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THE PHOTOREACTION CENTER OF *RHODOSPIRILLUM RUBRUM* MUTANT STRAIN F24.1

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Summary

Rhodospirillum rubrum strain F24.1 is a spontaneous revertant of nonphototrophic mutant F24 derived from wild-type strain S1. Strain F24 shows no detectable photochemical activity and contains, at most, traces of the photoreaction center polypeptides. Strain F24.1 has a phototrophic growth rate close to that of the wild-type strain (Picorel, R., del Valle-Tascón, S. and Ramírez, J.M. (1977) Arch. Biophys. Biochem. 181, 665—670) but shows little photochemical activity. Light-induced absorbance changes in the near-infrared, photoinduced EPR signals and ferricyanide-elicited absorbance changes indicate that strain F24.1 has a photoreaction center content of 7—8% as compared to strain S1. Polyacrylamide gel electrophoresis of isolated F24.1 chromatophores shows the photoreaction center polypeptides to be present in amounts compatible with this value. Photoreaction center was prepared from strain F24.1 and showed no detectable difference with that of strain S1. It is concluded that strain F24.1 photosynthesis is due entirely to its residual 7—8% of typical photoreaction center.

Introduction

Various mutants carrying specific defects in their photosynthetic system have been isolated from the Rhodospirillaceae (see Refs. 1 and 2 for recent

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Abbreviations: *P*-800 and *P*-870, the photoreaction center bacteriochlorophylls with absorption bands near 800 and 870 nm, respectively; *B*-880, antenna bacteriochlorophyll with an absorption band near 880 nm.

reviews). Several of those strains are still photochemically competent because mutation has affected only secondary electron transfer [2,3], while others lack photoreaction center altogether. Mutants of the latter group obtained from *Rhodopseudomonas sphaeroides* have made an important contribution to our understanding of the role and structure of the photoreaction center [4—6]. We recently reported the isolation of similar nonphototrophic mutants from *Rhodospirillum rubrum* [7]. From one of those mutants, strain F24, we obtained a spontaneous revertant, F24.1, which grew phototrophically at a rate close to that of the wild type but which showed no evidence of *P*-800 or of any photochemical activity. This led to the suggestion that this strain contains a photoreaction center carrying an alteration, perhaps of *P*-800 [7]. However, it is also possible that photosynthesis in the revertant is due to a small residue of unaltered reaction center which we could not detect in the previous studies [7]. The present work was aimed at discerning between those alternatives.

Our findings are that isolated chromatophores of strain F24.1 contain a small but significant amount of photoreaction center with spectral and electrophoretic properties that are indistinguishable from those of similar preparations from wild-type strain S1. According to all appearances, this small amount of photoreaction center is sufficient to permit the relatively high growth rate of this strain.

Materials and Methods

The isolation of mutant derivatives F24 and F24.1 from wild-type strain S1 of *R. rubrum* has been described before [3,7]. Lascelles [8] growth medium used in the cultures was supplemented with 2 g/l yeast extract. Unless otherwise indicated, the phototrophic strains (S1 and F24.1) were grown under photoanaerobic conditions and nonphototrophic strain F24 was cultured in the dark under low oxygen tension [9]. Cells were collected by centrifugation at 0—2°C, when the cultures reached 1.5—1.8 mg dry weight of cells per ml and contained between 2 and 4 nmol bacteriochlorophyll per mg dry weight. Chromatophores were extracted by grinding the cells with alumina powder [10]. The bacteriochlorophyll content of chromatophores was estimated from the absorbance at the peak near 880 nm, using an extinction coefficient of 153 $mM^{-1} \cdot cm^{-1}$ [11]. Photoreaction center was isolated from strain S1 by the method of Noël et al. [12] as modified by Vadeboncoeur et al. [13]. Possibly because of its small amount in that strain, photoreaction center from strain F24.1 was found to be more easily prepared with the method developed for strain G9 [13]. For the same reason, such preparations were still contaminated by other membrane proteins, as shown by sodium dodecyl sulfate gel electrophoresis. Further purification was obtained by precipitating the DEAE-cellulose column eluate (in 10 mM Tris-HCl, pH 7.5) with 35% saturated ammonium sulfate. This step was carried out as described in Ref. 13. The polypeptide constituents of chromatophores and of photoreaction center preparations were resolved by polyacrylamide gel electrophoresis in the presence of urea and sodium dodecyl sulfate [14].

