Cloning a Gene Encoding a Light-Harvesting I Polypeptide from *Ectothiorhodospira* sp.

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Trying to detect the genes coding for light harvesting II polypeptides of the purple bacteria *Ectothiorhodospira* sp. a gene corresponding to a light harvesting I polypeptide was cloned. This paper discusses the probable reasons of this result. The sequence of this polypeptide underlines the strong similarity with light harvesting complexes from bacteria such as *Rhodospirillum molischianum* and *Chromatium vinosum.*

Introduction

The primary processes of photosynthesis involve light absorption, excitation energy transfer and primary charge separation across the photosynthetic membrane. In purple photosynthetic bacteria, light energy is gathered by an extensive system of light harvesting (LH) complexes ensuring the efficient funnelling of excitation energy towards the photochemical reaction centers where the transduction into chemical potential energy takes place (Sunström and van Grondelle, 1996). The LHI or B880 is present in all purple bacteria and is intimatly associated with the reaction center (Sundström and van Grondelle, 1996; Cogdell, 1986; Picorel and Gingras, 1988). The LHII or B800-850 (Feick and Drews, 1978; Clayton and Clayton, 1981; Doi *et al*., 1991; Walker *et al.*, 1991) and LHIII or B800–820 (Hayashi and Morita, 1980; Cogdell *et al*., 1983) are arranged more peripherally (Monger and Parson, 1977).

All of these antenna complexes are supramacromolecular assemblages constituted of polypeptides (α/β) and pigments; bacteriochlorophyll (BChl) and carotene. They are spectrally characterised by one or two strong near infrared (NIR) electronic absorption bands arising from the *Qy* transition of the BChl *a*. Structural analysis of crystallised peripheral antenna complexes of purple bacteria has revealed a highly symmetric arrangement of the pigments (for review see Leupold and al., 2000). The active assembly consists of two concentric cylinders of 9α/9β helical protein subunits in *Rhodopseudomonas* (*Rps.*) *acidophila* (McDermott *et al.*, 1995) or 8α/8β in *Rhodospirillum* (*Rs.*) *molischianum* (Koepke *et al.*, 1996) that hold the pigment molecules.

An advantageous method to determine the sequence of these polypeptides consists in extracting them after running a purified complex from a SDS page gel. On the basis of the N-terminal sequences determined from the different extracts some primers are designed. Then, using these primers in a PCR process could permit to clone all of the genes coding for a type of polypeptide.

In this work, this method was used in order to fish the genes corresponding to the *Ecthothiorhodospira sp.* LHII family. Unexpectedly a LHI type gene was cloned. Considering the cause of this singular result as potentially instructive the methodology was discussed. The comparison of the sequence of this gene with LH genes from other bacteria indicated a strong homology with *Rs. molischianum* and *Chromatium* (*Cm.*) *vinosum*.

Materials and Methods

Antenna preparation

The photosynthetic bacterium *Ectothiorhodospira* sp., on deposit with the ATCC as No. 31751,

Abbreviations: BChl, bacteriochlorophyll; NIR, near infrared; LH, light harvesting; B800, 800-nm absorption band; B850, 850-nm absorption band; LDAO, lauryl dimethylamine N-oxide.

was grown photosynthetically as described by Lefebvre *et al.*(1984). The LHII antenna complex was isolated as in Ortiz de Zarate and Picorel (1994) with modifications (Buche and Picorel, 1998).

Gel electrophoresis

SDS-PAGE was performed with a Mini Protean apparatus (Bio-Rad). The resolving gel contained 15% acrylamide. Several concentrations in SDS (from 0 to 10%) and in urea (from 0 to 8 M) were tested in the sample buffer.

Peptide sequences

The low molecular weight proteins were extracted from the gel by diffusion into an aqueous solution. The analysis of the N-terminal sequence has been carried out automatically by the Edman degradation in a Beckman LF3000 equipped with an analyser of PTH-amino-acids (Gold System, Beckman, Fullerton, USA).

Primer design

The first primer has been designed on the basis of the N-terminal sequence of a β-polypeptide of *Ectothiorhodospira* sp.: MNDNSISGLTEEQAK. Only the LTEEQAK part of this sequence has been chosen in order to avoid the primer degenerescence as much as possible. Combined with the Kpn sequence we obtain the following primer: CCACGGTACC(C/T)TNACNGA(A/G)GA(A/G) CA(A/G)GCNAA. A second primer was designed from the following N-terminal sequence: SEYRP KRESNPSDDWK. The NPSDDWK part was transcribed (anti-N) in combination with the BamH1 sequence giving CCACGGATCC(T/C) TTCCA(A/G)TT (A/G)TTNGANGG

Cloning and sequencing of the light-harvesting gene

The genomic DNA was extracted using a kit from *Quiagen.* The PCR reactions were carried out such as previously described (Hunter *et al.*, 1992) by using an annealing temperature of 42 °C. The PCR products were cloned using the Puc19 vector. Their sequences were established using the simple chain processing. This modality consists in sequencing a DNA fragment by means of a single reaction. The M13 universal primers have been used for this reaction. The automatic system of sequencing was the PE ABI Prism (SYSTEM GENOMIC, Mannheim, Germany).

