GENETIC TRANSFER OF THE CAPACITY TO FORM BACTERIOCHLOROPHYLL-PROTEIN COMPLEXES IN *RHODOPSEUDOMONAS CAPSULATA*

Gerhart DREWS, Roland DIERSTEIN and Arne SCHUMACHER
Institut für Biologie II der Universität Freiburg, D-7800 Freiburg, West Germany

Received 15 June 1976

1. Introduction

Reaction center (RC) and light harvesting (LH) complexes are major constituents of the bacterial photosynthetic apparatus [1,2]. RC of *Rhodopseudomonas* (R.) *capsulata* and of *R. sphaeroides* contain three proteins (molecular weights 28, 24 and 21 kD) and RC bacteriochlorophyll (bchl) [3,4]. The LH-bchl of these species was proposed to be associated with one or more proteins of about 10 kD [5–10]. It has been shown that the amounts of both RC bchl and LH-bchl correlate with their respective proteins, under various physiological conditions. The nature of co-regulation of the biosynthesis of the two non-covalently bound constituents of membrane subparticles is at present not understood. Recently a bacteriophage-like particle (GTA) with linear and double-stranded DNA (molecular weight $3.6\ 10^6$) was discovered ([13] and B. Marrs, personal communication), which efficiently transfers genes between various strains of *R. capsulata* [14,15]. In this study the transfer of genes for bchl, proteins, and carotenoids, resulting in reconstitution of a functional photosynthetic apparatus, will be described.

2. Materials and methods

The following strains of *R. capsulata* were used: Z1, W1 and J1 (Department of Microbiology, Indiana University, Bloomington); 37b4, A1a$^+$, A1a$^-$ and YS (Lehrstuhl für Mikrobiologie, Universität Freiburg, West Germany); Y142, Y9 and Y5 (Department of Biochemistry, St. Louis University, St. Louis, Missouri). They are described elsewhere [16–19]. When not otherwise stated, the photosynthetically active strains (Pho$^+$) were grown anaerobically in the light, the photosynthetically inactive ones (Pho$^-$) were cultivated semiaerobically in the dark. The genes were transferred by incubation of a cell-free donor strain filtrate (1 h, 35°C) with a suspension of mutant strains deficient in formation of a functional photosynthetic apparatus or carotenoid synthesis. We followed the methods described in refs. 15 and 20. However, the medium YPS was replaced by the synthetic medium RCVB [17]. The isolation of intracytoplasmic membranes has been described [10]. The membrane proteins were solubilized by sodium dodecylsulfate (SDS) and separated by polyacrylamide gel electrophoresis (PAGE) using a 10 to 15% polyacrylamide step gradient gel [21]. The photooxidative killing of strains missing highly unsaturated carotenoids [22] was performed by simultaneous irradiation with white light (about 1000 fc from incandescent bulbs) with active aeration of anaerobically light precultivated suspension of bacteria (P. Weaver, personal communication). Symbols: Bch$^+$, Crt$^+$ = bacteriochlorophyll and carotenoid positive phenotypes, *bch* = genotype. Transferants were symbolized by donor and recipient; the arrow indicated the direction of gene transfer: e.g. A1a$^+$→W1.

3. Results

In the first group of experiments genes of Pho$^+$ donor strains were transferred to the Pho$^-$ mutants YS, W1 and A1a$^-$. By this process strain W1, missing bchl and colored carotenoids (Bch$^-$ Crt$^-$ Pho$^-$),

North-Holland Publishing Company – Amsterdam

became a Bch$^+$ Pho$^+$ Crt$^-$ strain whether the donor was a Crt$^+$ or Crt$^-$ strain (table 1). The in vivo absorption spectra of intracytoplasmic membranes of investigated transferants gave no indication of the presence of carotenoids. The position and relative heights of the infrared absorption maxima at 800 nm and 850–880 nm were comparable with spectra of other carotenoidless strains of *R. capsulata* [16,19] and *R. sphaeroides* [22].

The strain W1 was not only Bch$^-$ and Crt$^-$ but was found to be defective in formation of RC (28 and 20.5 kD) and LH-proteins. The Pho$^+$ transferants were recovered in both bchl and protein synthesis. The membrane protein patterns in the PAGE of all studied W1 Pho$^+$ transferants as well as A1a$^+$ [18,19] showed all three RC-bands, but only the second fastest migrating LH-protein band. Representative patterns of membrane proteins of A1a$^+$ and of one strain of W1 (37b4→W1) are given in fig.1.