Absorption spectra were obtained with a Hitachi spectrophotometer (model 356) and stored in a digital signal averager (Tracor Northern NS-570) which

also performed baseline substraction. Light-induced absorbance changes in the near-infrared were monitored with the same spectrophotometer, except for the changes at 1245 nm for which a Cary 14R spectrophotometer was used. In the Hitachi spectrophotometer, actinic illumination (120 $W \cdot m^{-2}$) was provided by a slide projector with a broad-band interference filter (Balzers K3). The photomultiplier tube was shielded from scattered light with complementary narrow band interference filters (Balzers BIR and Schott PIL-1). In the Cary 14R spectrophotometer, the phototube was shielded from scattered actinic light by a Baird Atomic 1ϕ8444 interference filter. All assays were performed with saturating actinic light.

The EPR spectra were recorded on a Bruker Spectrospin, model B-ER-418, operating in the X band at 9.7 GHz, equipped with a rectangular cavity of the TE 102 type. Modulation frequency of the magnetic field was 100 kHz and microwave power was 4 mW. The sample was in a (300 μl) rectangular quartz cell. A No 1180 Nicolet computer with a LAB11W program was used to improve the signal-to-noise ratio and to substract the dark baseline. Illumination was provided by a slide projector through 5 cm of water and Balzers Kristal-glässer JR-filter. Light intensity was saturating.

Fluorescence induction curves were obtained with the following arrangement. The chromatophore suspension in a 1 cm $\times$ 1 cm cuvette was illuminated by the collimated beam of a 450-W xenon arc through 5 cm of water, a Corning 9-54 glass filter and an Ealing electrochemical shutter. Fluorescence was monitored at 90° through a Schott RG 10 cut-off filter by a Hamamatsu R663 photomultiplier tube run at 900 V. After suitable amplification, the signal was displayed on the screen of a Tektronix D11 storage oscilloscope.

Results

The absorption spectrum of chromatophores from *R. rubrum* shows two distinct near-infrared bands: a major band centered around 880 nm (its exact location depends on the particular strain), due mainly to light-harvesting bacteriochlorophyll, and a lesser band near 800 nm corresponding to *P*-800 of the photoreaction center [5]. The absence of most of the latter spectral constituent in strains F24 and F24.1 [7] indicates that both mutants carry phenotypic alterations in their photoreaction center.

Because of its high growth rate [7] and despite its apparent lack of *P*-800, strain F24.1 would be expected to show light-elicited signals which would reflect the operation of its photoreaction center. We set out to check this possibility by different methods. Photoinduced spectral changes in isolated chromatophores was the first of these methods. When the chromatophore suspension (F24.1) was placed far from the photomultiplier tube in the Hitachi spectrophotometer, interference from fluorescence was minimized and a small but reproducible light-induced change could be detected. The light-minus-dark difference spectrum of chromatophores from strain F24.1 shows the typical reversible bleaching and blue-shift of *P*-870 and *P*-800 that are associated with photoreaction center activity in strain S1 (Fig. 1). However, another optical change centered at 905 nm, an absorbance increase which probably reflects a bathochromic shift of the far red band [15], can also be observed. The light-

