and found to be functional by complementation in yeast and *E. coli* (Niyogi and Fink 1992). The overexpression of the *Ruta graveolens* AS α isozymes in *E. coli* revealed the presence of a tryptophan feedback-insensitive AS α 1 and a sensitive AS α 2 enzyme (Bohlmann et al. 1996). Transformation of a 5-methyl tryptophan-resistant tobacco gene (*ASA2*) into *Astragalus sinicus* (a forage legume) resulted in an increased level of tryptophan (Cho et al. 2000). An *Arabidopsis* feedback-resistant AS α gene (a mutated *ASA1*) was transformed into *Catharanthus roseus* providing hairy roots with increased levels of tryptophan, tryptamine and the indole alkaloid lochnericine (Hughes et al. 2004). Relocating a native tryptophan feedback-insensitive gene from the nucleus to the plastid genome resulted in transplastomic tobacco plants with greatly increased tryptophan levels but normal phenotype and fertility, showing the advantage of plastid transformation compared to nuclear transformation (Zhang et al. 2001). Replacing aspartate with asparagine at a certain position in

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Fig. 1 The biosynthetic pathway of chorismate/ isochorismate derived-C6C1 compounds. 1 chorismate pyruvate-lyase, 2 *p*-aminobenzoate synthase, 3 anthranilate synthase, 4 chorismate mutase, 5 isochorismate synthase. The dashed lines with + and - indicate feedback activation and inhibition respectively. A dotted line means multi-step reactions



A. thaliana (Li and Last 1996) or *Oryza sativa* AS α (Tozawa et al. 2001), resulted in lower sensitivity for tryptophan inhibition. Sensitivity for tryptophan inhibition can also be due to a mutation in a regulator gene of the AS gene's expression (Ishikawa et al. 2003). The genes encoding the rice plastidial AS β subunits have been characterized (Kanno et al. 2004). Both AS β subunits are assembled with the mature forms of the AS α subunits.

Salicylic acid

Salicylic acid (SA) has several roles in plants (Raskin 1992) including the induction of systemic acquired resistance (SAR) as response to pathogens. SA-dependent SAR is characterized by the increase of SA and its conjugates and pathogenesis related (PR) proteins (Ryals et al. 1996).

SA in plants is thought to be derived from the phenylalanine pathway by cinnamic acid chain

shortening, either through a β -oxidative or a non-oxidative pathway. Some steps have been identified, others not yet (Verberne et al. 1999). The enzyme (benzoic acid 2-hydroxylase) converting benzoic acid (BA) into SA has been identified (Leon et al. 1995). The non-oxidative pathway to BA does not function in cucumber (*Cucumis sativus*) and *Nicotiana attenuata* (Jarvis et al. 2000). In microorganisms, SA biosynthesis involves isochorismate synthase (ICS, EC 5.4.99.6), converting chorismate into isochorismate, and isochorismate pyruvate lyase (IPL) providing SA (reviewed by Verberne et al. 1999). Verberne et al. (2000) suggested that plants may utilize this pathway and they introduced the microbial-isochorismate SA pathway into tobacco resulting in increased-SA levels and enhanced resistance to tobacco mosaic virus. Wildermuth et al. (2001) found evidence for a SA isochorismate pathway. The *Arabidopsis sid2* mutant unable to produce chloroplast-localized ICS1 exhibited a remarkable lower level of SA after infection and a reduced resistance against pathogens. Chong et al. (2001) showed that the SA accumulation in elicited

tobacco cells required de novo BA synthesis from *trans*-cinnamic acid, though, instead of BA, the benzoyl-glucose was the likely intermediate. The pathway from *trans*-cinnamic acid to SA via BA is involved in the stress-induced flowering of *Pharbitis nil* (Hatayama and Takeno 2003).

The catabolism of SA is mainly through glucosylation by SAglycosyl transferase, which occurs presumably in the cytoplasm and subsequently accumulated in the vacuoles. The uptake of SAG into vacuoles may involve different mechanisms in different plant species. For example, in soybean (*Glycine max*), the ATP-binding cassette (ABC) transporter is involved, whereas in the red beet it is the H⁺-antiport mechanism (Dean and Mills 2004). In a *Catharanthus roseus* cell suspension culture, SA was catabolized by a hydroxylation into 2,5-dihydroxybenzoic acid (gentisic acid) followed by a glucosylation of the newly introduced phenolic hydroxyl group. The 55 kDa hydroxylase and the 41 kDa regio-specific glucosyltransferase have been isolated by Shimoda et al. (2004) and Yamane et al. (2002).

