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# Light-harvesting complexes from purple sulfur bacteria *Allochromatium minutissimum* assembled without carotenoids

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ABSTRACT

Effect of carotenoid (Car) biosynthesis inhibitor diphenylamine (DPA) on purple sulfur bacteria *Allochromatium* (*Alc*) *minutissimum* cell growth has been investigated. Cell growth in the presence of maximum concentration of DPA results in practically complete suppression (~99%) of carotenoids (Cars) according to the spectrophotometric, HPLC and CD data. Phytoene does not replace the colored carotenoids in these cells. Also Phytoene does not accumulate in large amounts in the cells treated with DPA. A new method for calculating the content of Cars in the complexes from the cells with inhibited Car synthesis including the number of empty Car's "pockets" has been used. Our results together with published data devoted to DPA action on the cell growth of purple bacteria revealed that Phytoene was not accumulated in the cells treated with DPA. We have concluded that (i) DPA completely inhibits or strongly reduces synthesis of the colored Cars in the cells of purple bacteria, (ii) Phytoene is the main one among the trace amounts of the other Cars in the case of significant inhibition of Car biosynthesis (80–90% or higher).

The amount of the LH2 complexes presented in the membranes of *Alc minutissimum* was found to be little dependent on DPA. From DPA-grown cultures it was possible to isolate Car-less both the LH1 (as LH1–RC complex) and the LH2 complexes. Electronic absorption properties of BChl's were very similar to those isolated from the control cells. It is shown by HPLC data that the 100 LH2 complexes from cells of *Alc minutissimum*, in which the synthesis of Car was depressed, contained ~9 Car molecules and 5 Phytoene molecules. Thus, only nine (with 1 Car molecule per a complex) or less (if more than one Car molecule per a complex) of the 100 LH2 complexes contain molecules of Cars. It means that 90 or more LH2 complexes from each 100 ones are assembled without any Cars. This is in strong contrast with the previous results obtained with purple non-sulfur bacterium *Rhodobacter sphaeroides*, where the amount of LH2 presented in the membrane was directly correlated to the amount of the carotenoids synthesized (H.P. Lang, C.N. Hunter, The relationship between carotenoid biosynthesis and the assembly of the light harvesting LH2 complex in *Rhodobacter sphaeroides*, Biochem. J. 298 (1994) 197–205). Our results show that although the presence of Car molecules is important for the stability of the LH2 complexes the overall native structure can be maintained without any Cars at least in the case of purple sulfur bacteria.



## 1. Introduction

The first step of photosynthesis is absorption of the solar photons by the pigments bound to the specialized proteins which are known as antenna or light-harvesting (LH) complexes [1,2]. Here photon energy is transformed into excitation energy that migrates through the antenna system to the reaction centers, where the primary charge separation takes place [3,4]. In purple photosynthetic bacteria only a few different types of light-harvesting complexes are assembled, which deliver the excitation energy to a single type of the reaction center [3,5,6]. Light-harvesting complexes were named according to their absorption bands in the near infra-red regions as B800–850 and B870 (or 880) or LH2 and LH1, respectively. They are integral membrane proteins that bind two major classes of chromophores, namely bacteriochlorophyll (BChl) and carotenoid (Car) molecules [4,7]. The LH1 and LH2 complexes are an attractive model for studying the organization of membrane pigment–protein complexes and for the primary processes of photosynthesis [8–11].

The LH2 complexes from a number of nonsulfur bacteria are much more extensively studied photosynthetic objects as compared to the LH1 complex [12–14]. The structures of the LH2 complexes from *Rhodopseudomonas acidophila* and *Rhodospirillum molischianum* have been derived from X-ray crystallography to a resolution exceeding 2.5 Å [12,15]. Later the crystal resolution

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has been extended to 2.0 Å, and the structure of the surrounding detergent was elucidated [13,16]. The protein part of LH2 complexes consist of two low-molecular weight polypeptides (α and β), each spanning the membrane once. Depending on bacterial species, eight or nine αβ-pairs associate to form a ring-like structure within the membrane. Each αβ pair binds non-covalently three BChl molecules, two strongly interacting ones, (BChl-B850), located inside the membrane phase, and a monomeric one (BChl-B800), which is located on the outer side of the complex. The Car molecules (in all-trans configuration) are inserted between the α and β polypeptides, nearly parallel to their trans-membrane α-helices, *i.e.* perpendicular to the membrane. One end of the Car molecule is in van der Waals contact with BChl-B850, while the other one is exposed to periplasmic surface of the LH2 complex [12,13,15,17]. The single Car molecule has at least 15 contacts with different amino acid residues of both polypeptides. Most of interactions between the αβ pair in the membrane phase seem to be mediated by this molecule [17,18]. There is currently no evidence that LH2 complexes from sulfur bacteria have other organization, as they always consist of α and β low molecular weight polypeptides and each αβ pair binds non-covalently three BChl molecules and a Car molecule.

