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Time-resolved spectroscopy of the primary photosynthetic processes of membrane-bound reaction centers from an antenna-deficient mutant of *Rhodobacter capsulatus*

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The primary photosynthetic reactions in whole membranes of the antenna-deficient mutant strain U43 (pTXA6–10) of *Rhodobacter capsulatus* are studied by transient absorption and emission spectroscopy with subpicosecond time resolution. Extensive similarities between the transient absorption data on whole membranes and on isolated reaction centers support the idea that the primary processes in isolated reaction centers are not modified by the isolation procedure.

Introduction

The primary reactions in photosynthetic reaction centers (RCs) have been studied by a variety of absorption and emission experiments with high time-resolution [1–8]. In isolated RCs of purple bacteria a series of ultrafast processes was observed: On the longer time scale of 200 ps an absorption transient occurs which is correlated to the electron transfer (ET) from the bacteriopheophytin (H_A) to the quinone Q_A [3,4]. A faster transient absorption change with approx. 3 ps was observed and correlated to the decay of P*, the excited electronic state of the special pair P, the primary donor. Very recent emission experiments have shown that the 3 ps decay of P* seems to be a biphasic process with two time constants [8,9]. At all temperatures an additional subpicosecond component was detected in *Rhodobacter sphaeroides* having time constants of 0.3 ps to 0.9 ps for temperatures of 25 K to 300 K [10,11]. The same ultrafast component was found in RCs from *Rhodopseudomonas viridis* having a time constant of 0.65 ps at 300 K [5]. In addition, even faster sub-picosecond processes were observed at cryogenic temperatures where the transient absorption showed oscillations which were related to the motion of a vibronic wavepacket in the initial electron transfer [6,7].

Up to now there are no generally accepted explanations of the sub-picosecond reaction dynamics [12,13]. From our data, we favor a two-step model for the primary photosynthetic reaction where P* decays with a time constant τ_1 of approx. 3 ps into $P^+B^-_A$ and where the sub-picosecond kinetic τ_2 (approx. 0.9 ps) is associated with the electron transfer from $P^+B^-_A$ to $P^+H^-_A$ (The assignment of $\tau_3 = 200$ ps to the transition of $P^+H^-_A$ to $P^+Q^-_A$, on the other hand, is generally accepted). Regarding the sub-picosecond kinetic τ_2, one trivial explanation could not be ruled out: All published experiments where a sub-picosecond kinetic (namely τ_2) was observed, were done with detergent-treated preparations of isolated RCs [2,6,7,10]. It is not known whether the ultrafast absorption dynamics are altered by a preparation artifact during the isolation of the reaction centers from the chromatophores by detergents. Since it is well-established that detergents influence the spectral properties of the RC, an effect on the reaction dynamics is likely. Especially one could argue that the sub-picosecond kinetic τ_2 is exclusively caused by the chemical treatment during extraction of the RCs from the membrane.

It is the purpose of this paper to study the primary absorption and emission dynamics of RCs in whole membranes, which are not treated by detergents. A

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mutant of *Rb. capsulatus* was chosen which is free of light-harvesting antenna complexes. In this way, the experiments can be performed without the disturbing action of the antenna which would preclude the direct observation of ultrafast kinetic components.

Materials and Methods

In the experiment on *Rhodobacter capsulatus* we worked with the strain U43 (pTXA6–10) which is photosynthetically active, but free of light-harvesting complexes [14,15]. The genetic construction of this strain has been described by Dörge et al. [14]. The cells grew anaerobically in a turbidostate at low light intensities. Under these conditions the cell protein doubling time is 9.8 h, while at high light intensity the cell protein doubling time is 4.6 h. In cells grown at low light level, the concentration of photochemical reaction centers is high (1637 pmol RC/mg membrane protein). For comparison: at low light levels the concentration in wild-type cells of strain B 10 is 540 pmol RC/mg membrane protein. Membranes were isolated and purified by sucrose-gradient centrifugation and resuspended in Tris-HCl buffer (pH 7) at a concentration of 6.5 μM. The major membrane fraction was harvested and used for the experiments. The absorption spectrum of the membranes taken after the time-resolved experiment is presented in Fig. 1a. Absorption peaks occur at 863 nm (Q_y transition of the special pair P), at 801 nm (Q_y transition of monomeric bacteriochlorophylls), at 750 nm (bacteriopheophytin) and at 596 nm (Q_x of the bacteriochlorophylls). An additional absorption at 685 nm is apparently due to oxidized bacteriochlorophyll or to a precursor of the bacteriochlorophyll biosynthesis. This peak was observed in the membranes of at least two mutant strains lacking light-harvesting systems. Below 600 nm there is a pronounced rise in absorption due to scattering from the membranes. This is expected, since the membrane particles are much larger than the isolated RC.

