Biochimica et Biophysica Acta, 682 (1982) 354–363
Elsevier Biomedical Press

BBA 41203

PHOTOSYNTHETIC UNIT SIZE AND ELECTRON-TRANSPORT CHAIN IN A PHOTOREACTION CENTER-DEPLETED MUTANT OF *RHODOSPIRILLUM RUBRUM*

TED MAR, RAFAEL PICOREL and GABRIEL GINGRAS

Département de Biochimie, Université de Montréal, C.P. 6128 A, Montréal, Québec, H3C 3J7 (Canada)

(Received March 4th, 1982)

Key words: Reaction center; Bacterial photosynthesis; Electron transport; (R. rubrum)

The aim of this work was to explain the relatively fast growth of a mutant of *Rhodospirillum rubrum* (F24.1) which contains 7–8% of an apparently normal photoreaction center. We explored the double hypothesis that the size of its photosynthetic unit is larger than that of the wild type and that its electron-transport chain is organized in a network rather than in isolated loops. The first feature would allow faster growth under less than saturating light intensities and the second would allow faster maximal electron fluxes than would be predicted from the photoreaction center content. With respect to the first possibility, measurements of absorbance changes at 793 nm induced by short flashes of increasing intensity indicate that the photosynthetic unit of strain F24.1 is 5.6-fold larger than that of strain S1. The second possibility was verified by measuring relative electron fluxes at the photoreaction center in the two strains. This was established in the steady state from the amount of primary donor oxidized by a continuous light beam of increasing intensity. This electron flux was found to be about 70% as high in strain F24.1 as in strain S1. A more detailed study of the electron-transport chain indicated that cytochrome c_2 is by far the main secondary electron donor in strain F24.1. No evidence could be obtained for the existence of another secondary donor in that strain. The mole ratio of cytochrome c_2 to photoreaction center is about 6 in strain F24.1 as conpared to about 0.5 in strain S1. In strain 24.1, the pool of secondary donor appears to be collectively involved in the reduction of the oxidized primary donor. The replacement time at the photoreaction center of a first equivalent of oxidized cytochrome c_2 by a second equivalent of reduced cytochrome c_2 is less than or equal to 0.2 ms. The effect of the photoreaction center content on the size of the photosynthetic unit is discussed in terms of the different models proposed for the organisation of the photosynthetic unit. We propose that the electron-transport chain is organized in a network, perhaps by virtue of the lateral mobility of some of the electron carriers such as ubiquinone and cytochrome c_2.

Introduction

A strain (F24) of *Rhodospirillum rubrum* was recently described which is entirely devoid of photoreaction center and is therefore nonphototrophic [1]. From this parent strain, a spontaneous revertant, strain F24.1, was also obtained [1] which contains only about 7–8% of an apparently normal photoreaction center [2]. In spite of this small amount of photoreaction center, the photosynthetic growth rate of strain F24.1 has been reported to be comparable to that of the wild-type strain S1 [1]. These observations raised the problem of how such a small amount of photoreaction center can sustain a quasi-normal photosynthetic growth in the revertant. This problem was intrigu-

Abbreviations: HOQNO, 2-(*n*-heptyl)-4-hydroxyquinoline *N*-oxide; DCMU, 3-(3,4 dichlorophenyl)-1,1-dimethylurea; BChl, bacteriochlorophyll.

0005-2728/82/0000–0000/$02.75

ing, since there must be a selective advantage for strain S1 in possessing 12-times as much photoreaction center as strain F24.1.

In order to explain the relatively high growth rate of strain F24.1, we imagined two types of mechanisms which could possibly coexist. In the first one, the size of the photosynthetic unit would be increased because the smaller number of photoreaction centers would be served by an approximately constant number of BChl molecules. This adaptative response would insure a faster functioning of the electron-transport chain at low light intensity and thereby, presumably, a higher growth rate. At saturating light intensity, the maximal rate of electron transport is not determined by the photochemistry but rather by the molecular or supramolecular structure of the dark components of the electron-transport chain. We reasoned that if the electron-transport chain was assembled not purely in a linear sequence but rather as a network in which several elementary loops were interconnected, the result would be a higher electron-carrying capacity than possible if the electrons coming from one photoreaction center were confined to a single elementary loop. In this model, then, sufficiently high light intensities might be able to compensate for the lack of photoreaction centers, thus sustaining high electron fluxes. These are the two hypotheses that we set out to verify in this work.

The electron-transport chain of *R. rubrum* is known to be largely cyclic and to involve, besides the photreaction center, at least one *b*-type cytochrome, cytochrome *c*-560, and one *c*-type cytochrome, cytochrome *c*-420 [4,3]. No definite function has been attributed to the other two cytochrome *b* species that are known to be present in this organism [5,6]. It is generally agreed that cytochrome c_2 (*c*-420) donates electrons directly to the primary electron donor of the photoreaction center [7,8]. Strong evidence for this view was provided by the careful kinetic study carried out in whole cells by Van Grondelle et al. [4]. According to these authors, cytochrome *c*-420 donates electrons to 95% of the photoreaction center, the other 5% being reduced by cytochrome *c*-428, another *c*-type cytochrome. The major and minor components of the photoreaction center appear to be served by antennas of 30 and 300 BChl molecules, respectively. One hypothesis that we envisaged here is that strain F24.1 contains only this minor component.