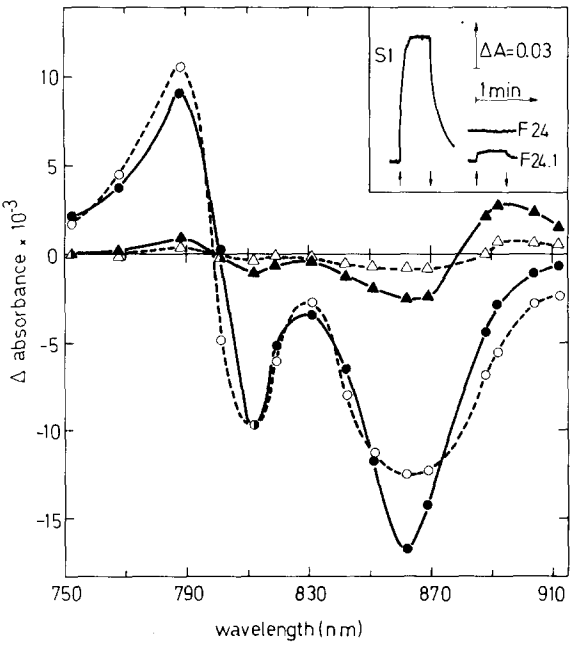


Fig. 1. Absorbance changes induced by continuous light in chromatophores from strains S1 and F24.1. Suspensions in 50 mM NaOH-Tricine (pH 8.0); A_{880} = 0.8. Circles, strain S1; triangles, strain F24.1; solid lines, no additions; dashed lines, with 5 μM gramicidin D. Insert (upper right). Kinetics of the change at 1245 nm; A_{880} = 20; light was turned on (↑) and off (↓) when indicated.

induced changes are greatly reduced by gramicidin D (Fig. 1) indicating that they are due mainly to an energized state of the membrane rather than to the photoreaction center itself [15]. The bathochromic shift also occurs in wild-type chromatophores, but is a comparatively much smaller fraction of the total

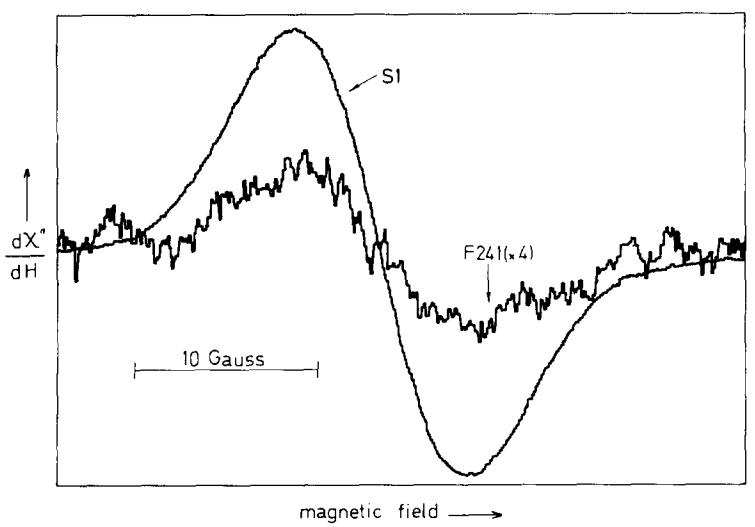


Fig. 2. Light-induced EPR spectra (g = 2.0026) of chromatophores from strains S1 and F24.1 at 20°C. Suspensions in 50 mM NaOH-Tricine (pH 8.0); A_{880} = 60.

light-induced change (Fig. 1). No light-induced signal was observed in non-phototrophic strain F24 (not shown). When assayed in the presence of gramicidin D, *P*-870 in strain F24.1 amounts to about 8% of that found in wild-type chromatophores.

However, the relatively high amplitude of the electrochromic shift found in strain F24.1 cast some doubts in our minds on the reliability of that estimate as an index of its photoreaction center level. To avoid this difficulty, we turned to the 1250 nm region of the spectrum, where the oxidized primary electron donor has a characteristic band and where absorption by antenna pigments is minimal. The extent of the light-induced change at this wavelength in F24.1 was about 7% of that observed in S1 chromatophores. No signal was observed with chromatophores from strain F24 (insert, Fig. 1).

