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Influences on tocopherol biosynthesis in the cyanobacterium *Synechocystis* sp. PCC 6803

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Summary

To elucidate influences on the tocopherol biosynthesis in cyanobacteria, wild type and mutant cells of a putative methyltransferase in tocopherol and plastoquinone biosynthesis of *Synechocystis* sp. PCC 6803 were grown under different conditions. The vitamin E content of cells grown under different light regimes, photomixotrophic or photoautotrophic conditions and varying carbon dioxide supplies were compared by HPLC measurements. The tocopherol levels in wild type cells increased under higher light conditions and low carbon dioxide supply. Photomixotrophic growth led to lower vitamin E amounts in the cells compared to those grown photoautotrophically. We were able to segregate a homozygous Δ*sll0418* mutant under photoautotrophic conditions. In contrast to former suggestions in the literature the deletion of this gene is not lethal under photomixotrophic conditions and the influence on tocopherol and plastoquinone amounts is diminutive. The methyltransferase encoded by the gene *sll0418* is not essential either for tocopherol or plastoquinone synthesis.

Introduction

Tocopherols as a group of lipophilic molecules exclusively synthesized by oxygenic photosynthetic organisms are collectively termed vitamin E. α-, β-, γ- and δ-tocopherol differ in the number and position of methyl groups on the chromanol ring. In most cases, α-tocopherol is the main vitamin E form in plant cells. The role of tocopherol in photosynthetic organisms has yet to be determined. Antioxidant, membrane stabilisation, intracellular signalling and cyclic electron transport around photosystem II are suggested functions of tocopherol (reviewed by Munné-Bosch and Alegre

Abbreviations: b, bubbled; Cm, chloramphenicol; DMPBQ, 2, 3-dimethyl-6-phytyl-1, 4-benzoquinone; glc, glucose; HGA, homogentisic acid; HPLC, high performance liquid chromatography; HPP, p-hydroxyphenylpyruvate; HPPD, p-hydroxyphenylpyruvate dioxygenase; MPBQ, 2-methyl-6-phytylbenzoquinone; ORF, open reading frame; PCR, polymerase chain reaction; PSII, photosystem II; s, shaken

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2002). In *Chlamydomonas reinhardtii* a protective function against photooxidative damage in photosystem II has been suggested (Trebst et al., 2002, 2004). The tocopherol biosynthesis in cyanobacteria is similar to the pathway in plants but differs in some aspects (Fig. 1). The conversion of *p*-hydroxyphenylpyruvate (HPP) to homogentisic acid (HGA) is catalyzed by *p*-hydroxyphyenylpyruvate dioxygenase (HPPD). HGA is the aromatic precursor for both tocopherol and plastoquinone biosynthesis in plants (Norris et al., 1998). The following step is the condensation of HGA and phytyldiphosphate and yields 2-methyl-6-phytylbenzoquinone (MPBQ) (Collakova and DellaPenna, 2001; Schledz et al., 2001). The cyclization of MPBQ leads to δ-tocopherol, which can be methylated to yield β-tocopherol. If methylation of MPBQ occurs before cyclization the two reactions create γ-tocopherol (Soll et al., 1985; Arango and Heise, 1998; Porfirova et al., 2002; Sattler et al., 2003) and the second methylation after cyclization produces α-tocopherol (Soll et al., 1980; d'Harlingue and Camara, 1985; Shintani and DellaPenna, 1998).

