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Reaction center complex from an aerobic photosynthetic bacterium, *Erythrobacter* species OCh 114

Ken-ichiro Takamiya, Koh Iba and Kazuo Okamura *

Department of Biology, Faculty of Science, Kyushu University 33, Fukuoka 812 (Japan)

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A photosynthetic reaction center complex has been purified from an aerobic photosynthetic bacterium, *Erythrobacter* species OCh 114. The reaction center was solubilized with 0.45% lauryldimethylamine *N*-oxide and purified by DEAE-Sephacel column chromatography. Absorption spectra of both reduced and oxidized forms of the reaction center were very similar to those of the reaction center from *Rhodopseudomonas sphaeroides* R-26 except for the contributions due to cytochrome and carotenoid. 1 mol reaction center contained 4 mol bacteriochlorophyll *a*, 2 mol bacteriopheophytin *a*, 4 mol cytochrome *c*-554, 2 mol ubiquinone-10, and carotenoid. The reaction center consisted of four different polypeptides of 26, 30, 32 and 42 kDa. The last one retained heme *c*. Absorbance at 450 nm oscillated with the period of two on consecutive flashes. The light-minus-dark difference spectrum had two peaks at 450 nm and 420 nm, indicating that odd flashes generated a stable ubisemiquinone anion and even flashes generated quinol. *o*-Phenanthroline accelerated the re-reduction of flash-oxidized reaction centers, indicating that *o*-phenanthroline inhibited the electron transfer between Q_A and Q_B. The cytochrome (cytochrome *c*-554) in the reaction center was oxidized on flash activation. The midpoint potential of the primary electron acceptor (Q_A) was determined by measuring the extent of oxidation of cytochrome *c*-554 at various ambient potentials. The mid-point potential of Q_A was −44 mV, irrespective of pH between 5.5 and 5.9.

Introduction

Erythrobacter OCh 114 is a species of 'aerobic photosynthetic bacteria', which can grow and synthesize bacteriochlorophyll only under aerobic conditions [1–3]. Its habitat is on the surface of sea weeds and on the sands of seashore [4]. The color of the intact cells is reddish-pink, due to the presence of carotenoid pigments [4]. The light-minus-dark difference spectrum of the membrane preparation from this bacterium suggested that reversible photooxidations of reaction center and cytochrome occurred as in the case of the traditional photosynthetic bacteria [5,6]. The shape of the light-minus-dark difference spectrum and absorption spectrum of chromatophores treated with iridic chloride in the near-infrared region [5] and separation of reaction center by polyacrylamide gel electrophoresis [7] suggested that the bacterium contained reaction centers similar to those of pur-

* Present address: National Center for Nervous, Mental and Muscular Disorders, Ogawa-Higashi-Machi, Kodaira, Tokyo 187, Japan.

Abbreviations: LDAO, lauryldimethylamine *N*-oxide; Q_A and Q_B, the primary and the secondary electron acceptor quinone, respectively; Cyt, cytochrome.

Correspondence: Ken-ichiro Takamiya, Department of Biology, Faculty of Science, Kyushu University, 33, Fukuoka 812, Japan.

ple photosynthetic bacteria. Photophosphorylation and light-induced CO_2 incorporation have also been observed in intact cells under aerobic conditions, but not under anaerobic conditions [8,9]. These facts suggest that although this bacterium has unique physiological properties, it has similar photosynthetic machinery to that of the traditional purple bacteria and that the machinery can operate under a certain condition.

One of the most important problems to be solved is whether this organism contains the same type of reaction center as that of the photosynthetic purple bacteria and how the electron transfer occurs in the reaction center. Here we have isolated a reaction center complex from *Erythrobacter* OCh 114 and characterized the electron transfer in the complex.

Materials and Methods

Erythrobacter OCh 114, originally supplied by Dr. Keiji Harashima of University of Tokyo, was cultured under vigorous aeration in the dark and harvested as described previously [10]. The cells, suspended in 50 mM Tris-HCl (pH 8.0), were disrupted through a French pressure cell and the membrane preparation was obtained as described before [10].

