

A Short Note on Carotenoid Biosynthesis

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Description

Two enzymes, Phytoene Desaturase (PDS) and carotene desaturase, catalyse carotene desaturation, an important step in the carotenoid biosynthetic pathway (Zeta Carotene Desaturase, ZDS). *Zdsfc*, a new *Ficus carica* carotene desaturase catalysing dehydrogenation of carotene into neurosporene and eventually lycopene, was cloned and expressed in *E. coli*. Rapid Amplification of CDNA Ends (RACE) was used to amplify the carotene desaturase (ZDS) gene from the fig tree, which spanned a 1746 bp Open Reading Frame (ORF) and encoded a 582-amino-acid-residue protein with an evaluated molecular weight of 64kD. A putative transit sequence for plastid targeting was found in the polypeptide's N-terminal region. *Zdsfc* showed remarkable resemblance with previously identified β -carotene desaturases from higher plant taxa based on phylogenetic and sequencing analysis.

Furthermore, sequencing analysis revealed that plant ZDSs are highly conserved. The predicted ZDS protein, termed *zdsfc*, likewise has a dinucleotide-binding domain at the N-terminus, followed by a conserved region found in other carotene desaturase sequences. These findings support the identification of our cloned *zdsfc* as a member of the ZDS protein family.

Carotenoids are pigments produced by plants, fungus, bacteria, and algae to shield them against the adverse effects of singlet oxygen and free radicals. Carotenoids are either primary or secondary metabolites in plants. Carotenoids can act as growth and development regulators, accessory pigments in photosynthesis, photo-protectors against photo-oxidative damage, or precursors of the hormone Abscisic Acid (ABA) as primary metabolites.

They also have a role in the colour of fruits and flowers, producing distinct yellow, orange, and red shades, and hence contribute significantly to plant-animal interactions. Furthermore, the hues of many carotenoid-accumulating fruits and flowers boost their attraction

and, as a result, their economic value. These pigments also act as antioxidants in human and animal diets, protecting both humans and animals. The major carotenoid metabolic route is well-known, and most carotenogenic species may share it. The condensation of two geranylgeranyl molecules, phytoene, is the initial step in this mechanism. A single soluble enzyme, Phytoene Synthase, (PSY) catalyses this two-step process. The first phytofluene is formed after two successive desaturations of phytoene, followed by carotene.

Phytoene De-Saturase (PDS) catalyses both of these processes, carotene desaturase (ZDS) catalyses two further desaturations, resulting in neurosporene and then lycopene, the symmetrical red carotenoid pigment. Many carotenoid biosynthetic genes have been found in plants and other organisms since the carotenoid biosynthesis gene cluster from *Erwinia uredovora* was cloned in 1990. The expression of recombinant *Ficus carica* lycopene beta-cyclase in *E. coli* produces carotene. The cloning and expression of *zdsfc*, a new *Ficus carica* carotene desaturase that catalyses the dehydrogenation of carotene into neurosporene and then lycopene, in *Escherichia coli*.

The *E. coli* culture containing *zdsfc* was able to convert carotene and neurosporene, two substrates of carotene desaturase, into neurosporene and lycopene, respectively, demonstrating the enzymatic activity of the recombinant ZDS we created *in vivo*. A phylogenetic tree indicating the evolutionary link between ZDS and other documented carotenoid Zeta Carotene Desaturases (ZDSs) from higher plants since the *F. carica* enzyme shared the same characteristics as other plant ZDSs.

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