Biochemical and Biophysical Research Communications 417 (2012) 393–398

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

HlPT-1, a membrane-bound prenyltransferase responsible for the biosynthesis of bitter acids in hops

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ARTICLE INFO

Article history:
Received 2 November 2011
Available online 7 December 2011

Keywords:
Aromatic substrate prenyltransferase
Acylphloroglucinol
Hop
Humulone
Lupulone
Xanthohumol

ABSTRACT

Female flowers of hop (*Humulus lupulus* L.) develop a large number of glandular trichomes called lupulin glands that contain a variety of prenylated compounds such as α- and β-acid (humulone and lupulone, respectively), as well as xanthohumol, a chalcone derivative. These prenylated compounds are biosynthesized by prenyltransferases catalyzing the transfer of dimethylallyl moiety to aromatic substances. In our previous work, we found HlPT-1 a candidate gene for such a prenyltransferase in a cDNA library constructed from lupulin-enriched flower tissues. In this study, we have characterized the enzymatic properties of HlPT-1 using a recombinant protein expressed in baculovirus-infected insect cells. HlPT-1 catalyzed the first transfer of dimethylallyl moiety to phloroglucinol derivatives, phlorisovalerophenone, phlorisobutyrophenone and phlormethylbutanophenone, leading to the formation of humulone and lupulone derivatives. HlPT-1 also recognized naringenin chalcone as a flavonoid substrate to yield xanthohumol, and this broad substrate specificity is a unique character of HlPT-1 that is not seen in other reported flavonoid prenyltransferases, all of which show strict specificity for their aromatic substrates. Moreover, unlike other aromatic substrate prenyltransferases, HlPT-1 revealed an exclusive requirement for Mg^{2+} as a divalent cation for its enzymatic activity and also showed exceptionally narrow optimum pH at around pH 7.0.

1. Introduction

Hop (*Humulus lupulus* L., Cannabinaceae) is a perennial and dioecious climbing plant and female plants of the species are cultivated world-wide for use as an essential ingredient of beer. Female flowers, also called hop cones, give the characteristic flavor and bitter taste to beer due to a variety of essential oils and aromatic compounds, which are biosynthesized and accumulated exclusively in yellow glandular trichomes, also designated lupulins, which develop at the basal part of hop cone bracts [1]. Among the secondary metabolites produced by the hop plant, prenylated acylphloroglucinols, conventionally called 'bitter acids', have received a large amount of attention because their characteristic bitter property is important for beer taste; moreover, their divergent biological activities, including radical scavenging activity, angiogenesis inhibition, and inducing effect for P450 enzyme, are beneficial for human health [2,3]. Hop cones also contain prenylated flavonoids, among which the major one is xanthohumol, a prenylated chalcone derivative, which has potential applications as a cancer chemopreventive agent [4].

The proposed biosynthetic pathway of bitter acids in hops, also called α- and β-acid (humulone and lupulone, respectively), are shown in Fig. 1, with the biosynthesis of xanthohumol illustrated in parallel. The aromatic core of humulone and lupulone is a phloroglucinol derivative formed by the condensation of an acyl-CoA and three molecules of malonyl-CoA via the function of valerophenone synthase [5]. There are three major phloroglucinol derivatives in hops according to the different sources of acyl-CoA, namely, phlorisovalerophenone (PIVP) biosynthesized from isovaleryl-CoA, which is the precursor for humulone and lupulone as representative bitter acids [5], phlorisobutyrophenone (PIBP) derived from isobutyryl-CoA leading to cohumulone and colupulone, and phlormethylbutanophenone (PMBP) given with 2-methylbutyryl-CoA, which is the precursor for adhumulone and adlupulone. After condensation with malonyl CoA, the resulting acylphloroglucinol derivatives undergo two or three prenylations with dimethylallyl diphosphate by an aromatic substrate prenyltransferase of hops. Mono-prenyl PIVP and di-prenyl PIVP (deoxyhumulone) are the key intermediates in the humulone and lupulone biosynthesis. Humulone is formed from deoxyhumulone

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0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.11.125