The data reported above seem to indicate that strain F24.1 contains a small amount of photooxidizable primary donor. Since they were obtained by optical methods, we thought it desirable to complement these data by an independent technique, namely EPR. Fig. 2 shows the light-induced $g = 2.0026$ signals obtained in chromatophore suspensions from strain S1 and from strain F24.1. The relative peak-to-peak amplitude of these signals is consistent with the optical data: in strain F24.1, it is about 8% of that of the wild type. No light-induced signal was observed in chromatophores from strain F24. Another important point is that the peak-to-peak derivative linewidth of these spectra is the same in strain F24.1 as in the wild type, about 9.5 G. This falls well within the range of previously reported values [16—18].

The signals induced by continuous illumination reflect the difference between the levels of oxidized primary donor in the light and in the dark steady states. Since that difference may not correspond to the total levels of primary donor — because the extent of its photooxidation may be limited by the availability of another constituent, such as an electron acceptor — we analyzed also the spectral changes which are elicited by oxidants in the dark. The spectra shown in Fig. 3 indicate that the *P*-800 shifts which are induced by potassium ferricyanide in revertant and wild-type chromatophores keep the same ratio as the light-induced changes. Therefore, it may be concluded that the residual photoreaction center levels of strain F24.1 are about 7—8% of those present in the wild-type strain. The spectra beyond 840 nm (not shown) could not be used for quantitative analysis because, in addition to *P*-870 bleaching, the oxidant elicited reversible decoloration of a part of antenna bacteriochlorophyll. Since this latter change represented a large fraction of the total change in F24.1 chromatophores, *P*-870 could not be reliably estimated.

Fig. 4 shows the induction curves of fluorescence emitted by antenna bacteriochlorophyll in chromatophores of the three strains used in this work. Since the photoreaction center quenches excitation of antenna bacteriochlorophyll [19,20], the high intensity of the fluorescence emitted by strains F24 and F24.1 may be explained by the low photoreaction-center content of both mutants. The ratio of fluorescence intensities when all the traps are closed is about 3.4—3.5 (F24/S1). This value is comparable to that obtained in chromatophores of *Rps. sphaeroides* strain PM8/strain Ga [19]. It is noteworthy also that, whereas no fluorescence induction is observed in strain F24 (cf. Ref. 19), its relative extent in strain F24.1 is consistent with the occurrence of a small