Spectroscopic measurements. Absorption and difference spectra were measured with a Shimadzu UV240 spectrophotometer. Flash-induced absorption changes due to reaction center bacteriochlorophyll and cytochrome were measured with a rapid single beam spectrophotometer (Union Giken RA 1201) as described previously [11]. Redox titration of the flash-induced oxidation of cytochrome were carried out with the same spectrophotometer as described before [11].

Sodium dodecylsulfate polyacrylamide gel electrophoresis was carried out according to the method of Laemmli and Favre [12]. The gel concentration was 12.5%. Reaction center solution, dissolved in 20 mM Tris-HCl buffer (pH 7.5) containing 0.1% Triton X-100, was lyophilized, then the dried reaction center was resuspended in the same volume of distilled water. The sample was treated with SDS (final concn., 3%) and β-mercaptoethanol (final concn., 1%) at 0°C for 30 min. After electrophoresis, the gel was stained with Coomassie brilliant blue (for protein) or stained in the dark with 3,3′,5,5′-tetramethylbenzidine (for heme detection) as described by Thomas et al. [13]. Without lyophilization the reaction center complex, occasionally failed to separate into subunits, the reason for this being unknown.

Other procedures. Ubiquinone-10 content was determined as described previously [14]. Ubiquinone-10 in the reaction center was extracted with 90% acetone and transferred to light petroleum ether layer followed by washing with 95% methanol. Washed ubiquinone solution was dried in vacuo and ubiquinone was dissolved in ethanol. The absorbance change at 275 nm before and after the addition of a few crystals of sodium borohydride was measured.

The contents of bacteriochlorophyll and bacteriopheophytin in the reaction center were determined by measuring absorbances of acetone/methanol (7 : 2, v/v) extract of reaction center at 770 and 747 nm, which are absorption maxima of bacteriochlorophyll and bacteriopheophytin in an acetone-methanol mixture, respectively. Millimolar absorption coefficients of bacteriochlorophyll at 770 and 747 nm and those of bacteriopheophytin at 747 and 770 nm in an acetone-methanol mixture were calculated from the absorption spectra of pure bacteriochlorophyll *a* and bacteriopheophytin *a*, prepared according to the method of Omata and Murata [15]. Millimolar absorption coefficients of bacteriochlorophyll *a* at 770 was assumed to be 76 [16] and that at 747 nm to be 46.3. The bacteriopheophytin *a* coefficients at 770 and 747 nm were 19.6 and 52.6, respectively. The content of the reaction center bacteriochlorophyll was determined by measuring absorbance difference at 864 nm between reduced and oxidized reaction center. $\Delta\varepsilon_{864}^{red-ox}$ of the reaction center bacteriochlorophyll was assumed to be 112 $mM^{-1} \cdot cm^{-1}$, according to Straley et al. [17].

Results

Purification of a reaction center complex

Membranes obtained as in Materials and Methods were resuspended in 20 mM Tris-HCl (pH 7.5)/0.1 M NaCl/1 mM phenylmethylsulfonylfluoride (bacteriochlorophyll concentration, 0.2

mM). To the suspension, LDAO was added dropwise from a 30% solution by stirring at room temperature in the dark, to give a final concentration of 0.45%. The mixture was incubated for 30 min and centrifuged at 300 000 × *g* for 2 h. The precipitates were resuspended in twice the volume of the same buffer and LDAO was added to give a final concentration of 0.45%. The mixture was incubated for 1 h at room temperature in the dark. The mixture was centrifuged at 300 000 × *g* for 2 h and the supernatant solution was applied to a DEAE-Sephacel (Pharmacia Fine Chemicals) column previously equilibrated with 20 mM Tris-HCl (pH 7.5)/0.1% Triton X-100/0.1 M NaCl. The column was washed with the same buffer solution until the eluent solution became colorless. Then the column was eluted with linear gradient of NaCl concentration (0.1 M–0.5 M) in 20 mM Tris-HCl/0.1% Triton X-100. Reaction center was eluted at a NaCl concentration of about 0.35 M. Reaction center-rich fractions were combined and diluted 5-times and again applied to the DEAE-Sephacel column. The column was washed and eluted with the same linear gradient solution of NaCl. Column chromatography was carried out at 4°C in the dim light. The yield of reaction center was about 20%.