In contrast to other Rhodspirillales such as *Rhodopseudomonas sphaeroides* [9] or *Chromatium vinosum* [10–12], in *R. rubrum* the pool of reduced cytochrome c_2 is smaller than that of photoreaction center. Van Grondelle et al. [4] reported, in fact, a mole ratio of 0.5 cytochrome *c*-420 per photoreaction center. They also reported a mole ratio of 1/6 cytochrome *c*-560 per photoreaction center. Since strain F24.1 is probably the result of a point mutation affecting the photoreaction center, it would be expected – if we discount possible pleiotropic effects – to contain a normal complement of cytochromes. This would entail different relative pool sizes of cytochromes to photoreaction center. Inasmuch as this point was directly relevant to our 'network hypothesis', we attempted in the present work to verify several of the photoreaction center-cytochrome c_2 relationships along with the main hypotheses presented above. This work has been presented elsewhere in preliminary form [13].

Materials and Methods

Strains S1 (ATCC No. 1170) and F24.1 of *R. rubrum* were cultivated anaerobically in the light for 24 h in the medium of Lascelles [14]. Strain F24.1 was grown in the light following a single transfer from a dark culture. The cells were collected by centrifugation and resuspended in a 2 : 1 (v/v) mixture of growth medium and Ficoll 400 (Pharmacia Fine Chemicals) in order to minimize light scattering. The cell suspension (A_{880} = 0.8–1.0) was made anaerobic by 5 min of a moderate bubbling with nitrogen gas containing less than 3 ppm of oxygen. It was kept anaerobic by layering paraffin oil on top of the sample cuvette.

Spectral changes were observed with a domestic single-beam flash instrument. The analyzing light was provided by a 650 W tungsten-halogen lamp driven by a constant-voltage power supply (Kepco JQE75). Its wavelength was selected by a 500 mm Bausch and Lomb monochromator. The photomultiplier tube (E.M.I. 9558 B) was protected from stray actinic light by a 135 mm Bausch and Lomb monochromator and suitable cutoff filters placed after the sample. The analyzing beam was ad-

mitted to the sample just before the onset of the actinic flash by means of an Ealing electromechanical shutter. Pulsed actinic light was provided by a Stroboslave type 1539 (General Radio Co.) and/or dye laser (Candela SLL 66) with respective flash durations of 3 μs and 100 ns. Continuous actinic light from a 600 W tungsten-halogen lamp was transmitted to the sample through suitable cutoff filters and a light guide. The photomultiplier output was amplified (Keithley model 127) and stored in a Biomation 202S signal averager. Synchronisation was assured by a Digitimer (type 3290, Devices Instruments).

Results

a. Size of the photosynthetic unit

The photosynthetic unit may be defined as the average number of antenna BChl molecules which are associated with the one-electron oxidation of the primary electron donor. Its size, U, is given by $(BChl/P)\cdot\phi$, where $BChl$ is the total number of antenna BChl molecules, P the total number of photoreaction centers and ϕ the quantum yield of the primary photoreaction when light is absorbed by antenna BChl. Instead of attempting to determine the absolute photosynthetic unit size of strain F24.1, we estimated its relative size with respect to that of wild-type strain S1. To do this, we worked with cell suspensions of both strains adjusted to equal BChl concentrations and in which we measured the ratio of oxidized to total primary electron donor as a function of actinic light intensity. The actinic light consisted of flashes of short duration compared to the reduction time of oxidized primary donor. Fig. 1 shows the variation of $(\Delta A/\Delta A_{max})_{793}$ as a function of flash intensity. Comparing the initial slopes gives the relative size of the photosynthetic units: it is found to be 5.6-times larger in strain F24.1 than in strain S1. As a consequency, saturation occurs at lower light intensities in the revertant.

b. Electron-turnover rate and electron flux at the photoreaction center

Since the oxidation of the photoreaction center is monoelectronic [15,16], the number of electrons passing through one photoreaction center per unit

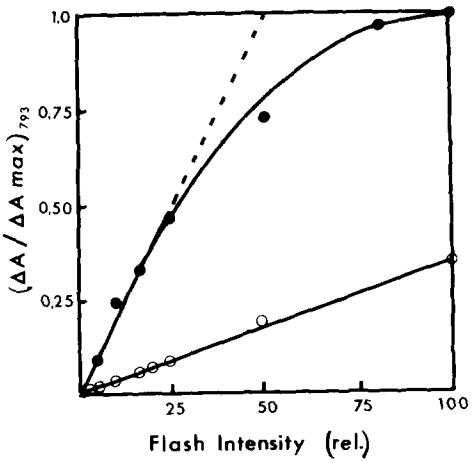

Fig. 1. Oxidation of the primary donor *R. rubrum* as a function of the relative light intensity of short flashes: F24.1 cells (●), S1 cells (○). The 3-μs flashes were given at intervals of 10 s. Each data point is an average of 64 experiments.