Results and Discussion

The starting point of our work consisted in finding the primers permitting to detect the genes corresponding to the LHII polypeptides (of the LHII complex) of *Ectothiorhodospira* sp. We tried to extract these polypeptides from a SDS-PAGE gel in order to establish the sequence of the first N-terminal amino acids. However, the problem was that the polypeptides of this complex are poorly soluble even in the presence of very strong chaotropic agents. Various compositions of chaotropes were tested without any success (not shown). The monomeric fraction remained much lower than 1% of the proteins corresponding to the treated LHII. Percentages as small as 0.1% were frequently estimated. In fact most of the proteins were eliminated during the centrifugation preceeding the electrophoresis or were found in the gel at higher apparent molecular weight indicating strong aggregation states of the LHII polypeptides. This observation agrees perfectly with the fact that the spectral properties of the complex stay stable in the presence of more than 8 M urea (Buche *et al.*, 2000). Very high concentration of NaOH are necessary to denature the complex (Buche and Picorel, 2001) whereas a reorganisation of the antenna by pH 2 is effective under strong chaotropic condition (Buche *et al.*, 2000; Buche, 2000). The consequence of this finding is that a low quantity of soluble proteins such as LHI (as lower as $\leq 0.1\%$) in the LHII preparation can significantly contaminate the monomeric fractions extracted from the polyacrylamide gel. We determined the N-terminal sequence of 4 polypeptides. Two of these proteins (SEYRASKPSNPRDDWKI and SEYRASKPSNPRDDWKI) have the characteristic of both *Ectothiorhodospira* (*E.*) *halophila* α1LHI and α2LHI with a short sequence including the strictly conserved Trp; WK and WKI respectively. More generally their sequences are also similar to that of *Rps. viridis* αLHI. Another polypeptide sequence (MNDNSISGLTEEQAK) shows a strong similarity with βLHII polypeptides of *Cm. vinosum* and *Rs. molischianum.* However, the SISGLTEEQAK part is also strongly similar

to the corresponding LHI sequence from a large variety of bacteria. The last sequence; (MNDNI) could be the beginning of a βprotein.

From these sequences we designed several primers and used them in PCR by testing various combinations of the genomic DNA. The PCR products were cloned and their sequences established. Only two primers (Fig. 1b,c) such as described in Materials and Methods, allowed us to obtain a DNA fragment related to an LH polypeptide (Fig. 2).

This fragment has 245 bp including a βLH sequence of 117 bp. Figure 2 compares the amino

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GGTACCTTGACAGAGGAGCAAGCGAAGGAATTCCCCGAGCAGTTCAAGACCACCTTCAC
  Kpn  L  T  E  E  Q  A  K  E  F  H. E  Q  F  K  T  T  F  T
CGTGTTCATGGCGCTTGCCGTTGCTGCCCACTTCCTCGTCTACGTGTGGAGACCGTTCT
  V  F  M  A  L  A  V  A  A  H  F  L  V  Y  V  W  R  P  F
ACTAAGAACGGTAACACCGCACGGTGATTCCGTGTGGCTTTGATTGAGTTTCAGTTGTA
Y ter
AAAAAAACTTGACAGGGTGATTTCAATGAGCGAATACAGAGCAAAGNGGCCGAGCAACC