Isolated RCs of *Rb. sphaeroides* were prepared according to the procedures given in Ref. 2.

The experimental set-up of the transient emission experiments has been described by Hamm et al. [8]. The salient features are: The lifetime of the long-wavelength emission was measured by up-conversion of the fluorescence light. The sample was excited by light pulses at 865 nm with an energy of 0.5 μJ (spot diameter 700 μm). The fluorescence light at a wavelength of 920 $\pm$10 nm was imaged onto a 1-mm-thick β-barium borate (BBO) crystal. Upconversion was performed by a properly delayed 3 μJ pulse at a wavelength of 840 nm. The time-resolution of the system was better than 400 fs.

The transient absorption data on *Rb. capsulatus* were measured with a redesigned sub-picosecond spectrometer [5]. A standard colliding pulse-mode-locked (CPM) dye laser was taken as a femtosecond pulse source at 620 nm. Single pulses from this laser were amplified in a multipass amplifier (rhodamine 101) pumped at 50 Hz repetition rate by a frequency-doubled, Q-switch Nd:YAG laser. The resulting recompressed pulses were split into (i), an excitation and (ii), a probing pulse to generate two efficient femtosecond continua.

(i) In the excitation branch, a second dye amplifier (styryl 15) in combination with an interference filter produced pulses 20 nm wide centered around 865 nm with durations of less than 150 fs. These pulses contained no spectral components below 850 nm avoiding excitation of the Q_y(B)-band.

(ii) In the probing branch, light was selected from the continuum by a special double-grating monochromator [16] which allowed to vary the spectral position and the spectral width. Subsequently, the properly attenuated probe beam was focused into the excited region of the sample. The accuracy of the measurements was substantially improved by the introduction of a reference probe beam, which passed an unexcited part of the sample. Pump and probe pulses had parallel polarizations. The width of the instrumental response function was around 300 fs.

The sample, about 0.3 ml of a suspension of membranes or RCs, was held in a cuvette with 1 mm path length under stirring (0.6 ml in a 2 mm cell were used in the upconversion experiment). The sample was adjusted to 50% transmission at 865 nm. The stirring in combination with weak excitation (less than 10% of the RC in the irradiated volume absorbed a photon per laser pulse) prevented double excitation of the RCs within the long photosynthetic cycle of approx. 1.8 seconds [15]. The delay line was scanned in linear steps up to delay times of 1 ps. Subsequently, the stepwidth was increased exponentially. In the figures the experimental data are displayed as points. For the theoretical modelling a sum of exponentials is convoluted with the instrumental response function.

Results

In a first set of experiments we address the decay of the excited electronic level P* of the special pair. Its population is investigated via the spontaneous emission or via stimulated emission (gain) in the long wavelength range of the special pair Q_y band. Fig. 1b displays the results of an up-conversion experiment, where the time dependence of the spontaneous emission is studied. This measurement yields directly the decay of the population of P* without interference of excited state absorption or ground state intermediates. The data points can be well-simulated by a biexponential model function: While the initial decay is domi-

nated by a process with a time constant of $\tau_1 = 4.5 \pm 0.3$ ps, an additional slow and weak process is found with a time constant of 90 ± 30 ps and a relative amplitude $A_{90}/A_{4.5}$ of approx. 0.1. A satisfactory fit of the experimental data does not require an additional time constant; e.g., when the analysis of the emission data is calculated on the basis of two time constants in the 2–15 ps range, the fit of the experimental data is not improved. However, the existence of an underlying 90 ps time constant makes it very difficult to prove or exclude an additional weak 11 ps kinetic component observed in isolated RC in Ref. 9. In the further modelling we therefore used one time constant (4.5 ps) in the 2–15 ps range.

