

INTERACTION OF VIOLOGEN DYES WITH CHROMATOPHORES AND REACTION-CENTER PREPARATIONS FROM *RHODOSPIRILLUM RUBRUM*

RAJNI GOVINDJEE, WILLIAM R. SMITH, JR.* and GOVINDJEE

Department of Physiology and Biophysics University of Illinois, Urbana, Illinois 61801, U.S.A.

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Abstract—Various low-potential viologen dyes enhance light-induced absorption changes in the near-infrared region (ΔA_{870}) in chromatophore preparations from *Rhodospirillum rubrum* in the presence of dichlorophenol indophenol and a high concentration of ascorbate (DCPIP + asc). An increase in ΔA_{870} was also observed in large reaction-center preparations from *R. rubrum* with viologens in the presence of ascorbate. Our results indicate that E'_0 of the primary electron acceptor X may be as low as -0.37 V, as suggested recently by P. A. Loach (1973, personal communication).

INTRODUCTION

Bacterial photosynthesis is generally visualized as involving a light-induced cyclic electron transport. In such a system the primary photochemical act occurring in a reaction center is the oxidation of a specialized bacteriochlorophyll (P870; $E'_0 = +0.44$ V) and the simultaneous reduction of an acceptor. Estimates of the midpoint potential of the primary acceptor range from -0.02 V (Loach, 1966) to -0.145 V (Cramer, 1969; Dutton and Baltschefsky, 1972) for *Rhodospirillum rubrum*. From the increase in bacteriochlorophyll fluorescence caused by the addition of reducing agents, it has been suggested (*cf.* Clayton, 1971) that the primary acceptor is completely reduced at about -0.1 V. The photoreduction of nicotinamide adenine dinucleotide (NAD^+) is explained by a thermodynamically reversed flow of electrons mediated by a high-energy intermediate (or ATP) generated in the photoact (Chance and Olson, 1960; Keister and Yike, 1967; Jones and Vernon, 1969). However, Nozaki *et al.* (1961) had earlier proposed a direct reduction of pyridine nucleotide by noncyclic light-induced electron flow. R. Govindjee and Sybesma (1970) suggested that younger cells of *R. rubrum* use an energy-linked reversal of electron flow to NAD^+ , whereas older cells can use an alternate noncyclic electron flow. Feldman and Gromet-Elhanan (1972), from O_2 uptake and ATP measure-

ments in the presence of methyl viologen and other dyes, concluded that both cyclic and noncyclic electron flow occur in chromatophores of *R. rubrum*. Independently, R. Govindjee and Sybesma (1972) suggested that the NAD^+ photoreduction by chromatophore preparations from *R. rubrum* is energy-linked when succinate is used as the electron donor, but that dichlorophenol indophenol (DCPIP) plus excess ascorbate (asc) catalyzes a noncyclic electron transport causing a direct photoreduction of the nucleotide. Photoreduction of NAD^+ by DCPIP/asc is reminiscent of noncyclic $NADP^+$ reduction by DCPIP/H₂ through the photosystem I reaction in green plants, which would imply the presence of an electron acceptor with a midpoint potential lower than that of $NAD^+/NADH$ couple. Therefore, it was considered worthwhile to check the interaction of low-potential viologen dyes in the DCPIP/asc system.

Data presented in this paper show that various low potential viologen dyes are indeed capable of enhancing the light-induced absorption changes in the near-infrared region (P870) in the presence of DCPIP/asc system. (An increase in ΔA_{870} was also observed with benzyl and methyl viologen in the presence of ascorbate, using large reaction-center preparations from *R. rubrum*). Our data are not inconsistent with the presence of a primary electron acceptor having an oxidation-reduction potential (E'_0) as negative as -0.37 volts, as recently reported by Loach and Hales (1973, personal communication; also *cf.* Seibert and DeVault, 1971).

*Present address: U.S.A. Surgical Research Unit, Brooke Army Hospital, Fort Sam, Houston, Texas.

MATERIALS AND METHODS

Rhodospirillum rubrum strain IV (Giesbrecht) was grown as described previously (Sybesma and Fowler, 1968). Chromatophore fractions were prepared from 4-5-day-old cultures by a method essentially similar to that used by Cusanovich and Kamen (1968). The chromatophore fractions were suspended in a sucrose-phosphate buffer (0.1 M phosphate plus 10% sucrose), pH 7.5, stored under vacuum in a refrigerator and used within 2 weeks of preparation. Large reaction centers were prepared according to the method of Smith *et al.* (1972).