Using mutagenesis (including transposon method) it is possible to interrupt the Cars' biosynthesis at any step and obtain the mutants without any colored Cars (so-called Car-less mutants), which accumulate the same amount of the precursors, or the mutants with over expression of the intermediate carotenoids [19,20]. For example, in the strain G1C of *Rhodobacter sphaeroides* Cars composition of the LH2 complex consists of Neurosporene (98%) and Lycopene (2%) [21]. It has been well documented that wild-type LH2 complexes are generally absent in the Car-less mutants of some non-sulfur photosynthetic bacteria [19,20,22–25]. If the B850 complex nevertheless survives in the Car-less mutants, it lacks generally the BChl-B800 molecule, and its lower energy electronic transition is shifted towards the red one [26]. The primary structure of its apoproteins is identical to those of the wild-type B800–850 complex except for positions of two amino acids. A correlation has been found between the product of Phytoene desaturase (CrtI) and assembly of the LH2 complex [19]. These observations led to the conclusion that Car molecules are important not only for light-harvesting and photoprotection, but also as regulatory and structural elements of the LH2 complex as well [19,20,25]. It was concluded that for the assembly of the LH2 complexes requires the presence of Car molecule, whereas the RC–LH1 complex can be successfully assembled in the absence of colored Cars.

Another method to reduce highly the content and the level of the Cars is inhibition of Car biosynthesis [27–29]. This method was generally applied to study Car biosynthesis in some cells of purple bacteria with DPA as the widely used inhibitor [30,31]. DPA inhibits the activity of the enzyme Phytoene desaturase according to the common point of view [30], replacing Car molecule by Phytoene and related precursors such as Phytofluene and ζ-Carotene. There are one none-sulfur species with the LH1 complex (*Rhodospirillum rubrum*) and two sulfur species (*Allochromatium vinosum*/strain D/and *Allochromatium minutissimum*, [old name *Chromatium*]) in which cells Car biosynthesis was inhibited by ∼95% [29,32–36]. It was well documented that in the cells of these bacteria grown in the presence of DPA the LH2 and LH1 complexes were assembled without any changes of their spectral and biochemical characteristics [29,37]. Nevertheless, Cars presented in every sample being investigated were easily determined using spectrophotometry.

In this work we have studied the influence of an inhibitor of Car biosynthesis on the antenna assembly in purple sulfur bacterium *Alc minutissimum*. We have found the grown conditions when Car biosynthesis can be reduced almost completely (>99%) by DPA. We have shown that the LH2 complex is still presented in intracytoplasmic membrane of *Alc minutissimum* in the absence of the colored Cars, and its spectroscopic and structural properties do not seem to depend on the presence of these molecules. This strongly suggests that different mechanisms underlie the structural stability of the LH2 protein of *Alc minutissimum* (and possibly of all purple sulfur bacteria) as compared to the equivalent antennae from purple non-sulfur bacteria.

## 2. Materials and methods

### 2.1. Cell growth and isolation of antenna complexes

The control cells of *Alc minutissimum* were grown phototrophically at 25 °C. Average illumination intensity was about 10 W $m^{-2}$ as previously described [34,36]. DPA (71 μM or 12 mg/L) was added to the growth medium for carotenoid biosynthesis inhibition. The temperature in growth chamber was reduced to 20 °C. Very active cell culture was used. Chromatophores were obtained from the whole cells after sonication as previously described [38].

LH2 complexes were isolated by preparative electrophoresis [39]. Parameters of the electrophoresis gel ($T$ = 6.2%; $C$ = l.96%; 11 mM Tris–Glycine buffer, pH 9.2) and the electrode buffer (11 mM Tris–Glycine buffer, pH 9.2 with 0.05% lithium dodecyl sulfate) were identical to those described previously with a slight modification [39]. The protein bands were extracted by passive diffusion into a detergent-containing buffer (0.05% DM, 10 mM Tris–HCl, pH 8.0).

### 2.2. Carotenoid compositions

Quantification of pigment content was performed as previously described [36,38,40]. Total pigment extracts from the samples were prepared using acetone/methanol mixture (7:2, v/v). The mixture of pigments was dried up by evaporation. The pigment extracts were analyzed by HPLC, using Spherisorb ODS2 C18 column (4.6 × 250 mm, Waters, USA). The HPLC equipment consisted of a LC 10ADvp pump with a FCV 10Alvp valve and a photodiode-array detector SPD-M20A (Shimadzu, Japan) working under LC-solution software. Following injection of the samples for the first 3 min, solution A (A = 73% acetonitrile/water (9:1), 27% ethyl acetate) was applied. Then applied a linear gradient of 0–100% of the solution B (B = ethyl acetate) for 35 min with the flow rate of 1.0 mL/min, and, finally, solution B for 5 min [36]. Full-time analysis of each extract was ∼45 min. The Cars were identified by comparison with authentic samples from various strains of bacteria according to their absorption spectra and retention time. The amounts of pigments were calculated using the same molar absorption coefficient at the wavelength of the peak for each color Car in the eluent (acetonitrile/water/ethyl acetate mixture) equal to 150 $mM^{-1}$ $cm^{-1}$. Corresponding coefficient for the Phytofluene equal to 75 $mM^{-1}$ $cm^{-1}$ and for the Phytoene equal to 50 $mM^{-1}$ $cm^{-1}$ was assumed. This assumption was made according to [31], since the molar extinction coefficient for many of Cars and their precursors have not been determined for the used mixture (acetonitrile/water/ethyl acetate). The same molar absorption coefficients were used for calculation of the ratio Phytoene/Car from the published data.

### 2.3. Spectroscopy

Room-temperature electronic absorption spectra were recorded using Gary 50 spectrophotometer (Varian, Australia) and CD spectra – using Mark Y CD spectrophotometer (Jobin-Ivon, France).