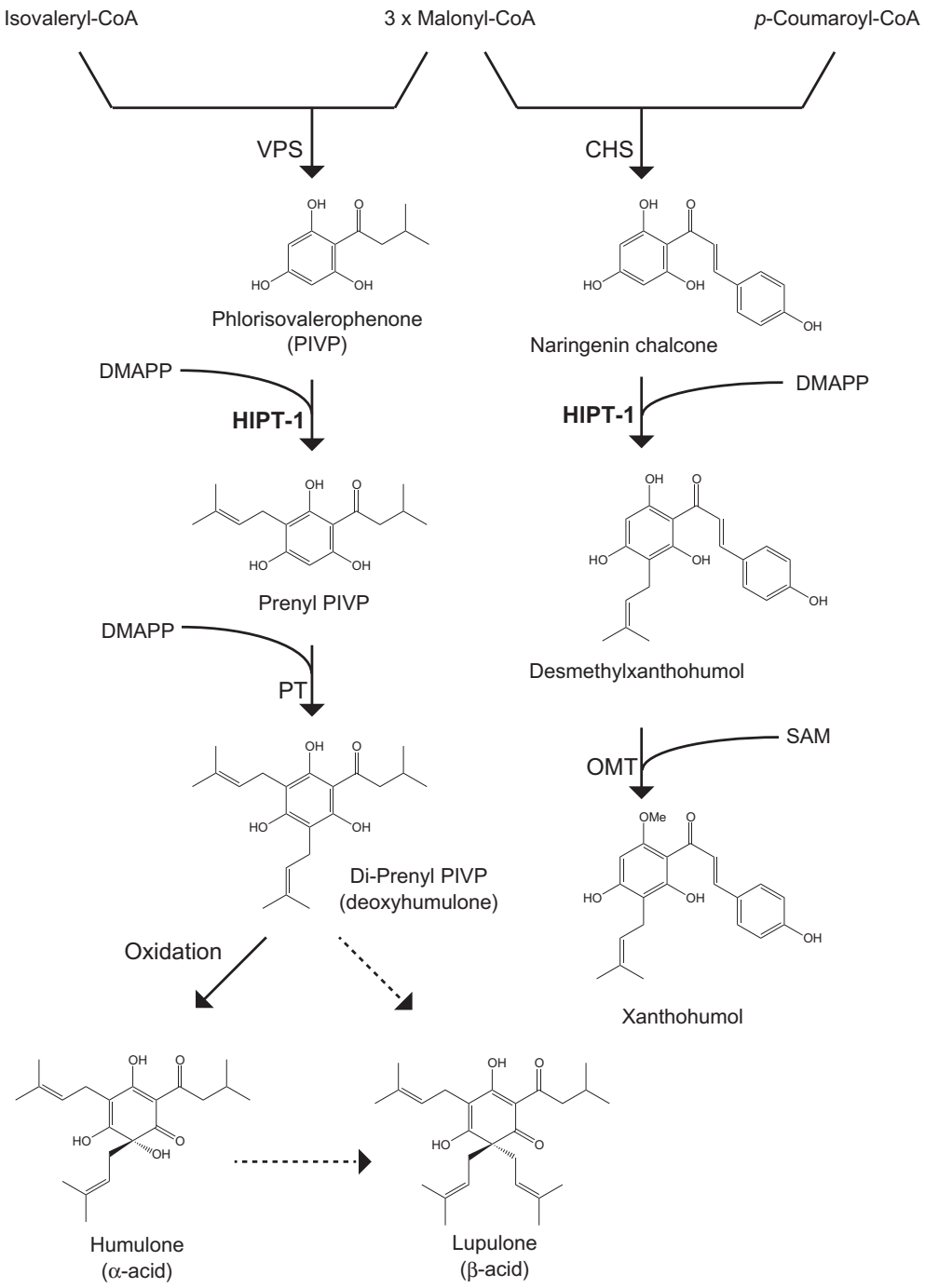

Fig. 1. Biosynthesis of bitter acids (humulone and lupulone) and xanthohumol in lupulin glands of hop. DMAPP, dimethylallyl diphosphate; VPS, valerophenone synthase; PT, prenyltransferase; CHS, chalcone synthase; OMT, *O*-methyltransferase; SAM, *S*-adenosylmethionine.

through oxidation [6], whereas lupulone, having three dimethylallyl moieties, is proposed to be synthesized either from deoxyhumulone or humulone by a further prenylation reaction [7]. In a manner similar to acylphloroglucinol derivatives, naringenin chalcone is biosynthesized via condensation of *p*-coumaroyl-CoA and three molecules of malonyl-CoA by chalcone synthase to yield the aromatic core of xanthohumol. *C*-prenylation of naringenin chalcone provides desmethylxanthohumol, which is then converted to xanthohumol by an *O*-methyltransferase in the presence of a methyl donor, *S*-adenosylmethionine [8]. In the biosynthesis of these secondary metabolites in hops, aromatic prenyltransferases play a crucial role for both phloroglucinol and flavonoid derivatives.

The plant prenyltransferases that recognize aromatic secondary metabolites are a new topic of research in plant molecular biology; the first flavonoid-specific prenyltransferase, naringenin 8-dimethyallyltransferase (SfN8DT), was identified in 2008, and thereafter a pterocarpan and isoflavonone-specific prenyltransferases, glycinol 4-dimethylallyltransferase (G4DT) and genistein 6-dimethylallyltransferase (SfG6DT), respectively, have been reported [9–11]. These enzymes are all divalent cation-requiring membrane-bound proteins, and those characterized to date have been localized in plastids. In our previous work on hops, we constructed a cDNA library from the lupulin gland-rich portion of female flower bracts, and randomly sequenced 11,233 EST clones, obtaining sequence information for 6613 non-redundant ESTs. Among them, a cDNA