time, R, is given by:

$$R = I\cdot(U\cdot\epsilon)\cdot(P/P_t) \qquad (1)$$

where I is the number of incident photons per unit time, $(U\cdot\epsilon)$ the probability that the photosynthetic unit would capture a photon and (P/P_t) the probability of the photoreaction center undergoing photooxidation. U is the photosynthetic unit size and ϵ the extinction coefficient of BChl in vivo. P and P_t are the concentrations of reduced and of total photoreaction center, respectively. The ratio of the electron-turnover rates of strain F24.1 and strain S1 is given by:

$$(R_F/R_S) = (U_F/U_S)\cdot(P/P_t)_F\cdot(P_t/P)_S \qquad (2)$$

To measure the relative electron-turnover rates in the steady state, we illuminated the cell suspensions with a continuous beam of actinic light. This induced absorbance changes of two kinds at 793 nm, one due to the oxidation of the primary donor and another due to light-scattering changes (unpublished results). To single out the first, we measured the fraction of the primary electron donor that was not oxidized by the continuous actinic light. This was done by means of a saturating laser flash of 100 ns duration which bleached the remaining reduced primary electron donor.

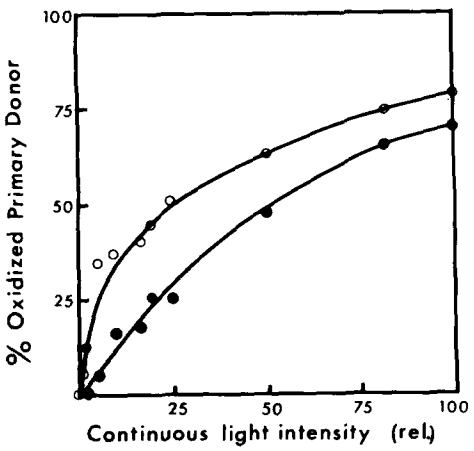

Fig. 2. Oxidation of the primary donor in *R. rubrum* as a function of the relative intensity of a continuous light beam: F24.1 cells (●), S1 cells (○). The ΔA_{793} produced by a saturating flash in the presence of continuous light of a given intensity was subtracted from the ΔA_{793} caused by the same flash alone. This difference divided by the ΔA_{793} elicited by a single saturating flash is the '% Oxidized Primary Donor' of the ordinate. Each data point is an average of 64 experiments using a 3 μs flash (strain F24.1) or of 8 experiments using a 100 ns flash (strain S1). The interval between the flashes was 10 s.

If the only difference between the two strains was the respective size of their photosynthetic units, the fraction of primary donor that is oxidized under weak continuous illumination should be greater in strain F24.1 than in strain S1. We obtain instead (Fig. 2) curves with a lower initial slope for strain F24.1 than for strain S1. Moreover, the shapes of these curves are different, indicating different rate-limiting steps in the two strains. If we now calculate the ratio of their electron turnover rates, R_F/R_S, according to Eqn. 2, we find that it varies from 6 at low light intensity to 9 at high light intensity. Thus, electron turnover is clearly faster in strain F24.1. However, it is total electron flux that is physiologically significant rather than the rate of electron turnover. The total electron flux is equal to the turnover rate at one photoreaction center times the total number of photoreaction centers. From the ratio of the electron-turnover rates, one finds that with only 7–8% of the photoreaction center content of strain S1, the maximal observed electron flux of strain F24.1 is about 60–70% that of the wild type. Discounting the remote possibility that electron transfer is faster in the mutant (see below), we think that this is strong support for the network model of elementary electron-transport chains.

c. Reduction kinetics of the primary electron donor

The faster turnover rate in strain F24.1 should be manifest also in the detailed kinetics of the electron-transport chain. Our attention was first focused on the reduction rate of the primary electron donor. Fig. 3 shows the reduction kinetics analyzed at 793 nm following a 100 ns flash of saturating intensity. Our first observation was that