Light-induced changes in absorbance at 870 nm were measured by a split-beam spectrophotometer in a 1 mm stoppered cuvette, as described earlier (Sybesma and Fowler, 1968). The reaction mixture for the measurement of light-induced absorbance changes contained 57 mM Tris buffer, pH 8.0, 0.85 mM MgCl₂, 7 mM K₂HOP₄, and 1 mg/ml bovine serum albumin. Either 3.2 mM succinate or 70 μM DCPIP and 50 mM ascorbate was added as the electron donor. Benzyl and methyl viologens (V320 and V440 respectively) were purchased from Mann Research Biochemicals. V550 (1,1'-Trimethylene-2,2'-dipyridylum dibromide, E₀' = -0.548V), V670 (1,1'-Trimethylene-5,5'-dimethyl-2,2' dipyridylum dibromide, E₀' = -0.671V) and V740 (1,1'-Trimethylene-4,4'-dimethyl-2,2'-dipyridylum dibromide, E₀' = -0.739V) were kindly supplied by Dr. B. Kok of Martin-Marietta Laboratories, Baltimore, Maryland. (These were initially supplied to Dr. Kok by Imperial Chemical Industries, Jealott's Research Station, G.B.; Kok *et al.*, 1965).

Oxygen uptake was measured using a Clark-type concentration electrode fitted into a cuvette containing the reaction mixture. Illumination was provided from a microscope lamp (Unitron P117V S8V 5A) through a Baird Atomic 870 nm interference filter (half-band width, 12 nm). The reaction mixture, for O₂ uptake measurements, was the same as used for light-induced absorbance changes; the concentration of ascorbate, however, was reduced from 50 mM to 14 mM. This was done in order to reduce the rapid dark O₂ uptake from the reaction mixture which resulted from the use of the higher concentration of

ascorbate. 0.2 M Dithiothreitol at a concentration of 5 μl/5 μg bacteriochlorophyll was also added to the reaction mixture only for oxygen uptake measurements, to decompose hydrogen peroxide formed during the reoxidation of the autooxidizable dyes.

For monitoring the accumulation of methyl viologen, anaerobic conditions were achieved by adding 10 mM glucose and 15 units per ml glucose oxidase, and the reaction mixture being left in a stoppered cuvette for 30 min in the dark.

RESULTS AND DISCUSSION

Chromatophores

P870 Change. Figures 1 and 2 show the time course of the light-induced absorbance changes at 870 nm in the absence and the presence of V440, with either succinate or DCPIP/asc as the donor system.

In the succinate system (Fig. 1), a light-induced decrease in absorbance at 870 nm is observed which represents the photooxidation of P870 (which must proceed with a simultaneous reduction of an electron acceptor X). The electrons return to P870⁺ via a cyclic pathway, presumably through a pool of secondary acceptors. The absorbance change reaches a steady-state level; a total reduction of P870⁺ occurs after turning off the light (see Fig. 1A). This sequence of photo-oxidation and subsequent reduction in the dark could be repeated quantitatively upon successive illuminations. Upon the addition of V440 (Fig. 1B), no change in the magnitude or rate of photooxidation could be observed; this indicates that either the reaction between the primary and the natural secondary acceptor is more rapid than the reaction between X⁻ and V440, or X⁻ cannot react with V440.

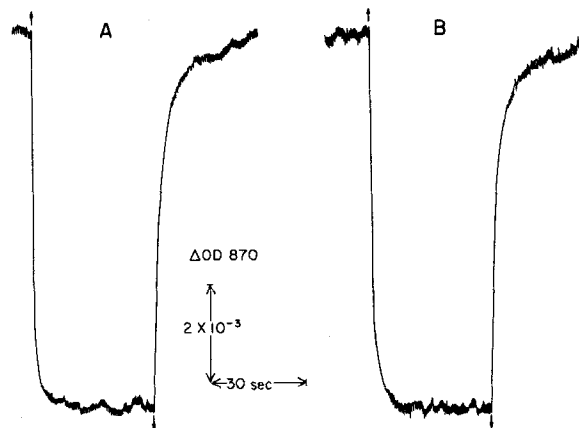


Figure 1. Time course of light-induced change in absorption at 870 nm in chromatophore fractions of *R. rubrum* in the succinate medium. (A), without methyl viologen; (B), in the presence of 0.4 mM methyl viologen. Intensity of the actinic light at 586 nm was 1.2 n Einsteins cm⁻²s⁻¹. ↑ indicates where the actinic beam was turned on and ↓ where it was turned off. 50 μg Bch 1/ml reaction mixture.

