LIGHT-DEPENDENT ATP FORMATION IN A NON-PHOTOTROPHIC MUTANT OF RHODOSPIRILLUM RUBRUM DEFICIENT IN OXYGEN PHOTOREDUCTION

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Summary : Intact cells and isolated membrane vesicles from a non-phototrophic mutant of Rhodospirillum rubrum carry out photophosphorylation at normal rates. In contrast, the same vesicles catalyze at decreased rates the photoreductions of oxygen and of tetrazolium blue by exogenous donors. Therefore it appears that the system which mediates these reactions, or part of it, is not required for photophosphorylation but is needed for the normal phototrophic growth of the organism. This conclusion is strengthened by the observation that the ability to catalyze the in vitro redox reactions is restored in vesicles prepared from a spontaneous, phototrophic revertant of the mutant.

The light-dependent electron transfer from reduced DCIP* and other artificial electron donors to oxygen (1), one of the reactions catalyzed by membrane vesicles (chromatophores) isolated from Rhodospirillum rubrum, is thought to be mediated by a part of the cyclic electron-transfer system responsible for photophosphorylation (2-4). Chromatophores from a non-photosynthetic mutant derivative of R. rubrum obtained in our laboratory are defective for the photoreduction of oxygen and also for the photoreduction of tetrazolium blue, another terminal electron acceptor in chloroplast and chromatophore reactions (5). In contrast, the same vesicle preparations and intact cells of the mutant catalyze photophosphorylation at normal rates, suggesting that at least a part of the system which mediates the photoreductions does not belong to the cyclic chain of carriers which supports the light-dependent ATP formation. These observations, which are reported here, suggest also that the system which reduces oxygen and tetrazolium blue in isolated chromatophores is required for the normal photosynthetic metabolism of R. rubrum.

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* Abbreviations used : DAD, 2,3,5,6-tetramethyl-p-phenylenediamine ; DCIP, 2,6--dichlorophenolindophenol ; TB, tetrazolium blue.

MATERIAL AND METHODS

The wild type strain (S1) of R. rubrum, a facultative phototrophic bacterium, and the culture medium were those used before (4). Non-phototrophic mutants were isolated after treatment of wild type cells with N-methyl-N'-nitro-N-nitrosoguanidine (6) and penicillin. Cells surviving penicillin treatment were plated on aerobic plates and the colonies resulting thereof were streaked both on aerobic and on anaerobic plates. Streaks failing to grow after 4 days on illuminated anaerobic plates were selected as possible non-phototrophic mutants and checked for phototrophic growth in liquid cultures (4). Aerobic liquid cultures were performed in erlenmeyer flasks filled up to 75% of their total capacity. The flasks were incubated at 30° C in the dark, and aeration was provided by girotory shaking. Cells were collected when the cultures reached 0.6 mg dry weight of cells per ml (about 3 nmoles of bacteriochlorophyll per mg dry weight) by centrifugation at room temperature (4 000 x g for 10 min). Chromatophores were prepared as described before (4) and their protein and bacteriochlorophyll contents were estimated using the methods of Lowry et al. (7) and Clayton (8), respectively.

For estimation of ATP levels in intact cells, cells were suspended in fresh growth medium at 0.87 mg dry weight per ml. Two 10-ml aliquots of the suspension were placed in open test-tubes (15 mm internal diameter), covered with 4 ml of paraffin oil and incubated in total darkness for 30 min at 30° C. 1 ml of 10 N HCl was added to one of the suspensions at the end of this incubation. The other suspension was illuminated for 30 s between two 100-W incandescent lamps and then received also 1 ml of 10 N HCl. The suspension was stirred during illumination with a small magnetic bar, taking care not to disturb the layer of mineral oil. The aqueous phases of the acid mixtures were centrifuged in the cold (3 000 x g for 10 min) to remove insoluble material and the supernatant was adjusted to pH 7.4 $\pm$ 0.02 with NaOH. The ATP content of the neutralized supernatant was estimated with the aid of a crude extract of firefly lanterns (9). Photophosphorylative activity of chromatophores was assayed in a 3-ml reaction mixture containing: 47 mM Tricine-NaOH (pH 8.0), 0.5 mM ADP, 5 mM HK_2PO_4, and 40-80 µg/ml of chromatophore protein. The mixtures, in open test-tubes (11 mm internal diameter), were placed in a water bath at 25° C, incubated for 3 min in the dark and then illuminated for 1 min as described in a previous report (4). At the end of illumination 0.3 ml of 10 N HCl were added to each assay tube and the mixtures were centrifuged in the cold (27 000 x g for 10 min). The supernatants were neutralized to pH 7.4 $\pm$ 0.02 and assayed for ATP, also by a luciferin-luciferase method (9). A duplicate of each assay mixture was kept in the dark and used to correct for background luminiscence.