Fig. 1 shows the absorption and oxidized-minus-reduced difference spectra of the purified

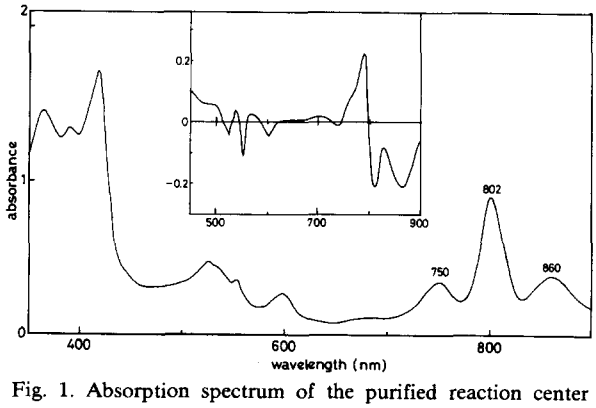

Fig. 1. Absorption spectrum of the purified reaction center complex. 3 μM reaction center was dissolved in 20 mM Tris-HCl buffer (pH 7.5) containing 0.1% Triton X-100. The inset shows the oxidized-minus-reduced difference spectrum. Reaction center concentration, 1.8 μM. The reaction center was oxidized by the addition of 10 μM ferricyanide, then excess ascorbate was added to the solution and the difference spectrum was taken.

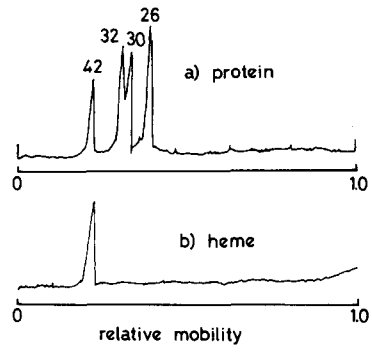

Fig. 2. Densitogram of SDS-polyacrylamide gel electrophoresis of reaction center complex. Details for SDS-polyacrylamide gel electrophoresis, see Text. (a) Protein staining with Coomassie brilliant blue; (b) heme staining with 3,3′,5,5′-tetramethylbenzidine. Numbers above bands are molecular mass in kDa.

reaction center complex. These spectra, as a whole, were very similar to those of the reaction center complexes from the purple photosynthetic bacteria. Absorption bands in the near-infra-red region indicated the presence of bacteriochlorophyll *a* and bacteriopheophytin *a*. Absorption peaks around 554 and 422 nm indicated the presence of cytochrome. The broad band around 520 nm was due to carotenoid pigments. The 860 nm band of the absorption spectrum was bleached by oxidation of the reaction center to give a trough at 864 nm in the difference spectrum. A trough at 605 nm in the difference spectrum was due to the oxidation of the reaction center bacteriochlorophyll. A trough at 554 nm in the difference spectrum was due to the oxidation of a *c*-type cytochrome (Cyt *c*-554).

Four subunits were detected after SDS-polyacrylamide gel electrophoresis with apparent molecular weight of 42 000, 32 000, 30 000 and 26 000 (Fig. 2). Heme staining indicated that the subunit with molecular weight 42 000 was a heme-containing polypeptide. Redox titration of cytochrome in the dark also showed the presence of only one type of cytochrome (Cyt *c*-554) and the midpoint potential was 330 mV at pH 7.0 (data not shown). The titration of flash-induced oxidation of Cyt *c*-554 gave the same midpoint potential. This value was in good agreement with 335 mV in chromatophores from the same organism [6]. The midpoint potential of the reaction center bacteriochlorophyll was measured by a

TABLE I

PIGMENT COMPOSITION OF REACTION CENTER COMPLEX FROM *ERYTHROBACTER* OCh 114

Values are the means of three determinations using different preparations.

