Preliminary

Characterization of an Aromatic Substrate Prenyltransferase, LePGT-1, Involved in Secondary Metabolism*[1]

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Introduction

Prenylated aromatic natural products widely occur in higher plants, particularly in some phanerogamic plant families, such as Guttiferae, Rutaceae, etc. For instance, *Mammea americana* (Guttiferae), an indeciduous tree native in tropical America, is used as an edible and aromatic plant, which contains many prenylated coumarins[1]. *Murraya paniculata* (Rutaceae), another indeciduous bush grown in south China and Indonesia, is utilized as a valuable wood resource and spice, and is also a resource of prenylated coumarins[2]. In addition, a large number of prenylated flavonoids were isolated from woody plants belonging to Moraceae and Leguminosae so far. There are many studies demonstrating that prenylated aromatic compounds show various biological activities, e. g., inhibitory effects on sulfotransferases[3] and on cyclic AMP-dependent protein kinase[4], and antiandrogenic activity[5]. It is also revealed that prenyl residues attached on the aromatic moiety largely contribute to their biological activities[5,6].

Contrary to phytochemical studies, little is known about biosynthetic enzymes responsible for the prenylation of those aromatic compounds, and cloning of genes encoding those prenyltransferase involved in secondary metabolism has been also desired. As the first example of such prenyltransferases, we isolated a cDNA that encoded *p*-hydroxybenzoate (PHB): geranyltransferase (LePGT-1) from a perennial medicinal plant, *Lithospermum erythrorhizon* (Boraginaceae)[7], which would be involved in the biosynthesis of a red naphthoquinone secondary metabolite, shikonin. From the viewpoint of molecular evolution this enzyme is presumably derived from prenyltransferase for ubiquinone (UQ) biosynthesis, whereas LePGT-1 shares relatively low homology with UQ biosynthetic prenyltransferases. In this report we demonstrate that LePGT-1 is, indeed, not involved in UQ biosynthesis but is specific for secondary metabolism, suggesting that LePGT-1 may be used as a model gene for secondary metabolic prenyltransferase that accepts aromatic substrates.

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Materials and Methods

The DNA sequence corresponding to the mitochondrial signal of the yeast prenyltransferase (COQ2) and the coding sequence of LePGT-1 were amplified by PCR using KOD-Plus DNA polymerase (TOYOBO). Both fragments were fused via PCR resulting in the chimeric DNA that was designated mtQ2-PGT-1. The whole sequence was confirmed by sequencing that no mis-incorporation by PCR took place. The chimeric DNA and wild type *coq2* gene as well as full-length cDNA of LePGT-1 were subcloned in a yeast shuttle vector pDR196, in which a strong constitutive promoter, PMA-1, is used to drive the foreign gene in yeast[8]. The resulting plasmids were introduced into yeast strain, W303-Δ*coq2*[7], by standard lithium acetate method.

Prenyltransferase activity was measured after differential centrifugation to obtain mitochondrial and microsomal fraction as described earlier[7]. Each yeast transformant was precultured in SD (-Ura) medium and grow to OD_{600} 1.0, and the aliquot (50 μl) was plated on SD medium containing either glycerol or glucose as the sole carbon source.

Results and Discussion

LePGT-1 shares relatively low amino acid identity (35%) with COQ2, which is the specific enzyme for UQ biosynthesis. COQ2 polypeptide is localized at inner membrane of yeast mitochondria, whereas LePGT-1 is localized in endoplasmic reticulum (ER) of plant. In order to assess whether LePGT-1 is actually involved in the secondary metabolism and not in UQ biosynthesis, we tried to complement the phenotype of *coq2* disruptant, which can not grow on the medium containing glycerol as the sole carbon source, whereas on glucose medium it shows the same growth rate as wild type yeast. The mitochondrial targeting signal of COQ2 is fused at the N-terminus of LePGT-1, and the fusion construct, mtQ2-PGT-1, was introduced into the *coq2* disruptant.

The enzyme activity of the chimeric protein to transfer the prenyl moiety from geranyl diphosphate (GPP) to PHB was confirmed in the mitochondrial fraction of the transformant (Fig. 1). The host strain, in which *coq2* gene is disrupted, gave no detectable prenyltransferase