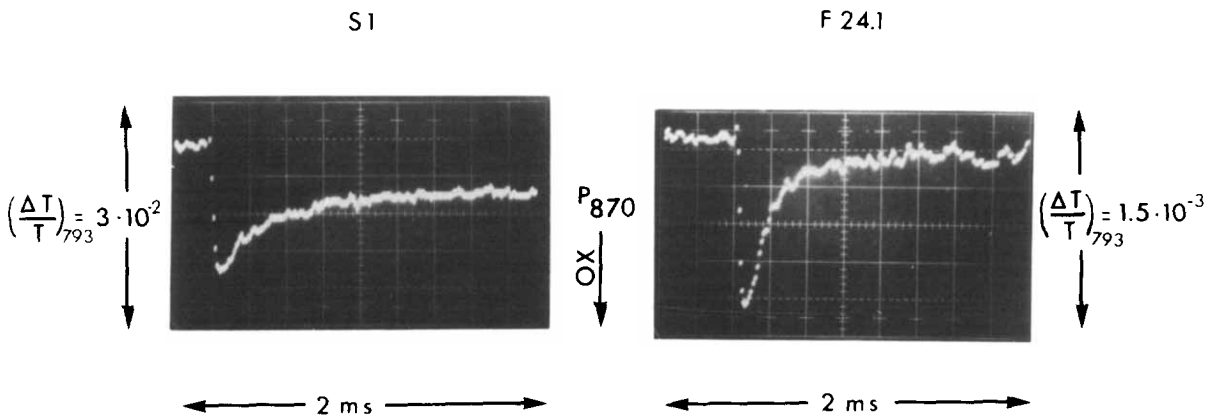

Fig. 3. Reduction kinetics of the primary donor in *R. rubrum* strains S1 and F24.1 after a single saturating flash. The flash regime and number of experiments were as in Fig. 2.

the photoreaction center content of strain F24.1 is about 7–8% of that of strain S1, in confirmation of earlier results obtained in the steady state [2]. It is apparent from Fig. 3 that the reduction kinetics are biphasic in both strains with a fast phase of about 0.1 ms half-lifetime. In strain S1, the slow phase has a half-life of about 20 ms. This slow component was not detected in strain F24.1; instead, a very slow component of several hundred milliseconds duration was apparent. The fast component accounts for about 50% of the recovery in wild-type cells but this proportion varies somewhat with the age of the culture. In strain F24.1, the fast component accounts for at least 90% of the recovery. We have not explored the nature of the very slow component.

Fig. 4 is another illustration of the recovery kinetics of the primary donor. After complete oxidation by a saturating flash, a second saturating flash can elicit further oxidation of the primary donor only inasmuch as it has been rereduced. According to Fig. 4, rereduction has a half-life longer than 10 ms in strain S1 but shorter than 0.6 ms in strain F24.1. Another important point is that the recovery kinetics after the second flash are slow in strain S1 but fast in strain F24.1. This behaviour is attributed to the fact that, while the fast component reflects the true oxidation rate of the prereduced fraction of secondary donor, the slow component reflects the reduction rate of oxidized secondary donor by the rest of the chain [4]. Accordingly, the results of Fig. 4 are consistent with the observations presented below (section e) that the mole ratio of secondary to primary donor is approx. 0.5 in strain S1 and 6 in strain F24.1.

d. The kinetics and stoichiometry of cytochrome c-420 and P-870 oxidoreduction reactions after a single flash

The next series of experiments was designed to identify the secondary electron donor in strain F24.1. A tempting hypothesis was that it might be cytochrome *c*-428 and, accordingly, that the photoreaction center remaining in strain F24.1 would be the small component described by Van Grondelle et al. [4]. In spite of a careful search, we were unable to find in the 428 nm absorbance change a kinetic component of any importance that would match the reduction kinetics of the primary electron donor.

We then turned our attention to cytochrome *c*-420. Our aim was to determine whether this cytochrome plays the same role in strain F24.1 as in strain S1 and, in this case, whether there might be other carriers donating in parallel with it. Our analysis was based on a comparison of the reduction rate of the primary electron donor as studied at 793 nm and of the oxidation rate of cytochrome as studied at 406 nm, an isosbestic wavelength for P-870. We also used the absorbance change at 419 nm which, in the first millisecond after the flash, is due almost exclusively to cytochrome *c*-420 and to

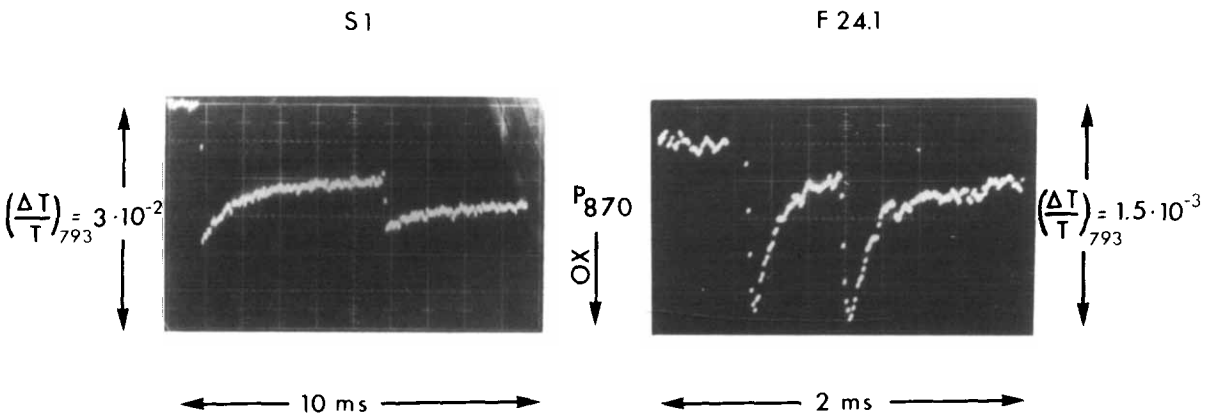

Fig. 4. Reduction kinetics of the primary donor of *R. rubrum* S1 and F24.1 in two consecutive-flashes experiments. The first and second flashes had respective durations of 3 μs and 100 ns. The traces obtained with strain S1 and with strain F24.1 are averages of 16 and 256 experiments, respectively.

the primary electron donor [4]. The oxidation kinetics of cytochrome *c*-420 matched the fast reduction phase of the primary donor: both had an exponential recovery with a half-life of about 0.1 ms. These observations are consistent with cytochromes c_2 being a secondary electron donor to P^+.