Component	Content (mol/mol RC)
Bacteriochlorophyll *a*	3.7 ± 0.2
Bacteriopheophytin *a*	2.0 ± 0.2
Cytochrome *c*-554	3.7 ± 0.2
Ubiquinone-10	1.7 ± 0.2
Carotenoids	not determined

flash-induced absorbance decrease at 605 nm in the higher ambient redox potential range (E_h 380–490 mV). $E_{m\,7}$ of the reaction center bacteriochlorophyll was 435 mV. This value was very close to that of the reaction center complex from purple photosynthetic bacteria. In Table I, the pigment and cytochrome composition is shown. The contents of bacteriochlorophyll *a*, bacteriopheophytin *a*, Cyt *c*-554 and ubiquinone-10 per reaction center were 4, 2, 4 and 2 mol, respectively. It has been reported that this bacterium contains only ubiquinone-10 as the quinone constituent [18]. The present result agreed with that reported previously [18]. The cytochrome content was determined by pyridine hemochrome method. Because the intensity of stain with Coomassie depends on polypeptides, it could not be decided how many apocytochromes were present in one reaction center complex. In fact, although there were four hemes per reaction center, the band height of 42 000 molecular weight was not as large as other bands (Fig. 2).

Photochemical properties of the reaction center complex

The effect of o-phenanthroline on the re-reduction of flash-oxidized reaction center bacteriochlorophyll. Time-courses of oxidation-reduction of reaction center were measured at 605 nm. The flash-oxidized reaction center was slowly re-reduced in the dark in the absence of *o*-phenanthroline ($\tau_{1/2}$ = 0.8 s, Fig. 3). In the presence of 10 mM *o*-phenanthroline the rate of the re-reduction was greatly accelerated ($\tau_{1/2}$ = 20 ms), suggesting that electron transfer from the primary electron acceptor quinone (Q_A) to the secondary electron acceptor Q_B was inhibited, and the rate of the charge recombination between the reduced Q_A and the oxidized reaction center was accelerated.

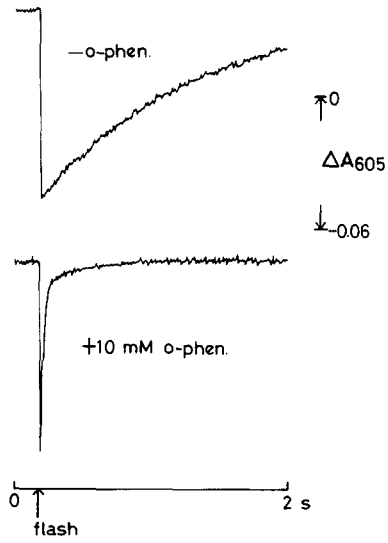

Fig. 3. Effect of *o*-phenanthroline on the rate of the re-reduction of oxidized reaction center. The 4.2 μM reaction center was dissolved in the same buffer as in Fig. 1. Absorbance changes due to oxidation and re-reduction after a single-turnover flash were measured at 605 nm.

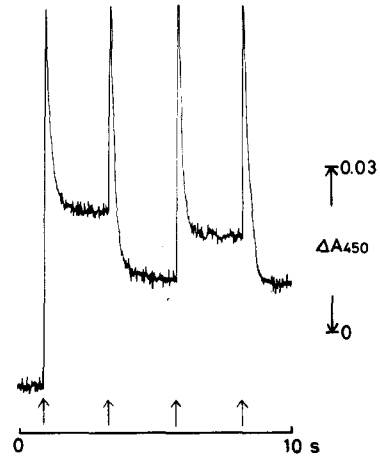

Fig. 4. Absorbance oscillation with two periods at 450 nm on successive flashes. 0.7 μM reaction center was dissolved in the same buffer as in Fig. 1. As an electron donor, 300 μM 2,3,5,6-tetramethyl-*p*-phenylenediamine was added. 30 μM ubiquinone-10 was added to the reaction center solution in an ethanol solution. Arrows on the time scale indicate when the flash was fired.