Light-dependent oxygen uptake by isolated chromatophores was estimated as previously described (4), using 50 µM DCIP. The reduction of tetrazolium blue was monitored with a Perkin Elmer spectrophotometer (model 356) in the split mode, following the light-dependent increase of absorption at 590 nm (5). The reaction mixture, in a cuvette of 1-cm optical path, contained: 40 mM Tricine-NaOH (pH 7.5), 50 µM tetrazolium blue, 50 µM DCIP (or 50 µM DAD), 1.7 mM sodium ascorbate, 27 mM glucose, 10 units of glucose oxidase (E C 1.1.3.4) and chromatophores as indicated. The mixture was covered with 0.5 of paraffin oil and the cuvette was placed in the spectrophotometer chamber at room temperature 21-23°C. Actinic illumination (64 W $\cdot$ m^{-2}) was provided by a tungsten-halide, 650-W source after filtration through a narrow-band interference filter (B-IR 888, Balzers, Liechtenstein). The photomultiplier was shielded from the actinic light with the help of complementary color and interference filters.

RESULTS

Strain F11, a derivative of R. rubrum selected for its inability to form colonies on illuminated, anaerobic agar plates, did not show detectable growth in liquid anaerobic cultures when incubated in the light (Table I). In contrast, cell suspensions of strain F11 in the same growth medium increased their mass after dark incubation when aeration was provided. The doubling time under aerobic conditions was similar to that of the parent strain and that of strain RF110, a spontaneous phototrophic revertant of F11 (Table I).

The synthesis of the photosynthetic apparatus of R. rubrum is repressed by oxygen. Nevertheless, aerobic pigmented cells of the microorganism can be obtained if the oxygen tension during growth is sufficiently low (10). Cultures of strain F11, grown under low oxygen, are normally pigmented. In fact, the bacteriochlorophyll and carotenoid bands in the absorption spectra of whole cell suspensions and of isolated chromatophores are identical to the corresponding bands in the parent and revertant strains (not shown). This observation indicates that the lack of phototrophic growth of the mutant is not due to a gross alteration of its photopigments.

In spite of their inability to grow in the light, intact cells of strain F11 increased

Table I. Doubling times and ATP levels of phototrophic and non-phototrophic strains of R. rubrum.

Strain	Doubling time (min)		ATP (nmoles/mg dry weight)	
	dark aerobic	light anaerobic	dark anaerobic	light anaerobic
S1 (parent)	230	240	0.6	6.3
F11 (mutant)	192	> 10.000	0.9	6.6
RF110 (revertant)	210	235	0.6	4.9

Increase of cell mass during growth was followed in a Klett-Summerson colorimeter with filter 66. Determination of ATP levels is described under Material and Methods.

their internal levels of ATP upon illumination to an extent similar to that reached by cells from the parent strain (Table I). The light-dependent ATP accumulation was tested in cell suspensions which had been previously subjected to a prolonged dark incubation (30 min) under a layer of paraffin oil. Since under those conditions the oxygen originally present in the suspension was used up in less than 5 min as monitored in the oxygen electrode (not shown) and the levels of ATP fell to very low values (Table I), the formation of ATP in the light could not be caused by the operation of the respiratory chain. Therefore photophosphorylation appears to be functional in the mutant.