The nearly monophasic complementary oxidoreduction kinetics of P^+ and of cytochrome *c*-420 seem to imply that, in strain F24.1, cytochrome c_2 is the sole main electron donor to P^+. To verify this point, we determined the mole ratio of oxidized cytochrome *c*-420 per reduced P after a 3 μs single saturating flash. Using $\Delta\epsilon_{419}^{red-ox}$ of 43.4 $mM^{-1} \cdot cm^{-1}$ for cytochrome c_2 [17] and $\Delta\epsilon_{793}^{red-ox}$ of 95.3 $mM^{-1} \cdot cm^{-1}$ for photoreaction center [18], we obtain a ratio of 0.95 mol cytochrome c_2 oxidized per mol primary donor. This is to be compared with a molar stoichiometry of 0.5 reported by Van Grondelle et al. [4] for strain S1. Our own results with strain S1 indicate a stoichiometry varying between 0.3 and 0.6 depending on the age of the culture.

e. The relative pool sizes of cytochrome c_2 and of photoreaction center

Since strain F24.1 is probably the result of a point mutation affecting the photoreaction center, it may be expected to contain a normal complement of cytochromes. Moreover, if our network hypothesis is correct, all the cytochromes present in the membrane should be availbale for electron transport. We attempted to verify this hypothesis in the particular case of cytochrome *c*-420. In one such experiment, the cells were incubated in the presence of HOQNO – which blocks the reduction of cytochrome c_2 [19] – and were submitted to a sequence of saturating flashes separated by intervals of 100 ms. At the same time, we monitored the 419 nm absorbance change. The result obtained with strain F24.1 is shown in Fig. 5. As expected, the amount of oxidized cytochrome c_2 is seen to accumulate at each successive flash up to a maximum. No such buildup of oxidized cytochrome *c*-420 was observed with wild-type cells placed under the same experimental conditions (not shown). Since in cells of strain F24.1 the 419 nm absorbance change elicited by the first flash corresponds to the oxidation of one cytochrome

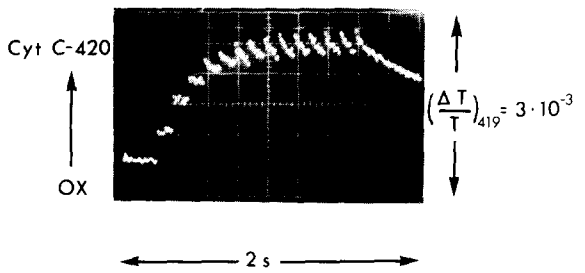

Fig. 5. Oxidation of cytochrome *c*-420 in *R. rubrum* F24.1 caused by a train of flashes in the presence of HOQNO. Each data point is the average of 64 experiments. The 3-μs flashes were given at intervals of 100 ms. Final concentration of HOQNO: 25 μM.

c-420 per photoreaction center, then the ratio of the maximal to the inital change should be a measure of the average total mole number of cytochromes that are available to reduce one oxidized primary electron donor. By this method, we find a mole ratio of 4 cytochromes *c*-420 per photoreaction center. This value is certainly underestimated. This is mainly due to the fact that at maximal accumulation of oxidized cytochrome *c*-420, the recovery of the 419 nm absorbance becomes too rapid to allow any further buildup with the flash frequency we use. We intend to study this phenomenon in subsequent work.

To obtain a more faithful estimate of the relative pool size of cytochrome *c*-420, we used another method. In this experiment, the maximal 419 nm absorbance change was followed as a function of the intensity of a continuous beam of actinic light interrupted by means of an electromechanical shutter. The contribution of the primary donor was subtracted (see legend to Fig. 6) from this signal to obtain the true contribution of cytochrome *c*-420. The ratio of this contribution to the absorbance change due to cytochrome *c*-420 after a single flash (section d) is a measure of the average mole ratio of total cytochrome *c*-420 available per primary electron donor. Under our experimental conditions, this value is 4.6. However, since the maximal intensity of the continuous light beam oxidized only 70% of the photoreaction center (see Fig. 2), this pool size must be corrected to 6.