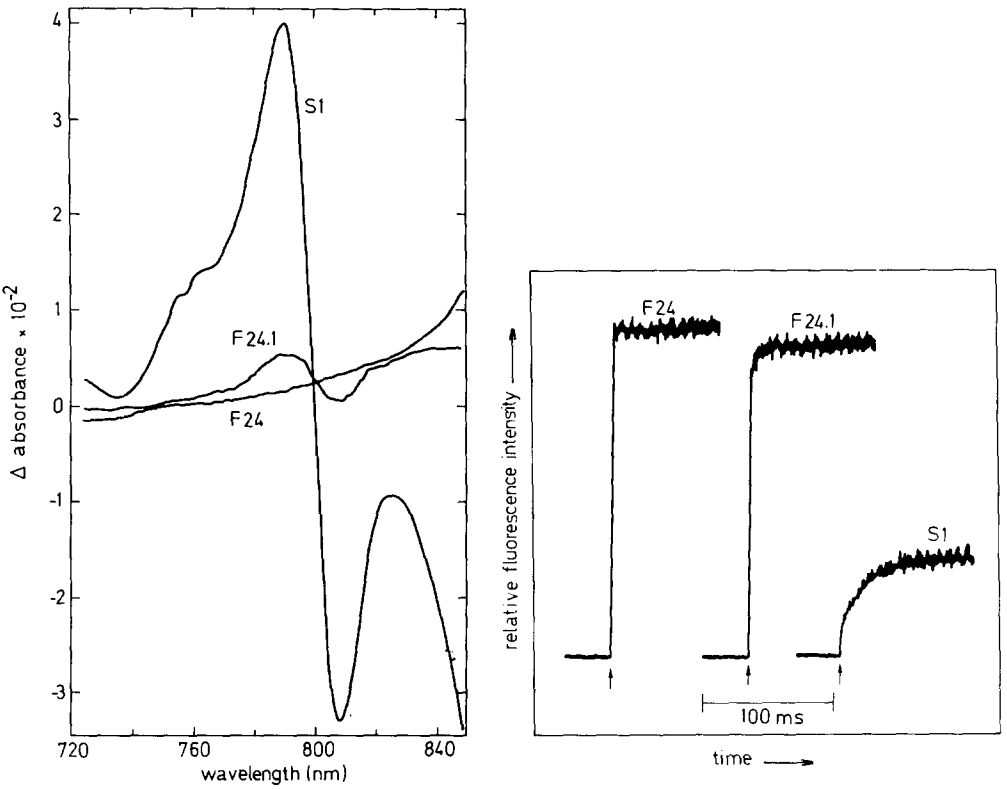
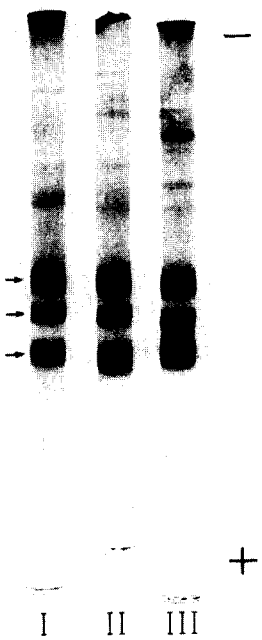


Fig. 3. Reversible absorbance changes induced by potassium ferricyanide in chromatophores from strains S1, F24 and F24.1. 2-ml aliquots of the suspension (A_{880} = 3) in 50 mM sodium phosphate (pH 7.0) were placed in each (the sample and the reference) cuvette. 15 µl of a 0.5 M ferricyanide/ferrocyanide mixture (50/1) were added to the sample and 15 µl of water to the reference. After 10 min in the dark, a first spectrum was scanned. Then, 15 µl of 1.5 M sodium ascorbate and 15 µl of water added to the sample and reference suspensions, respectively. A second spectrum was scanned 10 min later and used as baseline.

Fig. 4. Fluorescence induction curves in chromatophores from strains S1, F24 and F24.1. Suspensions in 50 mM NaOH-Tricine (pH 8.0); A_{880} = 0.1. The arrows indicate the turning on of the exciting light.

but distinct amount of photoreaction center [21].

The low level of photoreaction center in strain F24.1 and its complete absence from strain F24 may receive two types of interpretation: (1) the photoreaction center protein is present in the membrane but in partially (F24.1) or completely (F24) inactivated form; (2) an unaltered photoreaction center protein is present in low amounts (F24.1) or is absent (F24) from the membrane. We therefore performed polyacryalmide gel electrophoresis of the same quantities of chromatophores from strain S1, F24 and F24.1, as estimated on a bacteriochlorophyll basis. The gels of strains F24 and F24.1 showed no detectable bands or very faint ones at the level of the characteristic three polypeptides of strain S1 (not shown). The mutants either contain low levels of the three polypeptides or cannot incorporate these constituents into the membrane. They are to be compared, therefore, to *Rps. sphaeroides* mutants such as PM-8 [6]. For comparison purposes, we next set out to isolate



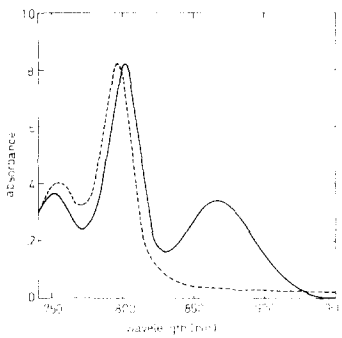


Fig. 5. Polyacrylamide gel electrophoresis of purified photoreaction centers from strains S1 and F24.1. I, S1 photoreaction center; II, S1 + F24.1 photoreaction center; III, F24.1 photoreaction center. Arrows indicate the three characteristic polypeptides.