Transient absorption data were taken at 20 selected probing wavelengths (λ_{pr}) between 640 nm and 940 nm. Here, only data at $\lambda_{pr} = 933$ nm, 809 nm, 798 nm, 790 nm, 789 nm, 788 nm and 683 nm will be presented. At first the part of the spectrum is treated where stimulated emission (optical gain) occurs due to the population of P*. Fig. 2a shows the transient absorption at a probing wavelength of 933 nm. The measurement exhibits an absorption decrease which is due to stimulated emission (gain). The gain decays with a time constant of 4.5 ps in full agreement with emission data given above. It should be kept in mind that the statistical scattering of the data points is larger in the absorption than in the emission experiments. Therefore, the modelling of the data with one time constant in the 2–15 ps range is fully adequate. At later delay times there is a weak absorbance change which cannot be assigned unambiguously to a 90 ps time constant. There

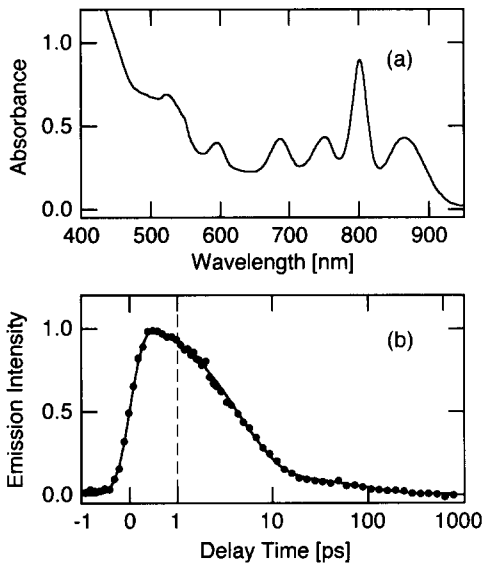

Fig. 1. (a) Absorption spectrum of the reaction centers of *Rb. capsulatus* strain U43 (pTXA 6–10) used in the time-resolved experiments. (b) Transient emission data from the RC after excitation at 865 nm. The data points are modelled by a biexponential model function (solid line) with time constants of 4.5 ps and 90 ps.

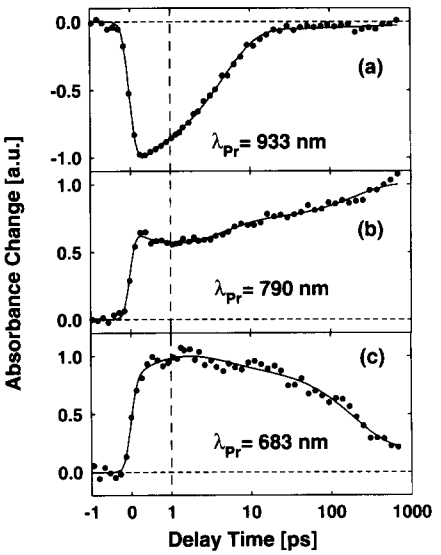

Fig. 2. Transient absorption data on RC of *Rb. capsulatus* (whole membranes) at probing wavelengths of 933 nm (a), 790 nm (b) and 683 nm (c). The solid line is a model function using time constants of 0.6 ps, 4.5 ps and 200 ps.

may also be some contribution from the electron transfer from the bacteriopheophytin H_A to the quinone Q_A which proceeds on the 200 ps time scale (see below) and which leads – at least in other reaction centers – to weak absorbance changes in the 930 nm range [4].

Fig. 2c shows transient absorption data taken at a probing wavelength of $\lambda_{pr} = 683$ nm, in the spectral range where the anions of bacteriochlorophyll and bacteriopheophytin strongly absorb. First, the absorption rises instantaneously at time zero followed by a subsequent weak sub-picosecond absorption increase and a weak decrease with a time constant of 4.5 ps. At later delay times the absorption decreases with a 200 ps time constant. The modelling of the data recorded at $\lambda_{pr} = 683$ nm contains a weak sub-picosecond component similar to that found in isolated RC. It should be mentioned that, due to the reduced accuracy caused by scattering of the membranes, the existence of such a component cannot be proven unambiguously at this wavelength. However, there are other wavelengths where a short kinetic component shows up very clearly.

