Functional analyses and metabolic engineering of plant prenyltransferase genes

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Ubiquinone (UQ) is a lipid-soluble electron carrier in the respiratory chain. It mediates electron transfer from NADH dehydrogenase (Complex I) to succinate dehydrogenase (Complex II) and further to the bc1 complex (Complex III) at the inner mitochondrial membrane (1). In humans, cellular UQ contents, particularly in heart cells, peak at age 20 then decreases (2). UQ therefore has been efficacious as a medicine to improve heart function. UQ supplementation recently has been reported to have mild symptomatic benefits for patients with Alzheimer (3), Parkinson (4), and Huntington (5) diseases. Furthermore, food supplements and cosmetics that contain UQ as an antioxidative have become very popular in the world market. However, only little is known about its physiological role *in planta*.

UQ is distributed in various compartments as well as in the mitochondria; the nucleus, plasma membrane, Golgi vesicles, and lysosomes, in which UQ is thought to function as an antioxidant (6) or an electron transporter (7). In yeast and arabidopsis, UQ biosynthesis is believed to take place in the mitochondria, but there are reports of UQ biosynthesis in such other subcellular areas as the endoplasmic reticulum (ER) and the Golgi apparatus in both rat liver (8) and spinach (9). A key reaction step in UQ biosynthesis is the prenylation of *p*-hydroxybenzoate (PHB); i.e., coupling of the aromatic substrate and isoprenoid chain, which is presumed to be rate-limiting for UQ production (Fig. 1). PHB: polyprenyltransferases (PPT) which catalyze this reaction are membrane-bound proteins. These occur widely from bacteria to humans and form a gene family. The length of the isoprenoid side chain varies with the organism. For example, *Saccharomyces cerevisiae* has UQ6, *Escherichia coli* UQ8, and *Schizosaccharomyces pombe* UQ10 as does humans. UQ chain length specificity reportedly is determined by the product specificity of prenyldiphosphate synthase (10), whereas PPTs have broad substrate specifity for prenyldiphosphates of different chain lengths (11). One reported exception is the LePGT involved in the biosynthesis of shikonin, a red naphtoquinone secondary metabolite in boraginaceous plants. It recognizes solely geranyldiphosphate as the prenyl substrate (12).

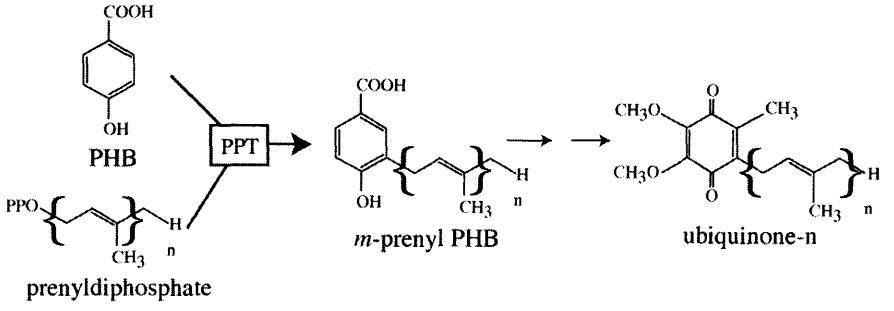

Fig. 1. Ubiquinone biosynthesis. PHB; *p*-hydroxybenzoate, PPT; PHB: polyprenyltransferase, *m*-prenyl PHB; *m*-prenyl-*p*-hydroxybenzoate.

The UQ contents in crop plants should be of interest in agricultural and food sciences, since this endogenous antioxidant may be quite beneficial for both consumers and producers, and thus the establishment of crops that produce UQ at high levels may be a new target of metabolic engineering. However, there has been no previous biosynthetic studies of UQ in crop plants, and therefore no biosynthetic gene is known, even in rice, which is both a model plant and a major crop that is cultivated worldwide. Moreover, the enzymatic properties of the PPT protein family have not been well characterized thus far. In this study, we characterized the *OsPPT1a* gene that encodes a rice PPT to clarify its biochemical properties as the key enzyme for UQ biosynthesis in rice. The deduced amino acid sequence of OsPPT1a contained a putative mitochondrial sorting signal at the N-terminus and conserved domains for putative substrate-binding sites typical of PPT protein family members. The subcellular localization of OsPPT1a protein was shown to be mainly in mitochondria based on studies using a green fluorescent protein-PPT fusion. A yeast complementation study revealed that *OsPPT1a* expression successfully recovered the growth defect of *coq2* mutant. A prenyltransferase assay using recombinant protein showed

that OsPPT1a accepted prenyl diphosphates of various chain lengths as prenyl donors, whereas it showed strict substrate-specificity for the aromatic substrate PHB as a prenyl acceptor. The apparent K_m values for geranyl diphosphate and PHB were 59.7 and 6.04 μM, respectively. Their requirement by OsPPT1a for divalent cations was also studied, with Mg^{2+} found to produce the highest enzyme activity. Northern analysis showed that *OsPPT1a* mRNA was accumulated in all tissues of *O. sativa*. These results suggest that OsPPT1a is a functional PPT involved in UQ biosynthesis in *O. sativa* (13).

In addition, we overexpressed the yeast *coq2* gene, which encodes PHB: hexaprenyltransferase (14), in order to increase the UQ10 content of tobacco. Because of the broad substrate specificity of COQ2, enhanced production of UQ10, which is native in tobacco, is to be expected because this enzyme functions as 'PHB: decaprenyltransferase' in tobacco. The COQ2 polypeptide deduced from the DNA sequence has a putative mitochondrial signal peptide at the N-terminus, as predicted by targetP (http://www.cbs.dtu.dk/services/TargetP/). In higher plants, however, little is known about the subcellular compartment of UQ biosynthesis. We engineered the subcellular localization of COQ2 in order to clarify whether there is mitochondrial or ER localization of this UQ biosynthetic enzyme by estimating its contribution to UQ production. The tobacco transgenic lines showed about a 6- fold increase in ubiquinone. COQ2 polypeptide, whose localization was enforcely altered to the endoplasmic reticulum, had the same or a greater effect as mitochondria-localized COQ2 on the increase in ubiquinone in both the yeast and tobacco transformants, indicative that the ubiquinone intermediate is transported from the endoplasmic reticulum to the mitochondria. Plants with a high ubiquinone level are more resistant to oxidative stresses caused by methyl viologen or high salinity. This is attributable to the greater radical scavenging ability of the transgenic lines as compared to the wild type (15).

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