The absorption change at 450 nm with the oscillation of two periods on successive flash activations. In the presence of 30 μM ubiquinone-10/300 μM 2,3,5,6-tetramethyl-*p*-phenylenediamine, absorption at 450 nm was increased on the first-flash activation, and on the second-flash activation the increased absorbance was decreased (Fig. 4). On the third flash, the absorbance was again increased. The spike after each flash was rapid oxidation and re-reduction, due to the reaction center bacteriochlorophyll. The difference spectrum (absorbance after first flash minus that in the dark) gave two positive peaks at 420 nm and 450 nm (Fig. 5). The shape of the difference spectrum was very similar to that of ubisemiquinone anion-minus-ubiquinone in the methanol solution [19]. The oscillation pattern with a period 2 and the difference spectrum suggested that after the first flash a stable ubisemiquinone anion was formed and the second flash reduced the anion to fully reduced ubiquinone. Therefore, it was implied that a two-electron gate mechanism operated in the electron transfer between Q_A and Q_B as in the case of the reaction center from purple photosynthetic bacteria [20,21].

Midpoint potential of Q_A. The midpoint potential of Q_A was determined by measuring the extent

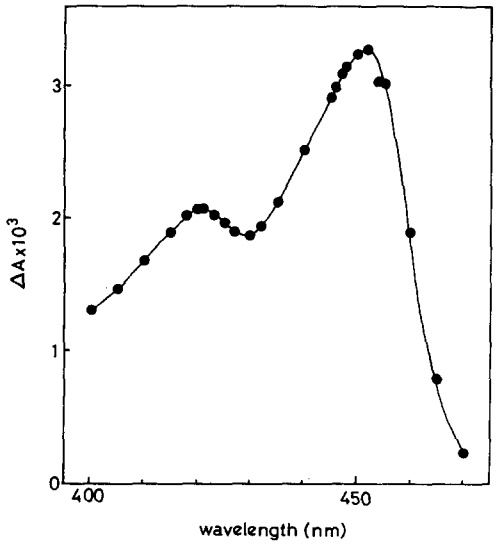

Fig. 5. Light-minus-dark difference spectrum on consecutive flashes. The absorbance differences between after first flash and before flash were measured at various wavelengths. Sample was the same as in Fig. 4.