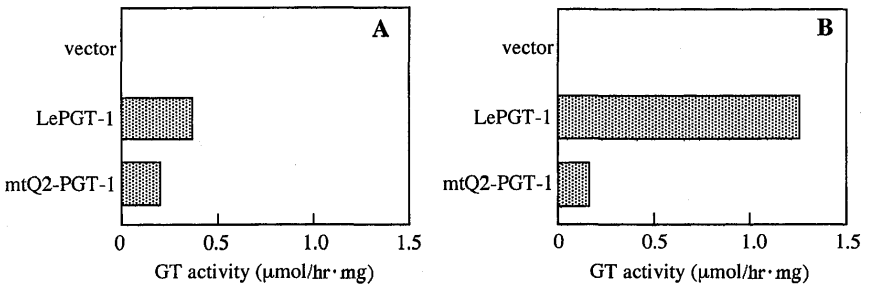

Fig. 1. Prenyltransferase activity of yeast transformants. A: Enzyme activity in mitochondrial fraction of yeast transformants, B: Enzyme activity in microsomal fraction of yeast transformants. Host strain is W303-*Δcoq2*, in which *coq2* gene is disrupted, and the transformant of empty vector is used as negative control.

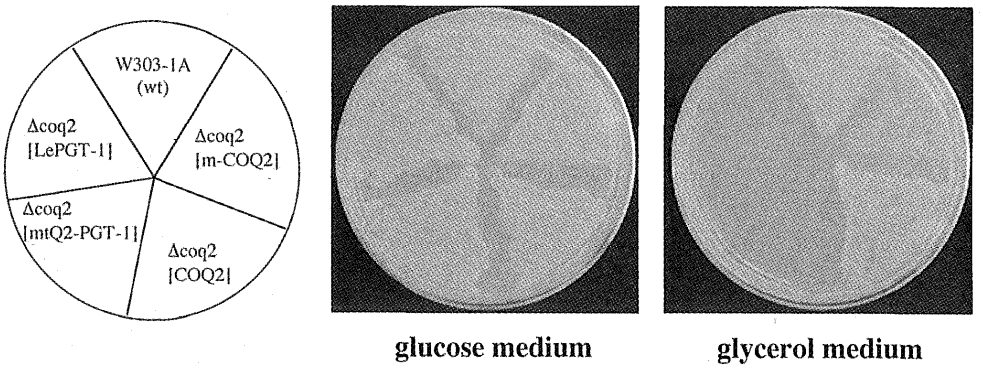

Fig. 2. Growth of yeast transformants on glycerol and glucose media. The yeast strain W303-*Δcoq2* is transformed with each plasmid and plated either on glycerol or glucose media. W303-1A is wild type yeast strain as a control. COQ2, full-length *coq2* gene; mtQ2-PGT-1, LePGT-1 fused to mitochondrial signal of COQ2 at the N-terminus; LePGT-1, full-length LePGT-1 cDNA; m-COQ2 is another COQ2 derivative which will be described elsewhere.

activity[7]. Full-length LePGT-1 showed high enzyme activity in the microsomal fraction, whereas low activity was also detected in mitochondrial fraction, which was probably due to the contamination of the microsomal membrane, as shown in our previous study[7]. The activity of mtQ2-PGT-1 in the mitochondrial fraction was, on the other hand, higher than that in the microsome, indicating that the mtQ2-PGT-1 polypeptide is actually localized in mitochondria as an active form.

The yeast strain that lacks in the ability to biosynthesize UQ is incapable of utilizing glycerol as the carbon source[9]. Thus we carried out the complementation study by use of the *coq2* disruptant to see whether the chimeric gene mtQ2-PGT-1 may complement the UQ biosynthesis, or not. Fig. 2 shows the comparison of cell growth of yeast transformants on glycerol and glucose media. The homologous gene COQ2 that encoded PHB: hexaprenyltransferase involved in UQ biosynthesis complemented the phenotype of W303-*Δcoq2*, whereas the plant prenyltransferase LePGT-1 did not. Similarly the mtQ2-PGT-1 polypeptide that is localized in mitochondria did not recover the cell growth of W303-*Δcoq2* on glycerol medium. HPLC analysis of these W303-*Δcoq2* transformants revealed that mtQ2-PGT-1-introduced yeast did not contain a detectable level of UQ, whereas an appreciable amount of UQ-6 was detected in COQ2 transformant as well as in wild type yeast (positive control). These results strongly suggest that LePGT-1 does not compliment the function of COQ2 to biosynthesize UQ, even if its subcellular localization is altered into mitochondria. The reason is probably because this prenyltransferase is very specific for geranyl diphosphate as the acceptable substrate[7], whose chain length is not sufficient to complete UQ biosynthesis, although further investigation is needed to clarify this point.

LePGT-1 cDNA is a typical aromatic substrate prenyltransferase involved in plant secondary metabolism. It is expected that this gene is utilized as a new molecular tool to isolate prenyltransferases that prenylate various aromatic substrates from various plant species, which contain prenylated coumarins and prenylflavonoids.

References

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