Volume 213, number 2, 245–248 FEB 04513 March 1987

Cloning, nucleotide sequence and transfer of genes for the B800-850 light harvesting complex of *Rhodobacter sphaeroides*

Mark K. Ashby, Shirley A. Coomber and C. Neil Hunter

Department of Pure and Applied Biology, Imperial College, Prince Consort Road, London SW7 2BB, England

Received 16 December 1986

The genes that encode the α and β polypeptides of the major light-harvesting complex of *Rhodobacter sphaeroides*, B800-850, have been cloned and sequenced through the use of oligonucleotides based upon the known polypeptide sequences. These genes, *puc*A and B, are transcribed in the order B, A, are of 150 and 164 nucleotides respectively, and are separated by a spacer region of 14 nucleotides. Transfer of these genes to mutant M21 lacking the B800-850 complex has been accomplished and absorbance spectra of recombinant strains M2181 and M2184 show that expression of *puc*A and B is comparable to levels found in the wild type.

Photosynthesis; Light harvesting; Gene cloning; Gene transfer; Photosynthetic bacteria

1. INTRODUCTION

The light-harvesting antenna of the photosynthetic bacterium *Rhodobacter sphaeroides* consists of the major complex, B800-850, arranged around a cluster formed by B875 antenna and approximately four reaction centres [1]. Within the photosynthetic membrane, approx. 1000 bacteriochlorophyll molecules are connected to form a domain for efficient energy transfer [2,3] and excitation energy is eventually trapped by a reaction centre where the primary photochemical event occurs.

The bacteriochlorophyll and carotenoid pigment of B800-850 and B875 are non-covalently bound to small polypeptides designated α and β. These simple antennae have been extensively characterised [4–6] and they are therefore attractive model systems for further studies involving manipulation of antenna structure through mutagenesis of *puc*A and B genes.

Here, the genes encoding the B800-850 α and β polypeptides, *puc*A and B, have been cloned and sequenced. These genes have been transferred to mutant M21 which lacks the B800-850 antenna, resulting in the restoration of a wild-type light-harvesting system.

Correspondence address: M.K. Ashby, Dept of Pure and Applied Biology, Imperial College, Prince Consort Road, London SW7 2BB, England

A preliminary report of this work was presented at the VII International Congress on Photosynthesis, Rhode Island, USA, August, 1986. Similar results were presented by P.J. Kiley, T.J. Donohne and S. Kaplan

2. MATERIALS AND METHODS

2.1. *Oligonucleotide probe*

A mixed 23-base oligonucleotide deduced from the published amino-terminal sequence of the α-subunit of the B800-850 complex [7] was made on an Applied Biosystems 380B synthesiser using methyl phosphoamidite. A 17-base mixed oligonucleotide was also made, but corresponding

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

to the opposite DNA strand. The oligonucleotides were radiolabelled using polynucleotide kinase; probing was as described by Woods [8].

2.2. *Molecular cloning*

A genomic library of *R. sphaeroides* DNA (average size 5 kb) was prepared in shuttle vector pNH2 (C.N.H., unpublished) in *E. coli* DH5. Plasmid DNA was prepared from positively hybridising clones using a Triton lysis method [10]. Following restriction analysis and Southern transfer [11] the nitrocellulose filters were probed with labelled oligonucleotides. DNA cloned was cloned into M13 mp18 and sequenced [12] using the universal 17-base primer and the mixed 17-mer (above).

2.3. *Mutagenesis*

Mutant M21, which lacks the B800-850 antenna complex, was isolated as a result of chemical mutagenesis of wild-type strain NC1B 8253 by *N*-nitrosoguanidine.

2.4. *Plasmid transfer*

Plasmids pMA81 and pMA84 were transferred from *E. coli* to mutant M21 using a mobilisation system based upon plasmid RP4 (C.N.H. and Olsen, J.D., in preparation). Amongst the pale background, some darker and larger colonies were obtained after 10 days of anaerobic photosynthetic growth and absorbance spectra of cultures derived from these colonies were used to investigate whether mutant M21 had acquired the B800-850 complex.

3. RESULTS AND DISCUSSION

3.1. *Cloning and nucleotide sequencing of* puc*A and B genes*

The sequence of the mixed oligonucleotide probe is shown in fig.1; it has a degeneracy of 2028. 6000 colonies of the gene library were screened and four positively hybridising clones were found which were used to prepare plasmid DNA (pMA81-4). pMA82, 83 and 84 had identical restriction patterns with *Eco*RI, *Hin*dIII and *Pst*I and had inserts of approx. 7 kb, whereas pMA81 had an insert of 3.75 kb (fig.2). Southern hybridisation revealed that a 1.1 kb *Bam*HI fragment of pMA81 must contain at least part of the

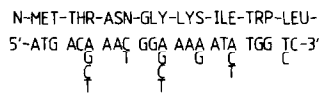

Fig.1. 23-base oligonucleotide deduced from the N-terminal sequence of the B800-850 α polypeptide.