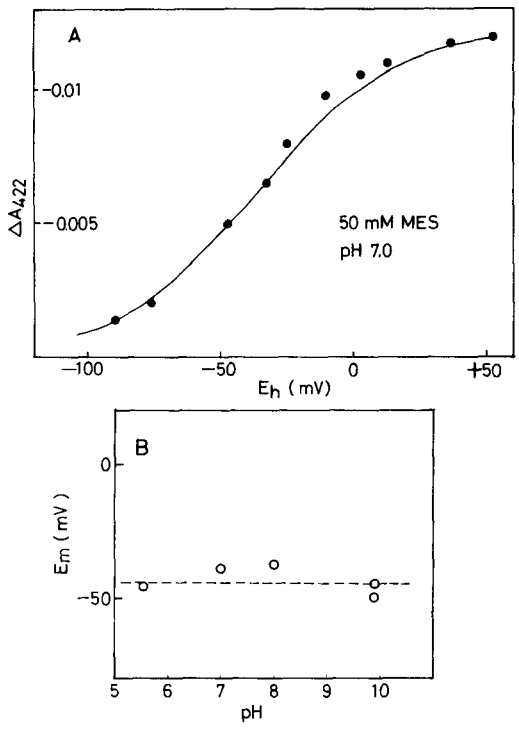

Fig. 6. (A) Redox titration of Q_A in the reaction center at pH 7.0. 0.5 μM reaction center was dissolved in the 50 mM morpholinoethanesulfonic acid buffer containing 0.1% Triton X-100. The extent of cytochrome oxidation by flash was measured at 422 nm. Titration was done in an anaerobic cuvette. Redox mediators used were 10 μM each of 2,3,5,6-tetramethyl-*p*-phenylenediamine, *N*-methylphenazonium methosulfate, *N*-ethylphenazonium ethosulfate and pyocyanine. The solid line is the theoretical Nernst curve with $n = 1$. (B) pH dependence of the midpoint potential of Q_A in the reaction center. Midpoint potential of Q_A was determined as in A at various pHs.

of flash-induced oxidation of cytochrome *c*-554 at various ambient potentials. Titration at pH 7.0 fit the theoretical Nernst curve with $n = 1$ with E_m value of −39 mV (Fig. 6A). There was no pH dependence of the midpoint potential between pH 5.5 and 9.9 (−44 mV, Fig. 6B). The pH independence of Q_A midpoint potential was in contrast to the pH-dependent midpoint potential of Q_A in the chromatophore membranes from the same bacterium [10]. The similar relationship of the Q_A midpoint potentials between chromatophores and isolated reaction center has been observed in the cases of purple photosynthetic bacteria [22,23], although reasonable explanations for this phenomenon have not been given yet. The midpoint potential of Q_A was slightly higher in the chro-

matophores of *Erythrobacter* than that of Q_A in the chromatophores from the traditional photosynthetic bacteria, but the midpoint potential of Q_A in the *Erythrobacter* reaction center was essentially the same as that of Q_A in the reaction center from purple photosynthetic bacteria.

Discussion

In spite of the physiological characteristics of *Erythrobacter* OCh 114 [2,4,8,9], reaction centers from this bacterium were very similar to that from the traditional purple photosynthetic bacteria with respect to absorption and difference spectra, subunit composition and characteristics of photochemical reaction in the reaction center complex. Present results indicated that the photochemical machinery of this bacterium was exactly the same as that of purple photosynthetic bacteria. This situation seems to be applied to other aerobic photosynthetic bacteria because light-minus-dark difference spectra of other aerobic photosynthetic bacteria had similar shape to that of the reaction center from *Erythrobacter* OCh 114 (Ref. 24 and Nishimura, M., personal communication). *Protaminobacter ruber* strain NR-1, another aerobic photosynthetic bacterium, is an orange-colored methylotrophic bacterium and its membrane fraction showed the reversible photooxidation of reaction center and photophosphorylation [24].

It is interesting that the midpoint potential of Q_A was slightly higher in chromatophores (35 mV at pH 7) from *Erythrobacter* than that of Q_A in chromatophores from the traditional purple photosynthetic bacteria [10], whereas the midpoint potential of Q_A in isolated reaction centers was essentially the same in both cases of *Erythrobacter* and of the traditional purple photosynthetic bacteria. Although we have no explanation why the midpoint potential of Q_A of this bacterium is slightly higher in the chromatophores than those of purple photosynthetic bacteria, we suggest that the high midpoint potential of Q_A is responsible for the inoperation of charge separation under anaerobic conditions. Under anaerobic conditions Q_A was easily reduced before flash activation, therefore no charge separation occurs [10].

In addition to the similarity of the photochemical machinery, a soluble *c*-type cytochrome which donates electrons to the photooxidized cytochrome *c*-554, cytochrome *c*-551 from this bacterium [25] had similar physicochemical properties and amino acid sequence to those of cytochrome c_2 and *Paracoccus denitrificans* cytochrome *c* (Okamura, K., unpublished data). These results suggest that *Erythrobacter* OCh 114 and purple photosynthetic bacteria have a common ancestor or *Erythrobacter* evolved from purple photosynthetic bacteria. In fact, Woese et al. [26] suggested by 16S rRNA analyses that *Erythrobacter longus*, another species of aerobic photosynthetic bacteria, was an isolated species of the α-subdivision in the phylogenic tree in purple bacteria. The α-3 subcluster of the α-subdivision contains two purple photosynthetic bacteria. From an evolutional point of view, aerobic photosynthetic bacteria may be the intermediates between strictly aerobic bacteria and strictly anaerobic photosyntheitc bacteria, as suggested by several authors [26–28]. And it might be said that aerobic photosynthetic bacteria locate closer to strict aerobic bacteria than the facultative anaerobic photosynthetic bacteria. In this respect, the photochemical machinery of aerobic photosynthetic bacteria seems to be only a 'left-over' of the evolutional processes.