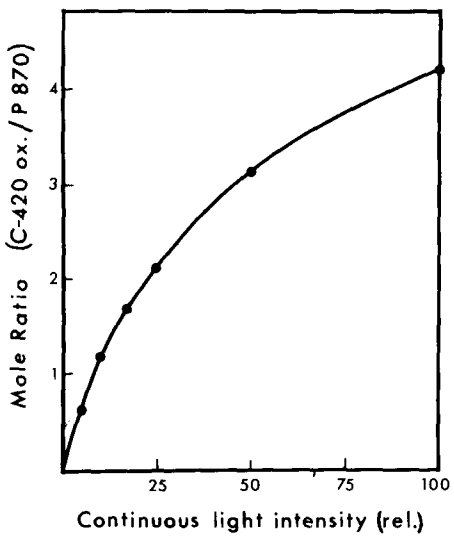

Fig. 6. Mole ratio of cytochrome *c*-420 oxidized per primary donor as a function of continuous light intensity in *R. rubrum* F24.1. This mole ratio was determined by dividing the amount of cytochrome *c*-420 oxidized in continuous light of a given intensity by the amount of cytochrome *c*-420 oxidized by a single saturating flash. The amount of cytochrome *c*-420 oxidized was obtained from the ΔA_{419} after correction for the participation of the primary donor. The extent of this participitation was taken to be the percentage of P-870 oxidized at each intensity (Fig. 2) multiplied by the ΔA_{419} due to the primary donor after a single saturating flash.

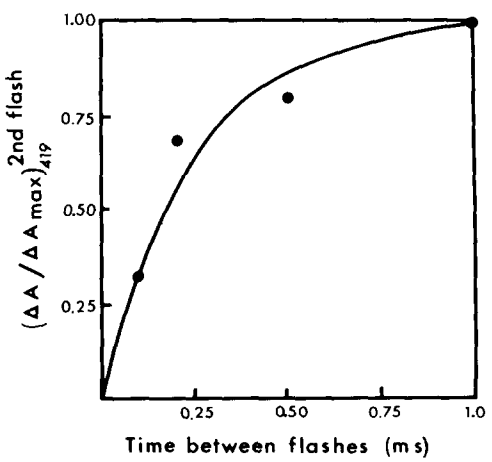

Fig. 7. Oxidation probability of a second equivalent of cytochrome *c*-420 as a function of the time interval separating first and second saturating flashes; *R. rubrum* F24.1. ΔA_{419} (max) was the absorbance change induced by a second flash given 100 ms after the first. The first and second flashes had respective durations of 100 ns and 3 μs. The groups of two flashes were separated by dark intervals of 10 s. Each point is the average of 512 experiments.

f. Replacement time of the first equivalent of reduced cytochrome c-420

The previous experiments have shown that the electron flux at the photoreaction center is of comparable magnitude in both strains S1 and F24.1 and that, in the latter, a pool of about 6 molecules of cytochrome c_2 is available for reduction of P^+. In order to gain a better idea of the organisation of this pool with respect to P, we sought to measure the replacement time of the first equivalent of cytochrome c_2 after its oxidation by a saturating light flash. In Fig. 7, we plotted the percentage of maximal change that is elicited by second flashes given at various time intervals after the first flash. The time required to obtain half of the maximal change is less than 0.2 ms. This half-replacement time is comparable to the reduction half-time of P^+ by cytochrome c_2.

Discussion

Our objective in this work was to understand the unexpectedly high growth rate of strain F24.1 with its 7–8% of the normal complement of photoreaction center. As outlined in the Introduction, our experiments were based on the double hypothesis that the photosynthetic unit is larger than normal and that the electron-transport chain is much faster than would be predicted from the photoreaction center content of the cells.

Size of the photosynthetic unit

The first hypothesis was confirmed by the finding that the photosynthetic unit of strain F24.1 is 5.6-times larger than that of strain S1 (Fig. 1). Hence, taking the photosynthetic unit of the wild type to contain 35 BChl molecules [20], we find, for strain F24.1, a size of about 200 BChl molecules. Since this increase is brought about by the selective removal of approx. 92% of the photoreaction center, we are forced to conclude that the photosynthetic unit of *R. rubrum* S1 is not an entity of predetermined size. Rather, it appears to result from the sharing of the antenna molecules among the photoreaction centers. This is consistent with the 'matrix' model of energy migration that has been shown to apply to strain S1 [21]. However, this sharing involves slightly less than one-half of the total antenna BChl in strain F24.1:

complete sharing in that strain would result in a photosynthetic unit of 400–500 BChl molecules. These results suggest that the photosynthetic unit of *R. rubrum* is approaching its maximal size in strain F24.1. In a series of *Chlamydomonas reinhardii* mutants deficient in the chlorophyll-protein complex CP I [22], extrapolation to a null content of CP I yields maximal Photosystem 1 unit size of 2.5/1 compared to the wild type.