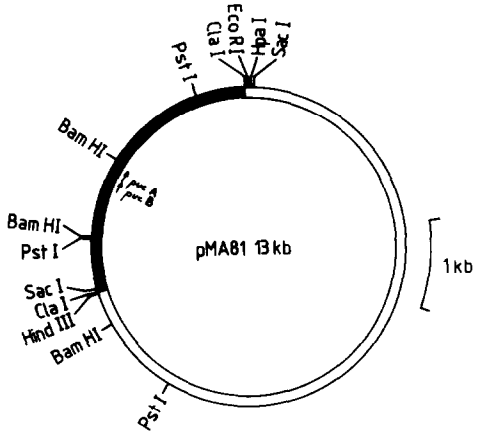

Fig.2. pMA81 showing 1.1 kb *Bam*HI fragment containing *puc*A and B genes.

*puc*A gene. After recloning into M13 mp18 and sequencing it was apparent that in one of the possible orientations, all of the *puc*A gene had been identified by comparison of the deduced polypeptide sequence with the amino acid sequence determined by Theiler et al. [7]. Through the use of a second oligonucleotide primer complementary to part of the *puc*B gene, the sequencing of both genes was completed and is shown in fig.3. Although the sequence was translated into six possible reading frames, one is presented which is in complete agreement with that published by Theiler et al. [7], with the exception of an exchange of isoleucine for leucine at position 37 in the β-chain in our sequence. This difference is not expected to have any effects on the conformation or function of the polypeptide (Theiler, R., personal communication), and we attribute it to a difference between our wild-type strain (NCIB 8253) and that used by Theiler (2.4.1). As has already been found for *R. capsulatus* [13], the genes are transcribed in the order *puc*B, A and each gene is preceded by sequences which are possible sites for ribosome binding [14]. These are indicated in fig.3. There is a space region of 14 nucleotides between the genes.

```
                                β ——► MET THR ASP ASP LEU ASN
GCCCTAGCGCACACCGTCGATTTACCATTGGAGACGCACATG ACT GAC GAT CTC AAC
CGGGATCGCGTGTGGCAGCTAAATGGTAACCTCTGCGTGTAC TGA CTG CTA GAG TTG

LYS VAL TRP PRO SER GLY LEU THR VAL ALA GLU ALA GLU GLU VAL HIS

AAA GTC TGG CCG AGC GGC CTC ACC GTT GCC GAA GCC GAA GAA GTT CAT
TTT CAG ACC GGC TCG CCG GAG TGG CAA CGG CTT CGG CTT CTT CAA GTA

LYS GLN LEU ILE LEU GLY THR ARG VAL PHE GLY GLY MET ALA LEU LEU

AAG CAA CTC ATC CTC GGC ACC CGC GTC TTC GGT GGC ATG GCT CTG CTC
TTC GTT GAG TAG GAG CCG TGG GCG CAG AAG CCA CCG TAC CGA GAC GAG

ALA HIS PHE LEU ALA ALA ALA ALA THR PRO TRP LEU GLY ***

GCG CAC TTC CTC GCC GCC GCT GCG ACC CCC TGG CTC GGC TGA TATGA
CGC GTG AAG GAG CGG CGG CGA CGC TGG GGG ACC GAG CCG ACT ATACT
                                                    ⌊

α ——► MET THR ASN GLY LYS ILE TRP LEU VAL VAL LYS PRO THR

GAGACTGACATG ACC AAC GGC AAA ATC TGG CTC GTG GTG AAA CCG ACC
CTCTGACTGTAC TGG TTG CCG TTT TAG ACC GAG CAC CAC TTT GGC TGG
⌊

VAL GLY VAL PRO LEU PHE LEU SER ALA ALA VAL ILE ALA SER VAL VAL

GTC GGC GTT CCG CTG TTC CTC AGC GCT GCC GTC ATC GCC TCC GTC GTT
CAG CCG CAA GGC GAC AAG GAG TCG CGA CGG CAG TAG CGG AGG CAG CAA

ILE HIS ALA ALA VAL LEU THR THR THR THR TRP LEU PRO ALA TYR TYR

ATC CAC GCT GCT GTG CTG ACG ACC ACC ACC TGG CTG CCC GCC TAC TAC
TAG GTG CGA CGA CAC GAC TGC TGG TGG TGG ACC GAC GGG CGG ATG ATG

GLN GLY SER ALA ALA VAL ALA ALA GLU ***

CAA GGC TCG GCT GCG GTC GCG GCC GAG TAA TGCTGCGCAAGCGCGGGCCTG
GTT CCG AGC CGA CGC CAG CGC CGG CTC ATT ACGACGCGTTCGCGCCCGGAC


CGGGCCCACGCCAGCCAGTCCGTGAGTCCGAGCAGGCCGGGA
GCCCGGGTGCGGTCGGTCAGGCACTCAGGCTCGTCCGGCCCT
```

Fig.3. Nucleotide and deduced amino acid sequence of the genes encoding B800-850 α and β polypeptides.