In *Synechocystis* sp. PCC 6803, the gene *slr0090* encodes the p-hydroxyphenylpyruvate dioxygenase (HPPD) and catalyzes the formation of homogentisate from HPP. Deletion of the orthologous gene in *Arabidopsis thaliana* leads to tocopherol and plastoquinone lacking mutants. The mutation is lethal (Cheng et al., 2003). In *Synechocystis* sp. PCC 6803 deletion of *slr0090* causes a tocopherol deficiency but does not impair plastoquinone biosynthesis (Daehnhardt et al., 2002). The protein encoded by ORF *slr1736* has been identified as phytyltransferase acting specifically in the tocopherol biosynthetic pathway. The knock out mutant shows markedly reduced levels of tocopherol but not of plastoquinone (Schledz et al., 2001). The next step of the pathway is catalyzed by a tocopherol cyclase encoded by *slr1737*. If this gene is deleted, tocopherol synthesis is blocked and the intermediate 2,3-dimethyl-6-phytyl-1,4-benzoquinone (DMPBQ) accumulates (Sattler et al., 2003). The γ-tocopherol methyltransferase which catalyzes the methylation of γ-tocopherol to α-tocopherol and of δ- to β-tocopherol has been

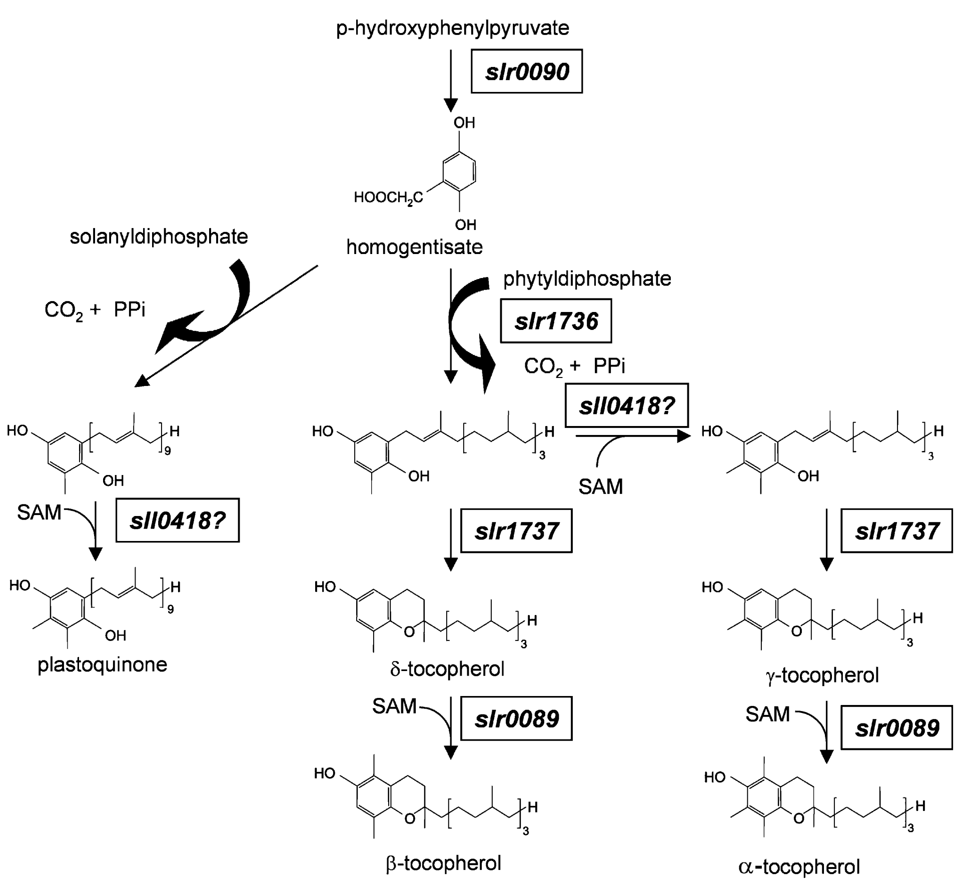

Figure 1. Tocopherol biosynthesis pathway with gene numbers of *Synechocystis* sp. PCC 6803 known to encode enzymes that perform steps in tocopherol and plastoquinone synthesis. Question marks indicate questionable functions. SAM = S-adenonsylmethionine (adapted from Cheng et al., 2003).