CCCTNCGTTTGGAAGGGATCC
               Bam
```

Fig. 1. PCR product including a βLH sequence.

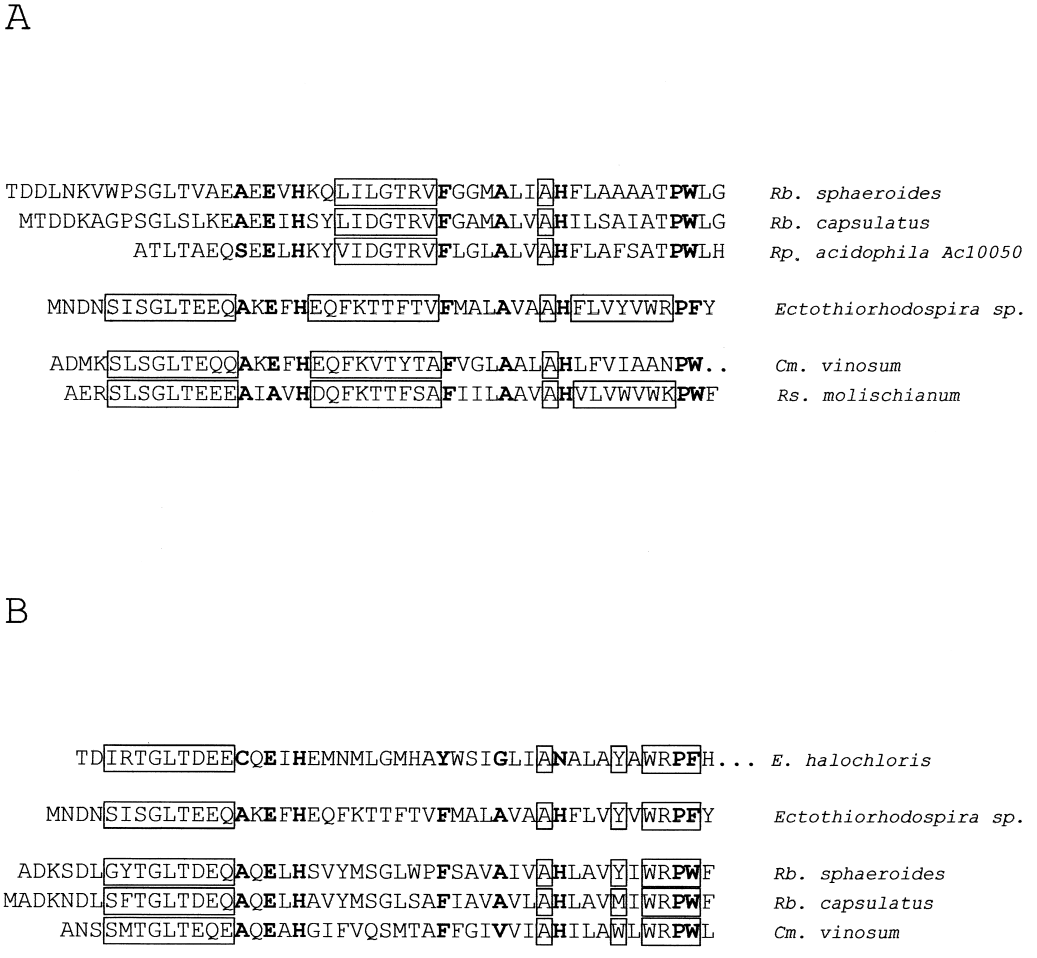

Fig. 2. Sequence comparison between the *Ectothiorhodospira* sp. βLH and a)the βLHII polypeptides,(b)the βLHI polypeptide of several purple bacteria.

acid pattern corresponding to this sequence with the βLHII amino acids sequence of two distinct groups of bacteria classified from the spectral or structural properties of their LHII complexes. A first set (group I) includes bacteria such as *Rhodobacter* (*Rb.*) *sphaeroides, Rb. capsulatus* and *Rps. acidophila.* The NIR spectral properties of the LHII antenna from this group include a broad exciton CD band corresponding to B800 and a very stable B850. For example under some acidic/chaotropic conditions B800 of *Rb. capsulatus* shifts specifically whereas B850 remains intact (Buche *et al*., 2001). The LHII of *Rps. acidophila* has a concentric arrangement of two hollow cylinders built from the transmembrane helices of nine α- and nine β-apoproteins (McDermott *et al*., 1995). Independently of the strictly conserved amino acids (in black in the figure) this group has some highly preserved sequences: The KI downstream from **W**, two sequence between **W** and **A** and an other one upstream of **H** which contains a W in position +9 from **H** and two other potential hydrogen donor, Y/(Y or W) in position +13, +14 from **H**. In the case of *Rb. sphaeroides* these amino acids have been shown to be H-linked to 850BChls (Fowler *et al*., 1992). This can explain the large stability of B850 in this group. It is of interest to observe that for *Cm. vinosum* and *Rs. molischianum*, which represent another group of bacteria (group II), this standard of structure does not exist. Although we could observe the presence of a Trp located in +11 of **H** in this group the structure close to **W** has not radically changed but two potential H donors located at −3 and +8 from **W.** could contribute to the stabilisation of B800. In fact B800 is more stable than B850 and both B800 and B850 shows a strong exciton CD signal. This group has an (αβ)8 configuration (Koepke *et al*., 1996). The LH sequence of *Ectothiorhodospira* sp. is very close to the corresponding sequences of this group in particular that of *Cm. vinosum* (Fig. 2). The sequences of group I contains a highly conserved K/S and LIDGTRV between **H** and **F** as well as a highly conserved A at −1 of **H**. Group II conserves this A (at −1 from **H)** but the LIDGTRV is substituted by other sequences. Several parts (especially the N terminal and central areas) of the *Ectothiorhodospira* sp. βLH sequence show strong similarities with the corresponding sequences of *Rs. molischianum* and *Cm. vinosum* polypeptides (Fig. 2). It is interesting to observe that the *Ectothiorhodospira* sp. LHII presents a (αβ)8 configuration (Oling *et al*., 1996) and spectral properties very close to that of *Cm. vinosum* and *Rs. molischianum* LHII (Ortiz de Zaratte and Picorel, 1998; Buche and Picorel, 1998, 2001; Buche, 2000).

In spite of the high homology between the group II LHII polypeptides and the βLH of *Ectothiorhodospira* sp. Figure 2 shows that its sequence corresponds to a LH**I** polypeptide. Its C-terminal sequence shows a typical LHI primary structure while the presence of **F** in the end position instead of **W** brings closer this sequence to that of LHI of *Ectothiorhodospira halochloris.*

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