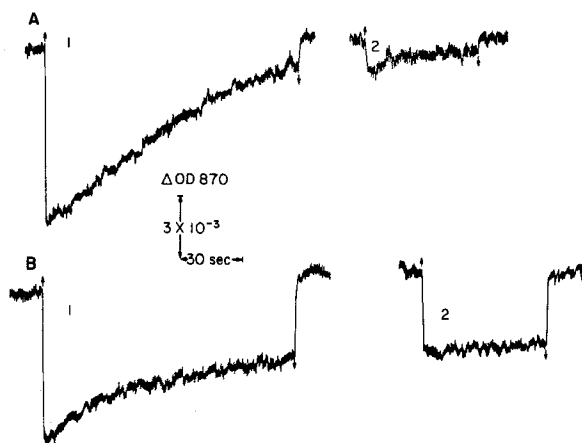


Figure 2. Time course of the light-induced change in absorption at 870 nm in chromatophore fractions of *R. rubrum* in DCPIP/ascorbate system. (A), without methyl viologen—(1) Actinic beam turned on immediately after aeration, (2) after 10 min in the dark; (B), in presence of 0.4 mM methyl viologen—(1) Immediately after aeration, (2) subsequent light exposure or after 10 min in the dark. Otherwise, as in Fig. 1.

When DCPIP/asc was used as the electron donor, the time course of the bleaching at 870 nm progressed as shown in Fig. 2A. The first light exposure (given when the sample was aerobic, i.e. immediately after putting the sample in the cuvette) induced a large initial bleaching, which decreased with time during the illumination (see Fig. 2A-1). Subsequent light exposures caused very little, or no, absorbance change (Fig. 2A-2). The large light-induced initial bleaching recurred when the sample was made aerobic again. Apparently, the initial aerobicity of the sample allowed the photoreduction of the primary acceptor X concomitant with the photooxidation of P870; with time, DCPIP/asc reduced the P870⁺ during illumination, thereby preventing the electrons from cycling back to P870⁺ from X⁻ or the pool of secondary electron acceptors; thus, the decrease in ΔA_{870} during illumination represents the filling up of the electron acceptor pools. The second light exposure, therefore, induced a very small change in absorption, due largely to the lack of oxidized electron acceptor (state of reaction center: P870, X⁻).

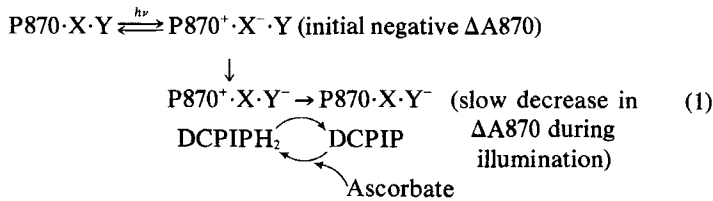
If, before the first light exposure, the sample was kept in the cuvette for 5–10 min in the dark and allowed to become anaerobic (this inference is based on the observation of a rapid dark oxygen uptake in the presence of 50 mM ascorbate), the resultant absorbance change was small and comparable to that seen in Fig. 2A-2. Thus it may seem that

ascorbate is performing a dual function in this system: (1) It keeps the DCPIP reduced at all times so that it can donate electrons to P870⁺, and (2) if the sample is kept in the dark for 5–10 min prior to illumination, ascorbate reduces the primary electron acceptor such that very little photooxidation of P870 (depending upon the amount of oxidized electron acceptor available) can occur. This can be understood if the suggested midpoint potential for X in *R. rubrum* is assumed to be -0.02 V (Loach, 1966). However, as will be seen from the effect of viologens on the photooxidation of P870, it seems that the primary electron acceptor is in the oxidized state (thus capable of accepting electrons) under these conditions. Alternatively, the extremely reduced absorbance change after a dark incubation, and under conditions in Fig. 2A-2, can be explained (see below) if we assign the titration value of -0.02 V to a pool of secondary electron acceptors (Y). Under conditions when Y is reduced* (i.e. after a preillumination, or 5–10 min incubation in the dark in the presence of ascorbate), the oxidation of X⁻ (produced concomitantly with the photooxidation of P870) is prevented and an extremely small ΔA_{870} is observed. To this effect, Loach (1973) has presented evidence suggesting that the previously reported value of -0.02 V (for E₆) represents the titration of a secondary electron acceptor in *R. rubrum*. On the basis of the electron paramagnetic resonance (EPR) studies, Loach and Hales (1973, personal communication) found that the primary electron acceptor (Loach and Hall, 1972) in photoreceptor complex preparations has a

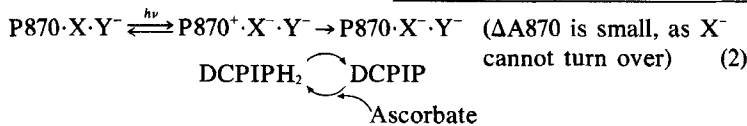
*Y⁻ cannot be oxidized through P870⁺, as the latter is reduced by DCPIP/asc and hence Y accumulates as Y⁻.

midpoint potential of -0.37 V; a similar midpoint potential for the quenching of phototrap activity was found for chromatophores and whole cells when they were kept under complete darkness and then subjected to a single short flash of light.