In Fig. 2b a probing wavelength of 790 nm is chosen. In this measurement a weak but distinct absorption decrease is observed during the first picosecond following the initial instantaneous absorption increase. After 1 ps the absorption rises again. By a fine tuning of the probing wavelength this fast kinetic component can be established quite convincingly. In Fig. 3 we used $\lambda_{pr} =$ 789 nm and measured the absorbance change during the early delay times with improved accuracy (increased number of data points). A simulation of the data with two time constants of 4.5 ps and 0.6 ps (solid line) describes the experimental points very well. Dis-

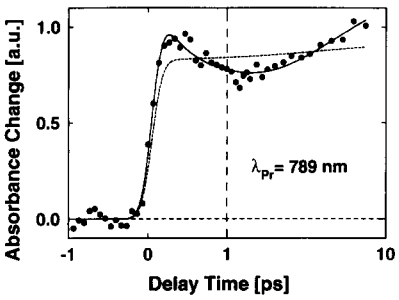

Fig. 3. Transient absorption (points) measured at a probing wavelength of 789 nm. The broken curve was calculated with a time constant of 4.5 ps while two time constants of 0.6 ps and 4.5 ps are used for the solid model function. The data show that the fast time constant is required in order to simulate the data points.

regarding the 0.6 ps time constant, the broken line results, which significantly deviates from the data points. This measurement clearly demonstrates that the fast sub-picosecond kinetic component, recently found in isolated RCs, is also present in RCs incorporated in whole membranes, i.e., in RCs which are not treated by detergents.

The substantial number of measurements at 20 selected probing wavelengths allows a more general comparison between isolated RCs of *Rb. sphaeroides* and membrane-bound RC of *Rb. capsulatus*. The overall transient behavior of both RCs is identical. At critical wavelength very small shifts of the order of a few nm occur. In order to support this statement, we show

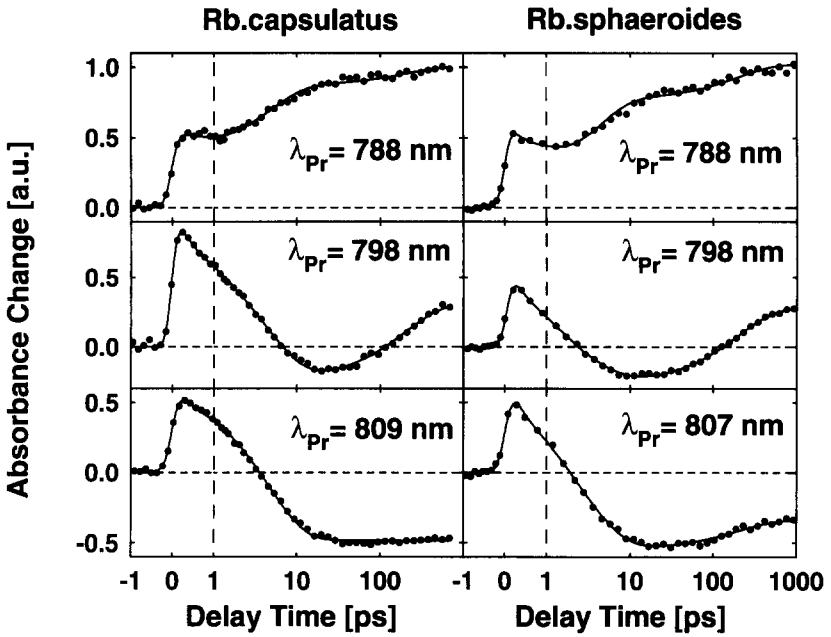

Fig. 4. Comparison of the transient absorption data of isolated RC of *Rb. sphaeroides* (right) and RC in whole membranes of *Rb. capsulatus* (left part). The probing wavelengths were taken in the Q_y absorption band of the monomeric bacteriochlorophylls. One observes a good qualitative agreement between the two types of reaction centers in this very complex spectral range.

TABLE I

Time constants deduced from transient absorption and emission experiments on reaction centers of various purple bacteria

RC	Ref.	τ_1 (ps)		τ_2 (ps)	τ_3 (ps)
Isolated RC of	5 and Dressler, K. et al.,	3.8 *		0.65	200
Rps. viridis	personal communication	2 **	7 **		
Isolated RC of	2,8,19	3.5 *		0.9	200
Rb. sphaeroides		2.3 **	7 **	0.9	200
	9	2.7 **	12.1 **		
Isolated RC of		3.4 *			209 ± 16
Rb. capsulatus	9,18,19	2.7 **	11.1 **		238 ± 3
RC in whole membranes of					
Rb. capsulatus	This work	4.5 *		0.6	200
		4.5 **	90 **		

* Monoexponential fit of the decay of P*.
** Biexponential fit of the decay of P*.