The restoration of photosynthetic capacity in the Pho$^-$ Bch$^-$ Crt$^-$ mutant A1a$^-$ [18] was very inefficient (table 1). This low efficiency may be the result of the presence of multiple mutations, since the A1a$^-$ strain differs from the wild type strain 37b4 in many phenotypic characteristics [18]. Besides defects in bchl synthesis, the protein patterns of A1a$^-$ showed modifications in the photosynthetic apparatus (fig.1). Consequently, membrane preparations of semiaerobically grown cells of A1a$^-$ and W1$^-$ do not show the characteristic precipitin line with anti-RC in the Ouchterlony test (data not shown).

The Bch$^-$ strain YS can synthesize carotenoids. However, the synthesis is not regulated as in wild type strains (unpublished results). Under low pO_2 only small amounts of carotenoids were formed. YS is a very stable mutant. We never have observed spontaneous revertants in controls of GTA experiments, indicating the possibility of a small deletion or cluster of mutations. The rate of conversion to Pho$^+$ phenotypes by GTA is relatively high (table 1). The in vivo spectra of transferants showed wild type character with low A_{880}/A_{860} nm ratio (table 2) and identical peaks in the carotenoid region (data not shown). The carotenoid synthesis is again coupled to bchl synthesis.

Table 1
Conversion of Pho$^-$ or Crt$^-$ *R. capsulata* mutants to Pho$^+$ or Crt$^+$ phenotypes by gene transfer agent (GTA)

Donor (phenotype in brackets)	Recipient	Pho$^+$ colonies per plate phenotype of Pho$^+$		
		Control	With GTA	Transferants
37b4 (wild type)	W1 (Bch$^-$ Crt$^-$)	1	11	Bch$^+$ Crt$^-$
J1 (Pho$^+$ Crt$^+$ Rif^R)	W1	1	70	Bch$^+$ Crt$^-$
A1a$^+$ (Bch$^+$ Crt$^-$)	W1	0	8	Bch$^+$ Crt$^-$
37b4	YS (Bch$^-$ Crt$^-$)	0	155	Bch$^+$ Crt$^-$
A1a$^+$	YS	0	85	Bch$^+$ Crt$^+$
W1$^+$ (Bch$^+$ Crt$^-$)	YS	0	10	Bch$^+$ Crt$^+$
Y142 (Bch$^+$ Crt$^+$ Pho$^-$)	YS	0	23	Bch$^+$ Crt$^+$
Z1 (Pho$^+$ Crt$^+$ As^R)	YS	0	100	Bch$^+$ Crt$^+$
A1a$^-$ (Bch$^-$ Crt$^-$)	YS	0	0	
37b4	A1a$^-$	0	2	Bch$^+$ Crt$^-$
Z1	A1a$^-$	0	6	Bch$^+$ Crt$^-$
J1	A1a$^-$	0	2	Bch$^+$ Crt$^-$
		Crt$^+$ colonies per plate		
		control	with GTA	
YS	W1$^+$	0	1	Bch$^+$ Crt$^+$
37b4	A1a$^+$	0	5	Bch$^+$ Crt$^+$
37b4	W1$^+$	0	5	Bch$^+$ Crt$^+$
Z1	A1a$^+$	0	27	Bch$^+$ Crt$^+$

Wild type strains have the capacity to synthesize bchl (Bch$^+$) carotenoids (Crt$^+$) and to grow photosynthetically (Pho$^+$). The values are means of 3 to 10 experiments. Per plate about $5 \cdot 10^7$ colony forming units of recipient cells were plated.