Since the matrix model does not predict any limit to the average number of BChl molecules that serve a photoreaction center, it is tempting to assume that all the BChl is involved in efficient transfer. Our results indicate that this assumption is not valid: we find a limit of 200 BChl molecules per photosynthetic unit. This limit may be purelely statistical and due to the fact that, on average, only 200 BChl molecules are involved in efficient energy transfer. Another possibility is that the membrane contains physical structures resulting from a supramolecular association between antenna holochromes and photoreaction centers. These structures would contain 200 BChl molecules plus 6 photoreaction centers in the wild type or plus one photoreaction center in strain F24.1. In the latter, about one-half of the particles would either be lacking altogether or at least be without a photoreaction center. The postulated existence of such supramolecular complexes in vivo finds support in the isolation of 'phototraps' from chromatophores [23] and in the in vitro reassembly of isolated photoreaction centers and antenna BChl protein [24,25]. We do not have sufficient information to make a choice between these two possibilities.

Rate of electron transport

In strain F24.1, the maximal observed electron flux at the primary donor is about 70% of that of strain S1 (Fig. 2). This is consistent with the observation that strain F24.1 grows almost as fast as strain S1 [1]. Since the revertant contains only about 7–8% photoreaction center with respect to the wild type, this can only mean that the electron flux is not limited by the number of 'complete' electron-transport chains. Therefore, instead of forming isolated loops, the electron carriers must be organized in a network. A similar conclusion was also reached for the electron-transport chain of the chloroplast after inactivation by DCMU [26]. Most likely, the existence of such networks is due to the lateral diffusion within the membrane of one or several of the electron carriers [13]. This phenomenon has recently been well documented in the case of the elctron-transport chain of the mitochondrial inner membrane [27].

Among the carriers that are most likely to have lateral mobility in the membrane are ubiquinone [27,28] and cytochrome c_2. In this article, we present some evidence for the mobility of the latter. Cytochrome c_2 is a small water-soluble protein [29] that has been shown, in *Rps. capsulata* and *Rps. sphaeroides*, to be located on the periplasmic side of the chromatophore membrane [30].

In strain F24.1, apparently all the electrons donated to P^+ come from cytochrome c_2. This is shown by the matching kinetics (0.1 ms half-time) for the 1 : 1 stoichiometric reduction of P^+ cytochrome *c*-420 after a first flash of light. However, we cannot exclude the posssibility proposed for strain S1 [4] that a minor (approx 5%) component of P^+ is reduced in parallel by another donor such as cytochrome *c*-428. This ambiguity is due to the rather poor signal-to-noise ratios that are obtained in strain F24.1, in spite of averaging. For lack of contrary evidence, we will assume, for the purpose of this discussion, that cytochrome *c*-420 is the only secondary donor in strain F24.1.

Strain S1 contains only approx. 0.5 cytochrome c_2 per photoreaction center. This results in the fast reduction of approx. one-half of the total amount of primary donor after its oxidation by a single saturating flash. The subsequent reduction of P^+ is slow and its rate appears to be controlled by the rate of reduction of cytochrome c_2 [4]. In strain F24.1, essentially all the primary donor that is oxidized by a saturating light flash is rapidly reduced by cytochrome c_2. In strain F24.1 but not in strain S1, a train of saturating flashes given at every 100 ms in the presence of HOQNO (Fig. 5) or of antimycin A (not shown) induces a buildup of oxidized cytochrome c_2. In principle, this buildup could be used to measure the amount of cytochrome c_2 available for reduction of photooxidized P. For reasons set forth in Results, the pool size of 4 cytochromes c_2 per photoreaction center obtained in this experiment is a minimum. The other method that we used (Fig. 6) leads to a more

reliable mole ratio of 6 cytochromes c_2 per P. This ratio is consistent with the notion that the total amount of cytochrome is the same in strains S1 and F24.1. But, more importantly, these experiments also show that a reservoir of 6 cytochrome molecules is collectively involved in the reduction of every P^+ center in F24.1. This conclusion is complementary to the suggestion based on kinetic simulation that every cytochrome molecule is shared amongst 4 or more P^+ in the wild type [4]. Since the photoreaction center undergoes no measurable rotational motion withing the seconds range [31], it is probably safe to assume that its lateral mobility is also very slow. The simplest explanation that we can propose for this sharing of electrons is that cytochrome c_2 diffuses along the chromatophore membrane. As pointed out above, some sort of a diffusional network model is also required to explain why the steady-state electron flux is so high in the photosynthetic chain of strain F24.1.

One may ask, then, why is the observed maximal electron flux 30% smaller in strain F24.1? However, the fluxes are expected to be similar only if the rate-limiting mechanisms are the same. Fig. 2 shows that this is not the case, since the light intensity curves have different shapes. Possible rate-limiting reactions could be reduction of cytochrome c_2 in strain S1 and diffusion of cytochrome c_2 to P^+ in strain F24.1. The average diffusional pathlength to P is 12-times larger in strain F24.1 than in strain S1.

The replacement time of the first equivalent of oxidized cytochrome c_2 at P should place useful constraints on diffusional models. Since it is similar to the reduction time of P^+ by cytochrome c_2, the replacement time of about 0.2 ms that we find (Fig. 7) may not reflect the true encounter mechanism between the two reactants. However, if the reduction kinetics are limited by the rate of diffusion, then this diffusion is a fast process; alternatively, as has been suggested from in vitro experiments, complex formation between the reactants might precede electron donation and be an important factor in the overall reduction rate [32]. Our results can be accommodated equally well by a purely diffusional model assuming fast diffusion rates or by a mixed model involving both diffusion and complex formation. In the second case, it is unlikely that more than two molecules of cytochrome c_2 would bind to the photoreaction center at any one time [33,34].