2,3-Dihydroxybenzoate

2,3-Dihydroxybenzoate (2,3-DHBA) is in microorganisms derived from isochorismate (Young et al. 1968). SA and 2,3-DHBA are precursors of siderophores such as enterobactin and pyochelone. This pathway involves ICS, 2,3-dihydro-2,3-DHBA synthase and 2,3-dihydro-2,3-DHBA dehydrogenase. 2,3-DHBA may derive from SA by hydroxylation (reviewed by Budi Muljono 2002). 2,3-DHBA is produced in *Catharanthus roseus* cell cultures after elicitation with fungal cell-wall preparations and parallels an increase in activity of ICS (Moreno et al. 1994). The ICS protein and its cDNA were obtained from *C. roseus* cell cultures (van Tegelen et al. 1999). This ICS has 57% homology with the ICS1 of *A. thaliana* and 20% homology with bacterial ICS (Wildermuth et al. 2001). A retrobiosynthetic study with *C. roseus* suspension cells fed with [1-¹³C]glucose confirmed the intermediacy of isochorismate in 2,3-DHBA biosynthesis (Budi Muljono et al. 2002).

p-Hydroxybenzoate

p-Hydroxybenzoate (4HB), a precursor of shikonin, is formed via the phenylpropanoid pathway (Loscher and Heide 1994). It is also a precursor of ubiquinones formed directly from chorismate by chorismate pyruvate-lyase (CPL) in bacteria or from both pathways in eukaryotic microorganisms (Meganathan 2001). The *ubiC* gene encoding CPL of *E. coli* was overexpressed in tobacco resulting in high CPL activity and increased level of 4HB as β -glucosides (4HBG, 0.52% DW) derived from the introduced pathway (Siebert et al. 1996). Using the same constructs, only 20% of the total 4HBG

produced in *Lithospermum erythrorhizon* employed this pathway (Sommer et al. 1999). Transformation using a strong (ocs)₃mas promoter did not change the level of 4HBG compared to the control cultures, but 73% of total 4HBG was derived from the introduced pathway (Kohle et al. 2002). Whilst, introducing this construct into tobacco and potato led to 5.1% (DW) of 4HBG in tobacco cell cultures and 4.0% DW in the leaves of potato shoots. These amounts correlated with CPL activity and are the highest for artificial secondary metabolites ever reached by genetic engineering in plants. It did not affect growth, proving the large capacity of the plastidial shikimate pathway (Kohle et al. 2003). *UbiC* without a transit peptide provided much lower levels of 4HB derivatives (Sommer and Heide 1998). In *L. erythrorhizon*, 4HBG was accumulated in vacuoles. The vacuolar transport of 4HB and of *p*-hydroxycinnamic acid in red beet, requires glucosylation and employs an H⁺-antiport mechanism, the same transport used by 5-hydroxychlorosulphuron (a herbicide)-glucoside (Bartholomew et al. 2002).

p-Aminobenzoate

p-Aminobenzoate (PABA) is the precursor of folic acids (folates). Folates are cofactors in “one carbon” transfer reactions as e.g. in the biosynthesis of some nucleotide bases (Scott et al. 2000). The conversion of chorismate into PABA in microorganisms is catalyzed by *p*-aminobenzoic acid synthase, EC 4.1.3.-. This enzyme consists of three subunits. The large subunit (aminodeoxychorismate synthase) encoded by *pabB*, converts chorismate into aminodeoxychorismate (ADC), the small subunit encoded by *pabA* is a glutamine amidotransferase and the third subunit (aminodeoxychorismate lyase) encoded by *pabC*, converts ADC into PABA and pyruvate (Viswanathan et al. 1995).

Sulfonamides are PABA analogues inhibiting dihydropteroate synthase (DHPS), the enzyme converting PABA into 7,8-dihydropteroate (Scott et al. 2000). DHPS is the key regulator of the folate biosynthetic pathway (Mouillon et al. 2002). The cDNA was recently purified and characterized from pea leaves. The presence of a putative mitochondrial transit peptide of 28 amino acids in the single copy gene, indicates the mitochondria as the site of 7,8-dihydropteroate synthesis (Rebeille et al. 1997), thus requiring transport of PABA across the plastidial- and mitochondrial membranes.

Conclusion

One should be very careful in extrapolating findings of C6C1 pathways in a plant e.g. *Arabidopsis* to other plants. It can not be excluded that particularly for secondary metabolites different localization and regulation of the pathways occurs in different plant species. Chorismate is biosynthesized in plastids, where also most of the enzymes discussed are localized. But

chorismate may be transported out of plastids and further converted in other compartments. For example, plants overexpressing microbial SA genes without plastidial signal sequence still produced small amounts of SA, thus requiring transport of chorismate. AS has also been proposed to have a plastidial and a cytosolic form, though evidence is lacking. The flux through the different branches is quite different with the CM pathway generally being the most active. Unraveling all the C6C1 pathways on the level of genes, proteins and intermediates including localization (transport) and regulation will be a major challenge for the coming years.

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