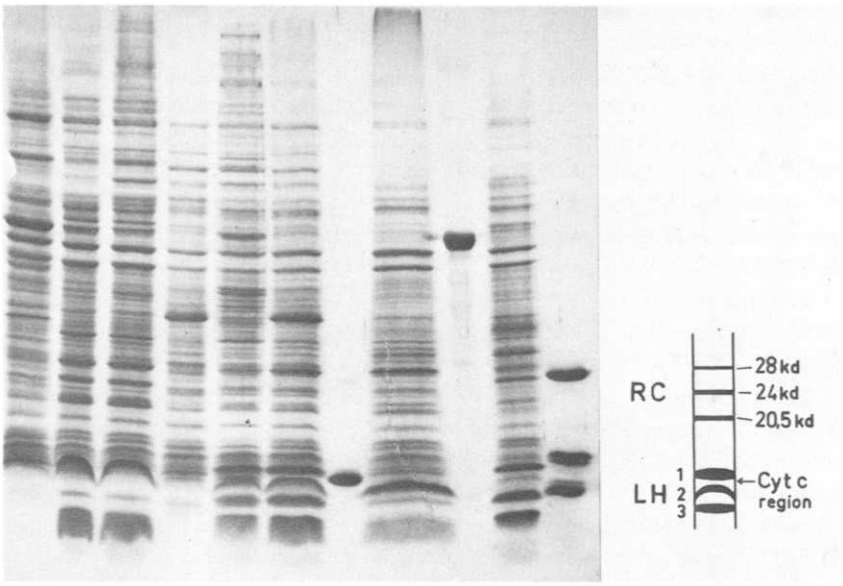

Fig.1. Protein pattern of membranes of *Rhodopseudomonas* capsulata strains after polyacrylamide gel electrophoresis. From left to right: Ala$^-$, Ala$^+$, Zi→Ala$^+$, YS, 37b4→YS, Z1→YS, Cyt.c, 37b4→W1, catalase, Y 142, chymotrypsinogen; scheme of protein pattern of the photosynthetic apparatus of wild type strains (37b4, J1, Z1).

The protein patterns of transferants (table 2) were the same as in the wild type parent strain 37b4 (fig.1). Solubilized YS membranes reacted positively with RC-antibodies, indicating that the RC proteins of YS were less extensively modified or suppressed than in A1a$^-$ or W1$^-$. In consequence it was possible to restore the photosynthetic capacity of YS by gene transfer from the Pho$^-$ Bch$^+$ Crt$^+$ strain Y142, but not by transfer from A1a$^-$ to YS (table 1).

In another group of experiments *crt* genes were transferred from a wild type donor to Crt$^-$ but Bch$^+$ Pho$^+$ strains. To separate Crt$^+$ transferants from blue-green phenotypes (Crt$^-$ Bch$^+$) those bacteria were selected, after gene transfer and expression, which

Table 2
In vivo infrared absorbance maxima of mutant strains and transferants and protein bands (PAGE) of light harvesting complexes of *Rhodopseudomonas capsulata*

Strain	Type of spectrum	I.R. maxima nm 1.	2.	Absorption ratio 2./1. IR peak;	A_{880}/A_{860}	LH bands PAGE
A1a$^+$	1	802	872	5.4	1.3	2
A1a$^+$→W1	1	805	872	4.9	1.1	2
37b4→W1	1	805	872	4.7	1.1	2
Z1→A1a$^+$	1	802	880	4.9	1.3	2
37b4→YS	2	800	860	1.6	0.5	1 2 3
A1a$^+$→YS	2	800	855	1.5	0.7	1 2 3
Z1→YS	2	800	857	1.5	0.5	1 2 3
Y 142	2	800	858	1.5	0.2	1 3
Y 5	2	800	858	1.5	0.2	1 3
Y 9	2	800	858	1.5	0.2	1 3
A1a$^-$		–	–	–	–	– –
37b4	2	802	855	1.5	0.5	1 2 3

are resistant to photooxidative killing [22]. One of these transferants, which received *crt* genes from Z1 (Z1→A1a$^+$) has carotenoid absorption maxima at 422, 472 and 505 nm in the in vivo spectrum. The positions of these peaks are different from those of the parent strain 37b4. Interestingly, the infrared region of the Z1→A1a$^+$ in vivo spectrum was similar to that of blue-green strains with a high A_{880}/A_{860} nm and longer λ IR absorption peak/shorter λ IR absorption peak (table 2), and different from the Pho$^+$ Crt$^+$ YS transferants (low peak ratio), in spite of the presence of carotenoids. However, the peak at 872 nm was shifted to 880 nm in Z1→A1a$^+$. In accordance with these characteristics of the IR-spectrum of Z1→A1a$^+$ only one LH-protein band was observed, at position 2 in PAGE (table 2, fig.1). The results show that only *crt* genes were transferred from Z1 to A1a$^+$ and the LH-complex remained unchanged. The membranes of mutants Y142, Y9 and Y5 (Pho$^-$ Bch$^+$ Crt$^+$) showed IR spectra similar to that of YS Pho$^+$ transferants (table 2). The protein pattern is characterized by the presence of LH-bands 1 and 3 but not 2 (fig.1). In the RC region bands at 28 and 24 kD seemed to be modified. An additional band of an apparent molecular weight of 31 kD appeared.