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References

1 Sato, K. (1978) FEBS Lett. 85, 207–210
2 Harashima, K., Shiba, T., Simidu, U. and Taga, N. (1978) Agric. Biol. Chem. 42, 1627–1628
3 Nishimura, Y., Shimadzu, M. and Iizuka, H. (1981) J. Gen. Appl. Microbiol. 27, 427–430

4 Shiba, T., Simidu, U. and Taga, N. (1979) Appl. Environm. Microbiol. 38, 43–45
5 Harashima, K., Nakagawa, M. and Murata, N. (1982) Plant Cell Physiol. 23, 185–193
6 Okamura, K., Takamiya, K. and Nishimura, M. (1983) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. 1, pp. 641–644, Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, The Netherlands
7 Shimada, K., Hayashi, H. and Tasumi, M. (1985) Arch. Microbiol. 143, 244–247
8 Okamura, K., Mitsumori, F., Ito, O., Takamiya, K. and Nishimura, M. (1986) J. Bacteriol., in the press
9 Shiba, T. (1984) J. Gen. Appl. Microbiol. 30, 239–244
10 Okamura, K., Takamiya, K. and Nishimura, M. (1985) Arch. Microbiol. 142, 12–17
11 Takamiya, K. and Hanada, H. (1980) Plant Cell Physiol. 21, 979–988
12 Laemmli, U.K. and Favre, M. (1973) J. Mol. Biol. 80, 575–599
13 Thomas, P.E., Ryan, D. and Levin, W. (1976) Anal. Biochem. 75, 168–176
14 Takamiya, K. and Dutton, P.L. (1979) Biochim. Biophys. Acta 546, 1–16
15 Omata, T. and Murata, N. (1983) Plant Cell Physiol. 24, 1093–1100
16 Clayton, R.K. (1966) Photochem. Photobiol. 5, 669–677
17 Straley, S.C., Parson, W.W., Mauzerall, D.C. and Clayton, R.K. (1973) Biochim. Biophys. Acta 305, 597–609
18 Harashima, K. and Nakada, H. (1983) Agric. Biol. Chem. 47, 1057–1063
19 Bensasson, R. and Land, E.J. (1973) Biochim. Biophys. Acta 325, 175–181
20 Verméglio, A. (1977) Biochim. Biophys. Acta 459, 516–524
21 Wraight, C.A. (1977) Biochim. Biophys. Acta 459, 525–531
22 Dutton, P.L., Leigh, J.S. and Wraight, C.A. (1973) FEBS Lett. 36, 169–173
23 Wraight, C.A. (1981) Isr. J. Chem. 21, 348–354
24 Takamiya, K. and Okamura, K. (1984) Arch. Microbiol. 140, 21–26
25 Okamura, K., Kisaichi, K., Takamiya, K. and Nishimura, M. (1984) Arch. Microbiol. 139, 143–146
26 Woese, C.R., Stackebrandt, E., Weisburg, W.G., Paster, B.J., Madigan, M.T., Fowler, V.J., Hahn, C.M., Blanz, P., Gupta, R., Nealson, K.H. and Fox, G.E. (1984) System. Appl. Microbiol. 5, 315–326
27 Broda, E. (1975) in The Evolution of the Bioenergetic Processes, pp. 93–106, Pergamon Press, Oxford
28 Dickerson, R.E. (1980) Sci. Am. 242, 98–110