measurements in the $Q_y(B)$ region, where the shape of the transient absorption data varies drastically with the probing wavelength (Fig. 4): On the left side we have plotted the data on *Rb. capsulatus*, while corresponding curves on isolated RC of *Rb. sphaeroides* are depicted on the right side. The comparison demonstrates that the basic properties of both Bchl-*a*-containing species are the same. Even here, at a highly complex spectral position, it seems that only weak wavelength shifts occur. From the data (e.g., at 809 nm) it is evident that the time constants found in *Rb. sphaeroides* and *Rb. capsulatus* are not exactly the same. Values of the time constants determined for RC of various purple bacteria are summarized in Table I.

Discussion

For a qualitative discussion we will compare the RCs of *Rb. capsulatus* bound to membranes with RCs isolated by detergents. Since isolated RCs from the investigated mutant strain were not available to us, we used own experimental results from isolated RCs of *Rb. sphaeroides* and data on isolated RCs of *Rb. capsulatus* (wild-type) for comparison [2, 8, 9, 17].

In isolated RCs of *Rb. sphaeroides* the decay of the excited state P* of the special pair shows a biphasic behavior with a dominating 2.3 ps and a weaker 7 ps component [8]. (In another publication [9] values of 2.7 ps and 12.1 ps were given for RCs of *Rb. sphaeroides* while 2.7 ps and 11.1 ps were reported for RCs of *Rb. capsulatus* wild-type). This biexponential time dependence was recently discussed in terms of several models [8,20]: (i), a functional heterogeneity with a fraction of RC having reduced primary reaction speed; (ii), a transiently-populated 'parking state', presumably a radical pair state involving a chromophore on the inactive pigment branch [8]; (iii), a branched reaction model with two reaction paths from P* to P^+H^- [9].

The analysis of the present emission data on membrane-bound RCs of *Rb. capsulatus* does not require two time constants in the 2–10 ps range. The fit is quite good with the one 4.5 ps time constant. However, there is the very slow 90 ps component observed in emission. Up to now we cannot rule out a functional heterogeneity with strongly perturbed RCs as origin of this slow time component. The parking model, on the other hand is not able to explain the slow component: A qualitative analysis of the experimental data using an additional parking state in the model leads to a very slow forward rate towards the active branch. This rate is not compatible with the observed rise of absorbance in the spectral range of the anion band of $P^+H_A^-$ at 683 nm. A parallel reaction model for the electron transfer from P* to $P^+H_A^-$ is not able to explain all time constants observed in our experiments. However, the special type of sample we used in our experiments could cause the 90 ps component: While we know that no free Bchl *a* is present in the membrane and no light-harvesting BChl was detectable by absorption spectroscopy and steady state bleaching of RCs, we cannot exclude that trace amounts of light-harvesting complexes (LH I) are present which are not directly connected to the RCs. They may have longer decay times and may be responsible for the 90 ps kinetic component. Finally, it should be mentioned, that the time constant of 4.5 ps observed in the membrane-bound RCs of *Rb. capsulatus* is somewhat longer than the time constant of about 3.4 ps observed in mono-exponential fits of isolated RCs of *Rb.capsulatus* and *Rb. sphaeroides* [2,18]. These differences in the time constants may be related to the special surrounding of the RCs in the various preparations.

The absorption data reveal a remarkable qualitative agreement between the membrane-bound RCs of *Rb. capsulatus* and the isolated RCs of *Rb. sphaeroides* (see, e.g., Fig. 4). This important finding strongly supports the idea that the primary reaction processes are not disturbed by the isolation procedure. In addition, a direct comparison with isolated RCs from wild-type *Rb. capsulatus* is possible: The large number of different probing wavelengths used in our experiments made it possible to calculate raw transient spectra of the sample for different delay times (data not shown). This allows a qualitative comparison to published sub-picosecond data of isolated RCs from *Rb. capsulatus* [18]: The overall shape of the difference spectra is the same as in Ref. 18. Moreover, the isosbestic points for the 600 fs and 15 ps transient spectra given in Ref. 18 at 765 nm and 798 nm, respectively, are well reproduced in our data where we obtain 765 nm and 792 nm. This observation gives strong evidence that the spectral properties of the RCs are not effected by the isolation procedure and by other pigments surrounding the RCs in membranes.

