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Functional analysis of spheroidene mono-oxygenase, CrtA, of the purple photosynthetic bacterium, *Rubrivivax gelatinosus*

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Introduction

In purple photosynthetic bacteria, carotenoids are bound to the pigment-protein complexes, *i.e.*, the reaction center complex and the light-harvesting 1 and 2 complexes. One of the two important functions of carotenoids in photosynthetic bacteria is the absorption of light energy with wavelengths ranging from 400 to 550 nm and the transfer of the absorbed energy to bacteriochlorophylls (Bchl). The other is photoprotection against the formation of singlet oxygen and other harmful free radicals.

In carotenoid biosynthesis, many purple bacteria synthesize spirilloxanthin and its precursors by the normal spirilloxanthin pathway. On the other hand, some species, such as *Rhodobacter* species, synthesize spheroidene and OH-spheroidene under photosynthetic conditions and keto-carotenoids, spheroidenone and OH spheroidenone, under semi-aerobic conditions by the spheroidene pathway (see Fig. 3) (Takaichi 1999). It is known that this oxidation is catalyzed by spheroidene mono-oxygenase CrtA in *Rhodobacter*. Keto-carotenoids have not been found from bacteria which have the normal spirilloxanthin pathway.

The purple photosynthetic bacterium, *Rubrivivax* (*Rvi.*) *gelatinosus*, has a unique carotenoid composition among purple bacteria (Takaichi and Shimada 1999). This bacterium synthesizes spirilloxanthin in addition to the major carotenoids, spheroidene and OH-spheroidene. The occurrence of these carotenoids is due to the unique characteristics of phytoene desaturase CrtI of this bacterium (Harada et al.

2001). In *Escherichia coli* with a phytoene background, CrtI of *Rvi. gelatinosus* changes phytoene mainly to neurosporene and about 10% of lycopene. Spheroidene and spirilloxanthin are synthesized from neurosporene and lycopene, respectively (see Fig. 3). We have also shown the production of spheroidenone and OH-spheroidenone as well as 2,2'-diketospirilloxanthin from spirilloxanthin via 2-ketospirilloxanthin under the semi-aerobic conditions (Takaichi and Shimada 1999).

To clarify the role of CrtA in mono-oxygenation of carotenoids in *Rvi. gelatinosus*, we cloned the crtA gene from the genome and constructed a crtA-deleted mutant.

Materials and Methods

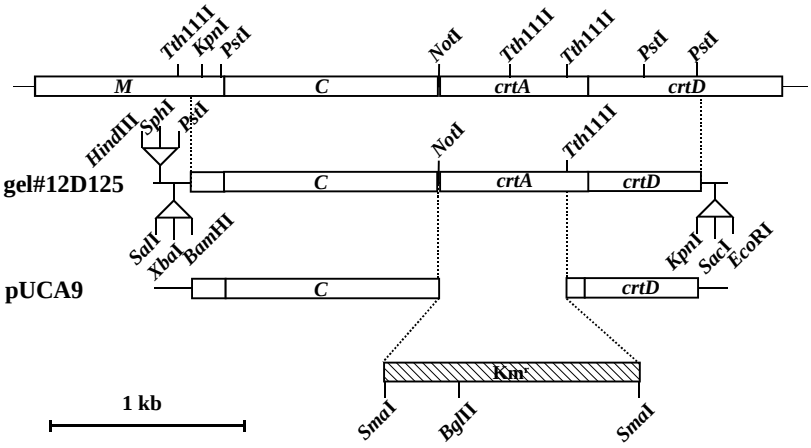

Fig. 1 Gene structure of the *Rubrivivax gelatinosus* wild type around the *crtA* gene and the plasmids gel#12D125 and pUCA9. The pUCA9 plasmid is a pUC119-based plasmid and used to construct the *crtA*-deleted mutant of *Rvi. gelatinosus* by double crossover recombination.

Rvi. gelatinosus strain IL144 was grown anaerobically under anaerobic-light or heterotrophic, aerobic or semi-aerobic dark conditions at 30 °C in PYS medium. The plasmid, gel#12D125, which contained the *crtA* gene of *Rvi. gelatinosus* was derived from the cosmid clone pgc#6 (Fig. 1) (Igarashi et al. 2001). Most of the *crtA* gene in the gel#12D125 plasmid was removed by *NotI* and *Tth*111I digestions and replaced by a kanamycin resistance gene, creating a plasmid named pUCA9 (Fig. 1). The pUCA9 plasmid DNA was introduced into the *Rvi. gelatinosus* wild-type cells by electroporation (Nagashima et al. 1996). Kanamycin-resistant cells were selected on PYS agar plates containing 50 μg/ml kanamycin and tested for resistance against ampicillin. The ampicillin-sensitive but kanamycin-resistant transformant was isolated as a ΔcrtA strain.

Pigments were extracted from the wet cells by acetone/methanol (7:2, v/v) using an ultra sonicator for several seconds. These pigments were dissolved in a small volume of chloroform/methanol (3:1, v/v) and directly analyzed by the HPLC

system equipped with a μ Bondapak C_{18} column eluted with methanol. Each carotenoid was identified by the retention times on HPLC and the absorption spectrum in the eluent by a photodiode array detector (Takaichi and Shimada 1999, Harada et al. 2001).

