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# Distribution of colored carotenoids between light-harvesting complexes in the process of recovering carotenoid biosynthesis in *Ectothiorhodospira haloalkaliphila* cells

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ABSTRACT

The processes of recovering colored-carotenoid (Car) biosynthesis in Car-less cells of the purple sulfur bacterium *Ectothiorhodospira haloalkaliphila* grown with diphenylamine (DPA-cells) have been studied. It has been found that (1) the rate of recovering colored-Car biosynthesis in the lag-phase is far ahead of the growth rate of the cells themselves; (2) several Cars (ζ-carotene, neurosporene etc.) act as intermediates in Car biosynthesis; (3) because filling the "empty" Car pockets in the LH1-RC complexes is faster than in LH2, available spirilloxanthin is preferentially incorporated into the nascent LH1-RC core particles; (4) as a consequence of the resulting lack of spirilloxanthin availability, the biosynthetic intermediates (anhydrorhodovibrin, rhodopin and lycopene) fill the empty nascent LH2 Car pockets. In the present report, we further discuss the process of colored Car incorporation into LH complexes during the recovery of Car biosynthesis in the DPA-cells of *Ect. haloalkaliphila*.



## 1. Introduction

The processes of harvesting and converting solar energy in phototrophic purple bacteria take place in two major structural components of the photosynthetic apparatus, i.e. in a light-harvesting antenna and a photochemical active reaction center (RC). The antenna is required to increase the efficiency of capturing solar energy, and usually consists of two types of light-harvesting pigment–protein complexes (LH1 and LH2) which are located in the intracytoplasmic membrane [1–3]. The two complexes have been isolated and well characterized [1,4–7]. In the near-IR region, the LH1 complex has the main absorption peak at ~865–890 nm, and the LH2 complex – the two main peaks at ~800 and ~850 nm [1,5,8]. The LH1 complex surrounds the RC, forming so-called LH1-RC core complex [9,10]. The ratio of the LH1 to the RC in the LH1-RC complex is constant (1:1) [11]. The LH2 complexes are found in most, but not in all purple bacteria [1,7]. A separate spatial high-resolution structure for the LH1-RC and the LH2 complexes has been defined [11–17]. Both types of the LH complexes in the purple bacteria are built on a similar modular principle [9]. The basic unit of each LH complex is a heterodimer consisting of two hydrophobic polypeptides (α and β) which bind non-covalently bacteriochlorophyll (BChl) and Cars [1,7]. Cars are additional pigments in bacteria, which are localized between α- and β-polypeptides in binding sites referred to as "Car pockets" [18,19]. *In vivo* they perform three main functions: (1) they harvest light in spectral regions where the BChl absorption is minimal; (2) they protect against potentially harmful BChl singlet- and derived triplet excited states as well as against reactive oxygen species; (3) they effectively stabilize the structure of LH complexes [1,20–23].

There are two major Car biosynthetic pathways for the purple bacteria: (i) the spirilloxanthin pathway (normal spirilloxanthin, unusual spirilloxanthin, spheroidene and carotenal pathways); (ii) the okenone pathway (okenone and R.g.-keto Car pathways) [24]. The Car composition in the cells of the purple bacteria was shown to vary depending on their type, age and growth conditions [25–28]. In the LH1 and the LH2 complexes, either identical or different types of Cars may be present. For example, spirilloxanthin predominated in the LH1-RC complex in *Rhodoblastus* (*Rbl.*) *acidophilus* strain 10050 and *Rhodopseudomonas* (*Rps.*) *palustris* strain 2.1.6, whereas rhodopin-glucoside and rhodopin were the main Cars in the LH2 complex from the same bacteria [29]. In *Rhodobacter* (*Rba.*) *sphaeroides,* spheroidene was predominantly associated with the LH2 complex, and spheroidenone was more abundant in the LH1-RC complex [30,31]. In contrast, the LH2 and LH1-RC complexes in *Rba. capsulatus* did not differ in their Car composition [32].

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It is well documented that the LH2 complexes do not assemble in Car-less mutants of the purple non-sulfur bacteria produced by classic mutagenesis or a transposon method [33,34]. For example, in the cells of *Rba. sphaeroides*, the LH2 complex was not assembled when a transposon was incorporated into the *crtB* gene [34]. A different method, however, with diphenylamine (DPA) as a Car biosynthetic inhibitor, does allow almost completely to inhibit Car biosynthesis in some purple bacteria (*Allochromatium* (*Alc.*) *vinosum*, *Alc. minutissimum*), with the assembly of the LH2 complex being kept at the level of normal cells [18,35,36].

Previously, we succeeded in inhibiting Car biosynthesis in one more purple sulfur bacterium (*Ectothiorhodospira* (*Ect.*) *haloalkaliphila*) and isolating Car-less LH2 complex from the cells of this bacterium [19,37]. In this study, we focus on the recovery of colored-Car biosynthesis in Car-less cells of *Ect. haloalkaliphila* and on the distribution of colored Cars between the LH1-RC and LH2 complexes in the course of this process.