Fig. 6. Near-infrared absorption spectra of photoreaction center purified from strain F24.1. Solid line, spectrum recorded without actinic light; dashed line, spectrum recorded under actinic light.

the photoreaction center of strain F24.1. Because of its small quantities in that strain, its purification required a few modifications of our usual procedure (Materials and Methods) including the solubilization of larger quantities of chromatophores. Polyacrylamide gel electrophoresis showed no difference in the migration pattern of the three typical polypeptides even when the preparations from strain F24.1 and from strain S1 were co-electrophoresed (Fig. 5). Fig. 6 shows the near-infrared absorption spectrum of the photoreaction center from strain F24.1: it is essentially identical with that of strain S1.

Discussion

The phenotypic properties of strain F24 indicate that it is a photoreaction-centerless mutant similar to those obtained from *Rps. sphaeroides* and *Rps. capsulata* in other laboratories [4,22,23]. To our knowledge, F24 is the first *R. rubrum* strain of this kind to be reported. The simultaneous absence of any photoreaction center and of the 800 nm band in chromatophores of strain F24 is a strong indication that this spectral component of *R. rubrum* is identical with *P*-800 of the photoreaction center. In fact, the absorption spectrum of strain F24 chromatophores can serve as a baseline to determine the *P*-800/*B*-880 ratio in chromatophores from strain S1. Using the extinction coefficients of these pigments [11,24] we obtain a value of about 1/30, which is in sub-

stantial agreement with the ratio for photoreaction center/*B*-880 measured by other methods (see Refs. 25 and 26, for example). Our interpretation is consistent with the results obtained with photoreaction-center depleted chromatophores [27,28] or with antenna-bacteriochlorophyll protein complexes [29] but is at variance with the observation by Sauer and Austin [30] of a small but distinct 800 nm band in such complexes.

In contrast to strain F24, its spontaneous phototrophic revertant, F24.1, contains low but significant levels of photoreaction center. All the methods we have used give or are consistent with an estimate of about 7—8% photoreaction center with respect to the wild type on a bacteriochlorophyll basis. Moreover, the photoreaction center of strain F24.1 carries no obvious features that distinguish it from that of the wild type. Absorption spectra of the reduced and oxidized forms of the primary donor are, to all purposes, identical. The same is true of EPR spectra the linewidth of which is about 9.5 G as previously reported by others for wild-type strains [16—18]. The size and the relative amounts of the three polypeptides also appear to be the same. Thus the only obvious effect of mutation is the considerable decrease of the photoreaction center/*B*-880 ratio, a parameter which in wild-type *R. rubrum* remains unchanged under diverse growth conditions [25]. It remains to be seen whether or not mutation is accompanied by some more subtle difference in the primary structure of the photoreaction-center protein or with alterations of some other constituents. Such knowledge might give us some clues as to the site and mechanism of these mutations.

Considering that the revertant significantly contains more photoreaction center than strain F24, from which it was selected for restoration of phototrophic growth only, and that we have found no evidence for another photoreaction center in strain F24.1, we feel that its photosynthesis is due entirely to its residual 7—8% of typical photoreaction center. This, however, raises the question of how such a small amount of photoreaction center can sustain a phototrophic growth rate close to that of wild-type strain S1 [7]. We have preliminary evidence suggesting that electron flux is of comparable magnitude in the transport chains of both strains. In the first place, the effective size of the photosynthetic unit of strain F24.1 seems to be much larger than that of strain S1. And secondly, the reduction of the photo-oxidized primary donor takes place with a shorter half-time in F24.1 than in the wild-type strain [31], so that its photoreaction center may have a faster turnover under continuous illumination.