Acknowledgements

This research was supported by the National Research Council of Canada and the Ministère de l'Education du Québec and the granting of a fellowship by the C.S.I.C. (Spain) to R.P.

References

1 Picorel, R., del Valle-Tascon, S. and Ramirez, J.M. (1977) Arch. Biochem. Biophys. 181, 665–670
2 Picorel, R., del Campo, F.F., Ramirez, J.M. and Gingras, G. (1980) Biochim. Biophys. Acta 593, 76–84
3 Ke, B. and Ngo, E. (1967) Biochim. Biophys. Acta 143, 319–331
4 Van Grondelle, R., Duysens, L.N.M. and Van Der Wal, H.N. (1976) Biochim. Biophys. Acta 449, 169–187
5 Dutton, P.L. and Jackson, J.B. (1972) Eur. J. Biochem. 30, 495–510
6 Niederman, R.A., Hunter, C.N., Mallon, D.E. and Jones, T.G. (1980) Biochem. J. 186, 453–459
7 Parson, W.W. (1974) Annu. Rev. Microbiol. 14, 41–59
8 Dutton, P.L. and Prince, R.C. (1978) in Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 525–570, Plenum Press, New York
9 Dutton, P.L., Petty, K.M., Bonner, H.S. and Morse, S.D. (1975) Biochim. Biophys. Acta 387, 536–556
10 Siebert, M. and De Vault, D. (1970) Biochim. Biophys. Acta 205, 220–231
11 Parson, W.W. and Case, G.D. (1970) Biochim. Biophys. Acta 205, 232–245
12 Van Grondelle, R., Duysens, L.M.N., Van Der Wel, J.A. and Van Der Wal, H.N. (1977) Biochim. Biophys. Acta 461, 188–201
13 Picorel, R., Mar, T. and Gingras, G. (1979) Abstracts 11th International Congress of Biochemistry, Toronto, p. 432
14 Lascelles, J. (1956) Biochem. J. 62, 78–93
15 Kuntz, I.D., Loach, P.A. and Calvin, M. (1964) Biophys. J. 4, 227–249
16 Cusanovich, M.A. and Kamen, M.D. (1968) Biochim. Biophys. Acta 153, 376–396
17 Bartsch, R.G. (1963) in Bacterial Photosynthesis (Gest, H., San Pietro, A. and Vernon, L.P., eds), pp. 475–494, Antioch Press, Yellow Springs
18 Van Der Rest, M. and Gingras, G. (1974) J. Biol. Chem. 249, 6446–6453
19 Nishimura, N. (1963) Biochim. Biophys. Acta 66, 17–21
20 Aagaard, J. and Sistrom, W.R. (1972) Photochem. Photobiol. 15, 209–225
21 Vredenberg, W.J. and Duysens, L.N.M. (1963) Nature 197, 355–357

22 Delepelaire, P. and Bennoun, P. (1978) Biochim. Biophys. Acta 502, 183–187
23 Hall, R.L., Chu Kung, M., Fu, M., Hales, B.J. and Loach, P.A. (1973) Photochem. Photobiol. 18, 505–520
24 Heathcote, P. and Clayton, R.K. (1977) Biochim. Biophys. Acta 459, 506–515
25 Drachev, L.A., Frolov, V.N., Kaulen, A.D., Kondrashin, A.A., Saminlov, V.D., Semenov, A. Yu. and Skulachev, V.P. (1976) Biochim. Biophys. Acta 440, 637–660
26 Siggel, U., Renger, G., Stiehl, H.H. and Rumberg, B. (1972) Biochim. Biophys. Acta 256, 328–335
27 Schneider, H., Lemasters, J.J., Höchli, M. and Hackenbrock, C.R. (1980) J. Biol. Chem. 255, 3748–3756
28 Hauska, G. (1977) in Proceedings of the 4th International Congress on Photosynthesis (Hall, D.O., Coombs, J. and Goodwin, T.W., eds.), pp. 185–196, The Biochemical Society, London
29 Horio, T. and Kamen, M.D. (1961) Biochim. Biophys. Acta 48, 266–286
30 Prince, R.C., Baccarini-Melandri, A., Hauska, G.A., Melandri, B.A. and Crofts, A.R. (1975) Biochim. Biophys. Acta 387, 212–227
31 Mar, T., Picorel, R. and Gingras, G. (1981) Biochim. Biophys. Acta 637, 546–550
32 Rickle, G.K. and Cusanovich, M.S. (1979) Arch. Biochem. Biophys. 197, 589–598
33 Rosen, D., Okamura, M.Y. and Feher, G. (1980) Biochemistry 19, 5687–5692
34 Overfield, R.E. and Wraight, C.A. (1980) Biochemistry 19, 3322–3327