4. Discussion

The foregoing results have shown that mutational defects in bchl synthesis and in formation of RC and LH proteins were restored simultaneously by gene transfer. We never observed, however, concomitant restoration of bchl and carotenoid formation. This is in agreement with results of other studies [14,17]. It remains to be studied whether genes for proteins of the photosynthetic apparatus are clustered together with the *bch* genes on the chromosome of *R. capsulata* or regulatory genes are linked with *bch* genes. The comparison of in vivo spectra of various mutant and genetic transferant strains with the respective protein patterns of their membranes has demonstrated a strong correlation between type 1 of spectra (peaks at 803 and 872 or 880 nm and high ratios A_{880}/A_{860} nm; table 2) and LH-protein 2. These characteristics were found in the presence (Z1→A1a$^+$) or absence (37b4→W1) of carotenoids (table 2). We propose that expression of LH-complex I [2] is dependent upon LH-protein 2. This is supported by the isolation of LH-protein 2-bchl complex from A1a$^+$ (unpublished results). The type 2 of spectra (IR peaks at 800 and 855–860 nm and a low A_{880}/A_{860} ratio) was correlated with the presence of LH-proteins 1 and 3 in Pho$^-$ strains as Y 142 or 1, 2 and 3 in wild type strains (table 2). It is not known whether protein 1 and 3 react with bchl to form LH-complex II [2]. In all investigated Pho$^+$ transferants (table 2) RC proteins and protein band 2 of LH complex were restored. This observation and the coordinated regulation of RC and LH complex I (unpublished results) suggest that RC and LH complex I form a structural unit in the intracytoplasmic membranes of *R. capsulata.*

Acknowledgements

The stimulating atmosphere and the support in the laboratories of Howard Gest, Dept. of Microbiology, Indiana University, Bloomington and June Lascelles, Dept. of Bacteriology, UCLA are greatly acknowledged by G.D. This research was supported, in part, by a grant from the U.S. National Science Foundation to Howard Gest and a grant from the Deutsche Forschungsgemeinschaft to Gerhart Drews.

References

[1] Thornber, J. P., Trosper, T. L. and Strouse, C. E. (1976) in The Photosynthetic Bacteria, (W. R. Sistrom and R. K. Clayton, eds.) Plenum, New York.
[2] Lien, S., Gest, H. and San Pietro, A. (1973) J. Bioenergetics 4, 423–434.
[3] Okamura, M. Y., Steiner, L. A., Feher, G. (1974) Biochem. 13, 1394–1410.
[4] Nieth, K. F., Drews, G. and Feick, R. (1975) Arch. Microbiol. 105, 43–45.
[5] Fraker, P. J. and Kaplan, S. (1972) J. Biol. Chem. 247, 2732–2737.
[6] Clayton, R. K. and Clayton, B. J. (1972) Biochim. Biophys. Acta 283, 492–504.
[7] Reed, D. W., Raveed, D. and Israel, H. W. (1970) Biochim. Biophys. Acta 223, 281–291.
[8] Clayton, R. K. and Haselkorn, R. (1972) J. Mol. Biol. 68, 97–105.
[9] Nieth, K. F. and Drews, G. (1975) Arch. Microbiol. 104, 77–82.
[10] Dierstein, R. and Drews, G. (1975) Arch. Microbiol. 106, 227–235.

[11] Takemoto, J. (1974) Arch. Biochem. Biophys. 163, 515–520.
[12] Dierstein, R. and Drews, G. (1974) Arch. Microbiol. 99, 117–128.
[13] Marrs, B. (1974) Proc. Natl. Acad. Sci. USA 71, 971–973.
[14] Yen, H.-C. and Marrs, B. (1976) J. Bacteriol. in press.
[15] Wall, J. D., Weaver, P. F., Gest, H. (1975) Nature 258, 630–631.
[16] Weaver, P. F., Wall, J. D., Gest, H. (1975) Arch. Microbiol. 105, 207–216.
[17] Wall, J. D., Weaver, P. F. and Gest, H. (1975) Arch. Microbiol. 105, 217–224.
[18] Drews, G. (1974) Arch. Microbiol. 100, 397–407.
[19] Nieth, K. F. and Drews, G. (1974) Arch. Microbiol. 96, 161–174.
[20] Solioz, M., Yen, H.-C., Marrs, B. (1975) J. Bacteriol. 123, 651–657.
[21] Laemli, U. L. (1970) Nature (London) 227, 680–685.
[22] Sistrom, W. R., Griffiths, M. and Stanier, R. Y. (1956) J. Cell. Comp. Physiol. 48, 473–515.