## 2. Materials and methods

### 2.1. Cultivation of cells and isolation of membranes

The cells of *Ect. haloalkaliphila* strain ATCC 51935$^T$ [38] were grown in white light with the intensity of 1200 l× on the modified Pfenning medium [39] at 26 ± 2 °C. To obtain cells with maximum inhibited synthesis of Cars (DPA-cells), DPA in a concentration of 71 μM was added into the growth medium, as described previously [19]. To obtain cells capable of de novo Car biosynthesis, DPA-cells were pelleted and resuspended in sterile growth medium, in the absence of the inhibitor. The growing cells were selected for their change in optical density at 650 nm after reseeding (the time of growth of each sample prior to selection is shown in parentheses). These DPA-cells served as an initial sample (Sample 1, 0 h). Then, 8 more samples were selected: Sample 2 (2 h), Sample 3 (4 h), Sample 4 (5 h), Sample 5 (10 h), Sample 6 (17 h), Sample 7 (25 h), Sample 8 (54 h) and Sample 9 (110 h). The Car composition of the selected samples was compared with the Car composition of the initial DPA-sample (Sample 1) and the DPA-untreated sample (Sample 10, control). The absorption spectra of cells were recorded using a Sintra 3030 spectrophotometer equipped with a sphere (GBC, Australia). The kinetics of cells growth was determined after measuring the change in optical density at 650 nm in a 1-cm cuvette using a Cary 50 spectrophotometer (Varian, Australia). Then, cells were disrupted by ultrasound, and membranes were obtained by the method of differential centrifugation [40]. LH complexes were isolated with preparative electrophoresis in polyacrylamide gel [5]. The membranes were solubilized with 2–2.5% of dodecylmaltoside. After electrophoresis, LH complexes were eluted and concentrated in an Amicon Ultra 50 K centrifugal filter unit (Millipore, USA).

### 2.2. Analysis of Cars

To extract Cars 9 ml of acetone–methanol mixture (7:2) were added to 1 ml of the complexes with optical density of 20–40 optical units under continuous stirring. 2–4 ml of petroleum ether and 20–25 ml of water were successively added to the extract obtained and mixed again. The extracted pigments were localized in the upper layer of the mixture. They were pipetted, transferred into a heparin vial and dried under argon flow. The obtained film of pigments was diluted in the acetone–methanol mixture (7:2) and 25 μl of the extract was injected into the column. The pigments were analyzed by HPLC as described previously [19], using an Agilent Zorbax SB-C18 (4.6 × 250 mm) column (Agilent, USA). The device for HPLC consisted of (1) a pump LC-10ADVP with a module FCV-10ALVP to make a solvent gradient on the side of low pressure, (2) a detector with diode matrix SPD-M20A and (3) a thermostat CTO-20AC (Shimadzu, Japan). The feed rate of the solvent was 1.0 ml/min. The analysis was performed at a constant temperature equal to 22 °C. The concentration of Cars was calculated with the program of LC-solution (Shimadzu, Japan) and the extinction coefficients [29]. The correction on the content of "empty" Car pockets was taken into consideration in each studied sample, as described in the report [19].

## 3. Results and discussion

Fig. 1 shows the absorption spectra of the DPA-cells and the control cells of *Ect. haloalkaliphila*. The absorption band maxima of the DPA-cells are located at 380, 594, 798, 856 and 892 (shoulder) nm, and those of the control cells are located at 380, 488, 515, 548, 594, 798, 852, 892 (shoulder) nm. The maxima at 798 and 856 (852) nm belong to the LH2 complex, and the maximum at 892 nm belongs to the LH1 complex. The maxima in the region of 400–570 nm, characteristic for Cars, are lacking in these cells. Thus, it is the Car-less DPA-cells that serve as an initial sample in which the two LH complexes assemble.

The active culture of the DPA-cells washed from the inhibitor was placed into a conventional growth medium. The cell growth time was 110 h. The growth curves of the DPA-cells were compared with those of the control cells. It was clearly seen that the two types of cells had approximately the same lag-phase of 12–14 h. The growth of the DPA-cells was slowed as compared to that of the control cells (Fig. 2). The DPA-cells reached the stationary phase ~42–46 h later than the control cells. Such retardation in the growth of the washed DPA-cells may be due to the influence of DPA which could remain in these cells, in spite of their washing before the start of the experiment. While DPA strongly affects bacterial cells, the effect of this inhibitor on other aspects of cell growth is still unknown. It may be: (i) the involvement in cell metabolism; (ii) the inhibition of some types of biosynthesis, including Car and ubiquinol biosynthesis; (iii) the decrease or increase in the synthesis of any protein, e.g. cytochrome; (iv) cell death [18,29]. In our case, the growth of DPA-cells may become slower because of the residual concentration of DPA which apparently has no significant effect on Car biosynthesis but may inhibit the processes associated with the cell growth or their metabolism. During the cell growth, the inhibitor diffuses into the growth medium where it is exposed to light and destroyed [41]. The slower growth of the DPA-cells is not probably due to the lack of Cars because the light-harvesting complexes are able to assemble in

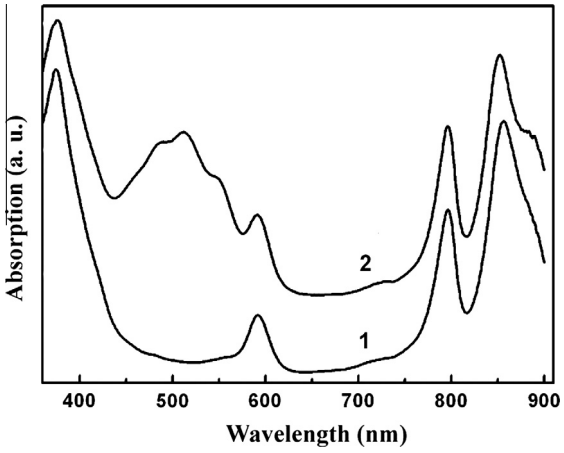


**Fig. 1.** The absorption spectra of the cells of *Ect. haloalkaliphila*: 1 – the DPA-cells; 2 – the control cells. The spectra are normalized by the BChl $Q_x$ band (590 nm).