Another important point is the fact that a sub-picosecond kinetic component with a time constant τ_2 of about 0.6 ps is needed to fit the experimental data in the $Q_y(B)$ region. It should be noted that – in agreement with all other measurements done by our group – the ultrafast kinetic is detectable only at special spectral positions: Independent of the specific type of RCs, these areas of detectability seem to be fixed to positions where the monomeric bacteriochlorophyll or its anion have absorption bands. Thus, we regard the sub-picosecond time constant τ_2 as an indicator for a substantial participation of the bacteriochlorophyll monomer in the primary charge transfer process.

In conclusion, the experimental data presented here do not show substantial differences of the transient behaviour of isolated and membrane-bound reaction centers. This indicates that it is well justified to use isolated reaction centers as a model system for the

study of primary electron transfer reactions in photosynthesis. However, slight differences of the time constants exist which deserve further investigation.

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References

1 Martin, J.-L., Breton, J., Hoff, A.J., Migus, A. and Antonetti, A. (1986) Proc. Natl. Acad. Sci. USA 83, 957–961.
2 Holzapfel, W., Finkele, U., Kaiser, W., Oesterhelt, D., Scheer, H., Stilz, H.U. and Zinth, W. (1989) Chem. Phys. Lett. 160, 1–7.
3 Kirmaier, C. and Holten, D. (1990) Proc. Natl. Acad. Sci. USA 87, 3552–3556.
4 Holzapfel, W., Finkele, U., Kaiser, W., Oesterhelt, D., Scheer, H., Stilz, H.U. and Zinth, W. (1990) Proc. Natl. Acad. Sci. USA 87, 5168–5172.
5 Dressler, K., Umlauf, E., Schmidt, S., Hamm, P., Zinth, W., Buchanan, S. and Michel, H. (1991) Chem. Phys. Lett. 183, 270–276.
6 Vos, M.H., Lambry, J.-C., Robles, S.J., Youvan, D.C., Breton, J. and Martin, J.-L. (1991) Proc. Natl. Acad. Sci. USA 88, 8885–8889.
7 Vos, M.H., Lambry, J.-C., Robles, S.J., Youvan, D.C., Breton, J. and Martin, J.-L. (1992) Proc. Natl. Acad. Sci. USA 89, 613–617.
8 Hamm, P., Gray, K.A., Oesterhelt, D., Feick, R., Scheer, H. and Zinth, W. (1993) Biochim. Biophys. Acta 1142, 99–105.
9 Du, M., Rosenthal, S.J., Xie, X., Di Magno, T.J., Schmidt, M., Hanson, D.K., Schiffer, M., Norris, J.R. and Fleming, G.R. (1992) Proc. Natl. Acad. Sci. USA 89, 8517–8521.
10 Lauterwasser, C., Finkele, U., Scheer, H. and Zinth, W. (1991) Chem. Phys. Lett. 183, 471–477.
11 Chang, C.-K., DiMagno, T.J., Chen, L.X.-Q., Norris, J.R. and Fleming, G.R. (1991) Proc. Natl. Acad. Sci. USA 88, 11202–11206.
12 Marcus, R.A. (1987) Chem. Phys. Lett. 133, 471–477.
13 Bixon, M., Jortner, J. and Michel-Beyerle, M.E. (1991) Biochim. Biophys. Acta 1056, 301–315.
14 Dörge, B., Klug, G., Gad'on, N., Cohen, S.N. and Drews, G. (1990) Biochemistry 29, 7754–7758.
15 Garcia, A.F., Mäntele, W., Gad'on, N., Tadros, M.H. and Drews, G. (1991) Arch. Microbiol. 155, 205–209.
16 Martinez, O.E. (1987) IEEE J.Q.E. 23, 59–64.
17 Chang, C.-K., Chen, L.X.-Q., Di Magno, T.J., Hanson, D.K., Nance, S.L., Schiffer, M., Norris, J.R. and Fleming, G.R. (1991) Chem. Phys. Lett. 176, 366–372.
18 Kirmaier, C. and Holten, D. (1988) Isr. J. Chemistry 28, 79–85.
19 Kirmaier, C., Holten, D., Bylina, E.J. and Youvan, D.C. (1988) Proc. Natl. Acad. Sci. USA 85, 7562–7566.
20 Müller, M.G., Griebenow, K. and Holzwarth, A.R. (1992) Chem. Phys. Lett. 199, 465–469.