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References

1 Saunders, V.A. (1978) Microbiol. Rev. 42, 357—384
2 Marrs, B.L. (1978) Curr. Topics Bioenerg. 8, 261—294

3 Del Valle-Tascón, S., Giménez-Gallego, G. and Ramírez, J.M. (1975) Biochem. Biophys. Res. Commun. 66, 514—519
4 Sistrom, W.R. and Clayton, R.K. (1964) Biochim. Biophys. Acta 88, 61—73
5 Clayton, R.K. and Sistrom, W.R. (1966) Photochem. Photobiol. 5, 661—668
6 Clayton, R.K. and Haselkorn, R. (1972) J. Mol. Biol. 68, 97—105
7 Picorel, R., del Valle-Tascón, S. and Ramírez, J.M. (1977) Arch. Biochem. Biophys. 181, 665—670
8 Lascelles, J. (1956) Biochem. J. 62, 78—93
9 Giménez-Gallego, G., del Valle-Tascón, S. and Ramírez, J.M. (1976) Arch. Microbiol. 109, 119—125
10 Del Valle-Tascón, S. and Ramírez, J.M. (1975) Z. Naturforsch. 30c, 46—52
11 Clayton, R.K. (1966) Photochem. Photobiol. 5, 669—677
12 Noël, H., van der Rest, M. and Gingras, G. (1972) Biochim. Biophys. Acta 275, 219—230
13 Vadeboncoeur, C., Noël, H., Poirier, L., Cloutier, Y. and Gingras, G. (1979) Biochemistry 18, 4301—4308
14 Van der Rest, M., Noël, H. and Gingras, G. (1974) Arch. Biochem. Biophys. 164, 285—292
15 Barsky, E.L. and Samuilov, V.D. (1973) Biochim. Biophys. Acta 325, 454—462
16 Androes, G.M., Singleton, M.F. and Calvin, M. (1962) Proc. Natl. Acad. Sci. U.S.A. 48, 1022—1031
17 Kohl, D.H., Townsend, J., Commoner, B., Crespi, H.L., Dougherty, R.C. and Katz, J.J. (1965) Nature 206, 1105—1110
18 Norris, J.R. and Katz, J.J. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 397—418, Plenum, New York
19 Heathcote, P. and Clayton, R.K. (1977) Biochim. Biophys. Acta 459, 506—515
20 Godik, V.I. and Borisov, A.Yu. (1977) FEBS Lett. 82, 355—358
21 Clayton, R.K. (1966) Photochem. Photobiol. 5, 807—821
22 Takemoto, J. and Lascelles, J. (1974) Arch. Biochem. Biophys. 163, 507—514
23 Yen, H.-C. and Marrs, B. (1977) Arch. Biochem. Biophys. 181, 411—418
24 van der Rest, M. and Gingras, G. (1974) J. Biol. Chem. 249, 6446—6453
25 Aagaard, J. and Sistrom, W.R. (1972) Photochem. Photobiol. 15, 209—225
26 Oelze, J. and Pahlke, W. (1976) Arch. Microbiol. 108, 281—285
27 Oelze, J. and Golecki, J.R. (1975) Arch. Microbiol. 102, 59—64
28 Cuendet, P.A. and Zuber, H. (1977) FEBS Lett. 79, 96—100
29 Drachev, L.A., Frolov, V.N., Kaulen, A.D., Kondrashin, A.A., Samuilov, V.D., Semenov, A.Yu. and Skulachev, V.P. (1976) Biochim. Biophys. Acta 440, 637—660
30 Sauer, K. and Austin, L.A. (1978) Biochemistry 17, 2011—2019
31 Picorel, R., Mar, T. and Gingras, G. (1979) Abstracts 11th International Congress of Biochemistry, Toronto, p. 432.