Results and Discussion

In the wild-type cells of *Rvi. gelatinosus* cultured under semi-aerobic conditions, 2,2'-diketospirilloxanthin and 2-ketospirilloxanthin were detected in addition to the major carotenoids, spheroidenone and OH-spheroidenone (Fig. 2A, peak 3, 5, 7 and 2, respectively). Spheroidene, OH-spheroidene and OH-neurosporene were also detected in the wild-type cells (Fig. 2A, peak 8, 4 and 6, respectively). In the ΔcrtA strain cells grown under semi-aerobic conditions, spheroidene, OH-spheroidene and spirilloxanthin were detected (Fig. 2B, peak 8, 4 and 10, respectively). In this strain, no keto-carotenoids were found. These results suggests that there is one *crtA* gene in *Rvi. gelatinosus* and it catalyzes the mono-oxygenation of not only spheroidene and OH-spheroidene but also spirilloxanthin. This is the first report that CrtA catalyzes the mono-oxygenation of spirilloxanthin. From these results, we propose the full carotenoid biosynthesis pathway in *Rvi. gelatinosus* (Fig. 3).

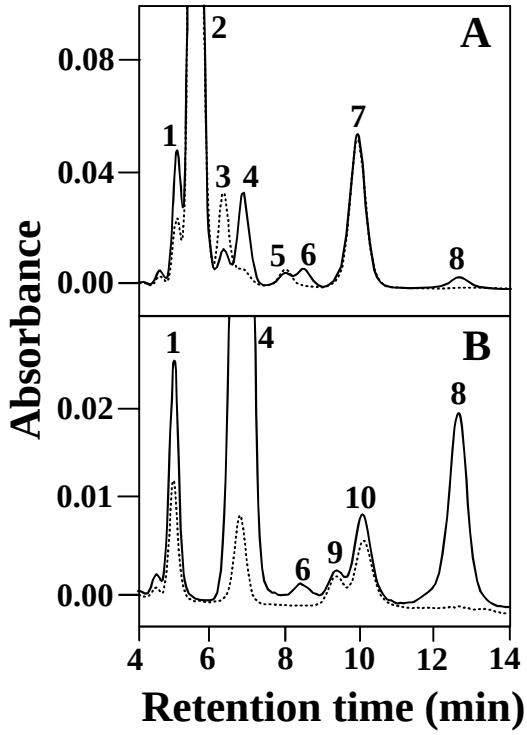

Fig. 2 Elution profile of HPLC of pigments extracted from the wild type (A) and the ΔcrtA strain (B) grown semi-aerobically. Absorbance at 452 nm (———) and 507nm (- - - -). Peak 1, bacteriochlorophyll *a*; peak 2, OH-spheroidenone; peak 3, 2,2'-diketospirilloxanthin; peak 4, OH-spheroidene; peak 5, 2-ketospirilloxanthin; peak 6, OH-neurosporene; peak 7, spheroidenone; peak 8, spheroidene; peak 9, *cis*-spirilloxanthin; peak 10, spirilloxanthin.

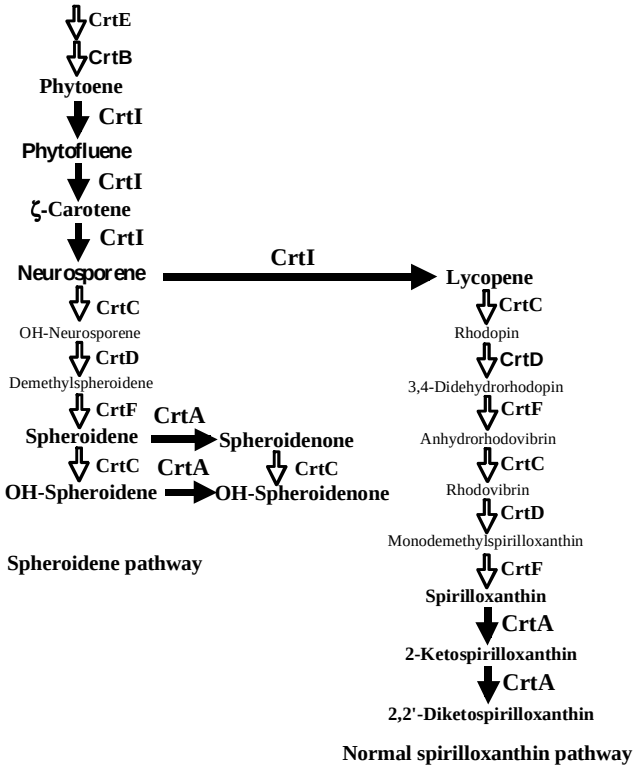

Fig. 3 Carotenogenesis pathway in *Rubrivivax gelatinosus*. Black arrows show the pathway determined in our works.

The significance of formation of keto-carotenoids has not been elucidated; why does mono-oxygenation of carotenoids occur only in species possessing spheroidene pathway? To obtain clues for this question, we have compared growth and viability of the ΔcrtA strain to the wild type. However, no significant difference was observed in the growth rate under semi-aerobic light conditions. It was possible that spirilloxanthin which was still synthesized in the ΔcrtA strain had photoprotective function, since the reaction center of *Rvi. gelatinosus* contains spirilloxanthin (Jirsakova et al. 1995). This was also suggested by a growth inhibition of a blue-green mutant (*crtI* gene-deleted mutant of *Rvi. gelatinosus*) under the semi-aerobic light condition which totally lacked in colored carotenoids. Experiments to obtain strains deficient in both spheroidenone and spirilloxanthin is under way.

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