Identification and characterization of *p*-hydroxybenzoate polyprenyltransferase gene involved in coenzyme Q biosynthesis in rice.

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Ubiquinone (UQ, coenzyme Q) is a lipid-soluble electron carrier required for the respiratory chain in the mitochondria of eukaryotic cells. Adding to the conventional clinical use for the improvement of heart function, UQ has also been recently used in cosmetics and food supplements to prevent the accumulation of active oxygen species.

Prenylation of the aromatic intermediate *p*-hydroxybenzoate (PHB) is a critical step in UQ biosynthesis. The enzyme that catalyzes this prenylation reaction is PHB polyprenyltransferase (PPT), which substitutes an aromatic proton at the *m*-position of PHB with a prenyl chain provided by polyprenyl diphosphate synthase. The rice genome contains three PPT candidates that share significant similarity with the yeast PPT (*COQ2* gene), and the rice gene showing the highest similarity with *COQ2* was isolated by RT-PCR and designated *OsPPT1a*. 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*.

REFERENCE

Ohara, K., et al. *Plant Cell Physiol.* **47 (5):** 581-590 (2006)