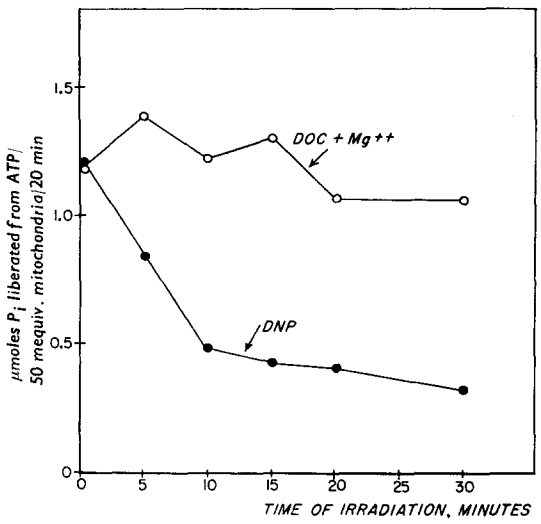

Fig. 1.

the other brought about by dislocation of components due to aging or DOC treatment. The latter pathway, involving reaction III, would presumably be irreversible.

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Photooxidation of cytochrome c by illuminated chromatophores of *Rhodospirillum rubrum* under anaerobic conditions

The photosynthetic process in bacteria is thought to involve the simultaneous production of an oxidizing and reducing power in the bacterial chromatophore under the the influence of light. This oxidizing and reducing power would result from the shifting of electrons in the organized pigment system of the chromatophore, and would be expressed in the bacterial cell as cellular oxidation–reduction reactions. It has been shown by the experiments of VERNON[1] and more clearly by the work of FRENKEL[2] that

the reducing power can be used by the chromatophores of *R. rubrum* for a photoreduction of pyridine nucleotides. The experiments described below reveal that it is also possible to demonstrate the oxidizing power produced by the chromatophore in terms of a photooxidation of cytochrome *c* under anaerobic conditions.

The *R. rubrum* chromatophores used in these experiments were prepared by sonic oscillation of whole cells in a medium which was 0.04 *M* in tris(hydroxymethyl)-aminomethane buffer, pH 7.4, and 10 % in sucrose, following which the particles from the centrifugal range of 25,000–60,000 × *g* were collected, washed once, and suspended in the same medium. When such chromatophores were exposed to light in the presence