3.2. *Transfer of* puc*A and B genes to mutant M21*

In order to test whether these genes could restore B800-850 to a mutant lacking this light-harvesting complex, mutant M21 was isolated. This mutant has been characterised by spectroscopy at 4 K [15] and the amount of B800-850 complex present has been determined by circular dichroism to be less than 1% (Van Dorssen, R., Van Grondelle, R. and C.N.H., unpublished). This figure is in agreement with results from hybridisation analyses of mRNA from M21 probed with the radiolabelled 1.1 kb *Bam*HI fragment purified from pMA81, where the level of transcription of *puc*A and B genes was at the limit of detection. Fig.4 shows three absorbance spectra, including a control in which the cloning vector alone (no insert) was transferred to the wild-type. This spectrum exhibits characteristic peaks at 800 and 850 nm (attributable to B800-850 antenna) and a shoulder at 875 nm (B875 antenna). M21, which lacks B800-850, shows only a peak at 875 nm and a small maximum at 800 nm which is due to some of the reaction centre

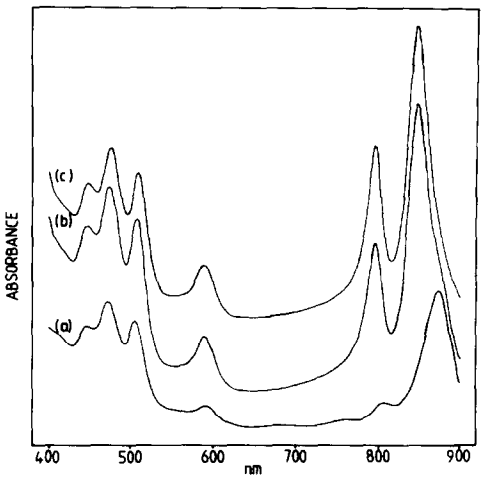

Fig.4. Absorbance spectra of (a) mutant M21 and (b) transconjugant strain M2181, with (c) wild type containing cloning vector pNH2 included as a control.

bacteriochlorophylls and not residual B800; specific mutation of reaction centre genes in M21 results in the disappearance of this absorbance peak (Hunter, C.N., unpublished).

The strain M2181 obtained from transfer of pMA81 into M21 has an absorbance spectrum closely resembling that of the wild type showing that the transfer and expression of *puc*A and B genes have been achieved. Preliminary measurements of fluorescence emission at 4 K indicate that the efficiency of energy transfer from B850 to B875 is comparable to that in the wild-type (Van Dorssen, R., Van Grondelle, R. and C.N.H., unpublished). We have noted that the transfer of pMA84 into M21 (not shown) also results in expression of *puc*A and B genes although at reduced levels, an observation that we ascribe to some difficulty in maintenance of pMA84 which has a relatively large insert.

ACKNOWLEDGEMENTS

We wish to thank the Science and Engineering Research Council (SERC) and the Nuffield Foundation for financial support. M.K.A. is a recipient of an SERC postgraduate studentship. The authors acknowledge the technical assistance of Dr S.J. Zara and Mr J. Olsen.

REFERENCES

[1] Vos, M., Van Grondelle, R., Van der Kooi, F.W., Van de Poll, D., Amesz, J. and Duysens, L.N.M. (1986) Biochim. Biophys. Acta 850, 501–512.

[2] Van Grondelle, R., Hunter, C.N., Bakker, J.G.C. and Kramer, H.J.M. (1983) Biochim. Biophys. Acta 723, 30–36.

[3] Hunter, C.N., Kramer, H.J.M. and Van Grondelle, R. (1985) Biochim. Biophys. Acta 807, 44–51.

[4] Drews, G. (1985) Microbiol. Rev. 49, 59–70.

[5] Zuber, H. (1985) Photochem. Photobiol. 42, 821–844.

[6] Kramer, H.J.M., Van Grondelle, R., Hunter, C.N., Westerhuis, W.H.J. and Amesz, J. (1984) Biochim. Biophys. Acta 765, 156–165.

[7] Theiler, R., Suter, F., Zuber, H. and Cogdell, R.J. (1984) FEBS Lett. 175, 231–237.

[8] Woods, D. (1984) Bethesda Res. Labs. Focus 6, no.3, 1–3.

[9] Hanahan, D. (1985) in: DNA Cloning Vol.1 – A Practical Approach (Glover, D. ed.) pp.109–135, IRL, Oxford.

[10] Grinsted, J. and Bennett, P.M. (1984) Methods Microbiol. 17, 123–131.

[11] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning – A Laboratory Manual, Cold Spring Harbour, NY.

[12] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.

[13] Youvan, D.C. and Ismail, S. (1985) Proc. Natl. Acad. Sci. USA 82, 58–62.

[14] Gibson, J., Stackebrandt, E., Zablen, L., Gupta, R. and Woese, C.R. (1979) Curr. Microbiol. 3, 59–64.

[15] Vos, M., Van Dorssen, R.J., Van Grondelle, R., Hunter, C.N., Amesz, J. and Duysens, L.N.M. (1986) Proc. VII Int. Congr. Photosynth., in press.