Cyclic photophosphorylation by chromatophores was assayed in the absence of exogenous redox cofactors in order to avoid the possibility of bypassing a part of the endogenous cyclic chain of electron carriers (11). As expected from the data obtained with intact cells, chromatophores isolated from strain F11 catalyzed light-dependent ATP formation at normal rates. Table II, which shows those results, shows also that mutant chromatophores had a decreased ability to mediate the photoreduction of oxygen by reduced DCIP as compared to chromatophores from both wild type and revertant strains. More stressed is the failure of F11 chromatophores to catalyze the photoreduction of a different acceptor, tetrazolium blue, by reduced DCIP. The

Table II. Photophosphorylation and oxygen photoreduction catalyzed by chromatophores from phototrophic and non-phototrophic strains of R. rubrum

Strain	ATP	Oxygen
	(nmoles per min per mg protein)	
S1 (parent)	143	85
F11 (mutant)	156	16
RF110 (revertant)	162	122

The assays were carried out as described under Material and Methods. The bacteriochlorophyll content of chromatophores was (in nmoles/mg protein): strain S1, 21; strain F11, 15; strain RF110, 23.

reaction was sustained however by chromatophores from the phototrophic strains (Fig. 1). The transient increase of absorption observed at the onset of illumination might be due to the transient accumulation of the oxidized form of DCIP, which also absorbs at the wavelength used to monitor tetrazolium blue reduction, 590 nm. This interpretation is supported by the observation that the transient change took place even in the absence of tetrazolium blue, but not when DAD sustituted for reduced DCIP as a donor (Fig. 1).

DISCUSSION

The results just described suggest that oxygen and tetrazolium blue are reduced by the same electron-transfer system of R. rubrum and that the alteration of this system is directly related to the lack of phototrophic growth of strain F11. The possibility that the defects are due to independent mutations is unlikely because strain RF110, selected only for phototrophic growth, recovered simultaneously the ability to catalyze the photoreductions. Unfortunately it is not possible to discard at the present moment the possibility that the phenotypic lesions are the result of a pleiotropic mutation affecting the normal expression of more than one gene. Only

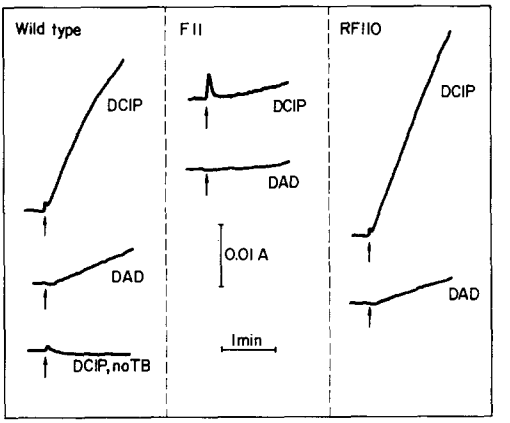

Figure 1. Photoreduction of tetrazolium blue catalyzed by chromatophores from phototrophic and non-phototrophic strains of R. rubrum. The reaction was followed as described under Material and Methods. Arrows indicate the onset of actinic illumination. Protein concentration in the assay mixtures was: wild type strain, 48 µg/ml; strain F11, 145 µg/ml; strain RF110, 44 µg/ml. Bacteriochlorophyll content of chromatophores was as in Table II.

the isolation and study of other related mutants may help to discern whether the presence of an active photoreducing system is an absolute requirement for the photosynthetic growth of R. rubrum.

At any rate it seems clear from the data of this report that the part of the electron-flow system which is defective in strain F11 is not directly involved in photophosphorylation, since this process is apparently normal in chromatophores and in intact cells of the mutant. This conclusion leads to inquire into the function of the oxygen photoreducing system in the intact cell. We have thought of a possible relationship between this system and the mechanism of NAD(P) photoreduction in whole cells of R. rubrum, a process which has been a matter of great controversy (11-13). However, our preliminary results on this respect are difficult to interpretate and a more thorough investigation is needed before conclusions can be drawn.

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