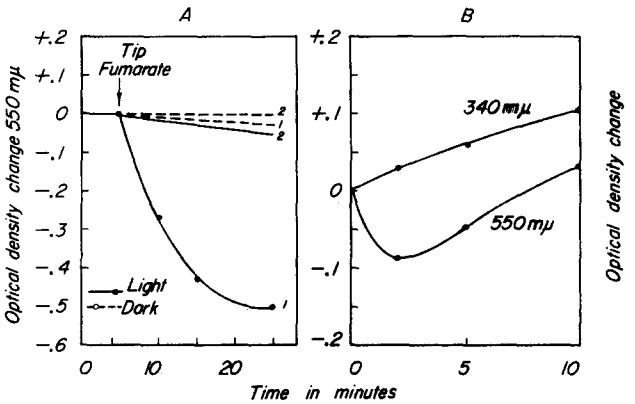

Fig. 1A. Photooxidation of reduced cytochrome *c*. The experiments were performed with Thunberg tubes adapted so that two square pyrex absorption cells (1 cm) were sealed to the tube through a T joint. Initially the main compartment of the adapted Thunberg tube contained 80 μmoles tris(hydroxymethyl)aminomethane, pH 7.4, 1.0 μmole $MgCl_2$, 3.0 μmoles DPNH, 4.0 mg cytochrome *c* and *R. rubrum* chromatophores containing 0.073 mg chlorophyll in a final vol. of 5.8 ml. The side arm contained 10 μmoles sodium pyruvate adjusted to pH 7.0, 5 μmoles KCN and 0.5 mg of twice-recrystallyzed lactic dehydrogenase. The adapted Thunberg tube was evacuated with a vacuum pump three times, with intermediate flushing with N_2. This procedure, plus the presence of excess DPNH, insured completely anaerobic conditions in the tube. The contents of the main compartment were incubated for 10 min to insure complete reduction of the cytochrome *c*, following which the contents of the side arm were tipped, thus removing the excess DPNH via the lactic dehydrogenase-pyruvate system. Following this, N_2 was introduced into the system again, 0.3 ml 0.05 *M* sodium fumarate added to the side arm, and the system re-evacuated as quickly as possible. The contents of the main compartment were thoroughly mixed and divided into two equal portions in the two absorption cells. One cell was covered with black cloth and aluminum foil, while the other cell was exposed to white light of approximately 1500 foot candles intensity at 25° for the period indicated. The curves designated by 1 represent the results of such an experiment, with the solid line representing the illuminated system and the dashed line representing the non-illuminated control. The arrow indicates the introduction of fumarate into the system by tipping, mixing the cell contents and redistributing into the two absorption cells. The curves designated by 2 represent an identical experiment with the exception that following the initial addition of pyruvate, lactic dehydrogenase and cyanide from the side arm, the cell contents were heated at 55° for 20 min before beginning the exposure to light.

Fig. 1B. Simultaneous photooxidation of reduced cytochrome *c* and photoreduction of DPN by *R. rubrum* chromatophores. The reaction mixture contained 80 μmoles tris(hydroxymethyl)-aminomethane, pH 7.4, 4 mg cytochrome *c* reduced by ascorbic acid, 3 μmoles DPN, 5 μmoles KCN, 1 μmole $MgCl_2$, 2 mg of photosynthetic pyridine nucleotide reductase from spinach and *R. rubrum* chromatophores equivalent to 0.072 mg chlorophyll in a final vol. of 6.0 ml. Initially the reduced cytochrome *c* was in the side arm of the adapted Thunberg tubes, and was tipped after the system was made anaerobic and just prior to illumination of one arm of the Thunberg tube at 1500 foot candles. The reported values are absorbancy (optical density) differences between the illuminated and non-illuminated systems.

Abbreviations: DPN, DPNH, oxidized and reduced diphosphopyridine nucleotide.

of reduced cytochrome *c* under strictly anaerobic conditions, no change was noted until fumarate was introduced into the system from the side arm of the adapted Thunberg tubes, as shown in Fig. 1A. Upon addition of fumarate, there was a rapid oxidation of cytochrome *c* in the light, with a dark control of identical composition showing no change. Heating the chromatophores destroyed this activity.

There have been previous reports of photooxidations catalyzed by *R. rubrum* chromatophores or whole cells. Thus, a photooxidation of cytochrome *c* by *R. rubrum* chromatophores was reported by VERNON AND KAMEN[3], but differed from the present experiment in that O_2 was strictly required. In the present experiments there was no O_2 present, as indicated by the lack of photooxidation before fumarate was added to the system. The requirement for O_2 in the photooxidation of cytochrome *c* reported by VERNON AND KAMEN prevented the assignment of such a photooxidation to the normal photosynthetic process in the cell.

The spectrophotometric experiments on whole *R. rubrum* cells reported by CHANCE AND SMITH[4] and by DUYSENS[5] demonstrated that light caused the cytochrome components of such cells to become more oxidized. The present experiment demonstrates the ability of chromatophores isolated from the cell to perform a similar reaction. The function of the fumarate is apparently to react with and remove the photochemical reducing power simultaneously produced, thus allowing the photochemical oxidizing equivalents to be used for cytochrome *c* oxidation.

The photooxidation of cytochrome *c* by *R. rubrum* chromatophores can be coupled to the photoreduction of DPN, as shown in Fig. 1B. By following the absorbancy changes at 340 mμ and at 550 mμ, a simultaneous oxidation of cytochrome *c* and a reduction of DPN by illuminated chromatophores was observed. The rate of DPN reduction was not as great as it would be with succinate, but proceeds at a moderate rate. The photooxidation of cytochrome *c* persisted for only a short period of time, following which a reduction became apparent. This subsequent reduction was the result of the DPNH being formed in the light interacting with the cytochrome *c* via the enzymic components of the chromatophore. This simultaneous photooxidation–photoreduction reaction was only observed with chromatophores which had been stored at 0° for a few days, since initially the DPN system would not support cytochrome *c* photooxidation.

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