The following reaction sequence explains the events in Fig. 2A-1:



where P870 is the reaction center bacteriochlorophyll, X the primary electron acceptor, and Y the secondary electron acceptor. Following a light exposure as in Fig. 2A-2, or after dark incubation, in which case Y is reduced by ascorbate in the dark, the reaction sequence could be represented as follows:



The effect of viologens on the infrared absorbance changes in the DCPIP/asc system is shown in Fig. 2B. Addition of V440 caused virtually no effect on the total bleaching at 870 nm and the initial time course, but after approximately 1 min there was no further change in ΔA870 and a steady-state level was reached (see Fig. 2B-1) during illumination. Subsequent light exposures induced a rapid bleaching which reached a substantial steady-state level. If the sample was kept in the dark prior to illumination for 5–10 min, the time course during the first light exposure was comparable to that shown in Fig. 2B-2. Similar results were obtained with V320 and V540, although the relative concentrations of the dyes required to obtain the maximum ΔA was different. The lower-potential dyes V670 and V740 had virtually no effect even up to 5 mM (the highest concentration used). Figure 3 shows the light-induced near-infrared absorbance change

as a function of concentration of the various viologen dyes. (The ΔA870 values shown in Fig. 3 were obtained with a time regimen represented by Fig. 2B-2).

For 50% stimulation of ΔA870 , $5 \mu\text{M}$ V320, $50 \mu\text{M}$ V440, and $0.5\text{--}1 \text{mM}$ V540 were required.

The fact that the initial ΔA870 and the time

course in Fig. 2A-1 and 2B-1 (in the absence and presence of V440) are the same can be explained as follows: Initially, when the sample was aerobic*, and Y was oxidized, and X^- could react with Y, the decrease in $\Delta\text{OD 870}$ during illumination must indicate the filling up of this pool. Once the secondary acceptor pool Y was all reduced, the reaction be-

tween X^- and Y^- could not occur and the viologen dyes could then accept electrons from X^- , at which point a steady-state level was reached (Fig. 2B-1),

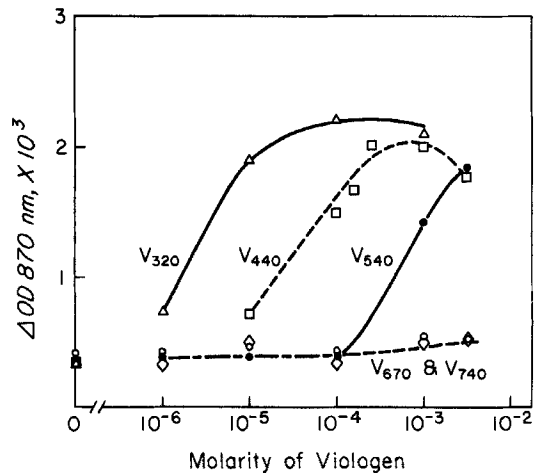
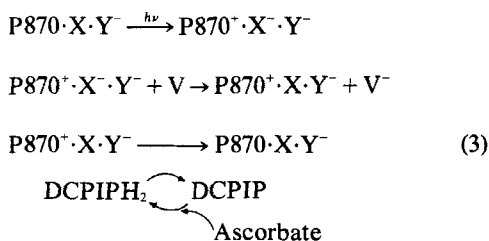


Figure 3. Light-induced change in absorbance (ΔOD or ΔA) at 870 nm in presence of various concentrations of indicated viologens, in the DCPIP/ascorbate system (for complete names of viologens, see text). The intensity of the actinic light at 586 nm was $1.2 \text{ n Einsteins cm}^{-2}\text{s}^{-1}$; $33.5 \mu\text{g}$ Bechl/ml reaction mixture.

*This was always the case immediately after putting the sample in the cuvette, but in about 5 min or so the sample became anaerobic, as would be expected by the observation of a rapid dark oxygen uptake in the presence of 50 mM ascorbate.

but in Fig. 2A-1 no such steady-state level was observed due to the absence of the viologen dyes. All this suggests is that the reaction between X^- and the natural electron acceptor Y is more rapid than X^- and viologen dyes. Thus, under conditions in Fig. 2B-2 or after 5–10 min incubation with ascorbate in the dark, viologen dyes (V) take over the function of increasing the turnover of X^- , since Y is kept in the reduced state by ascorbate. The following reaction sequence explains the events in Fig. 2B-2:



Under anaerobic conditions the reduced dye can accumulate or be reoxidized by P870^+ (if the rate of interaction of V^- with P870^+ is faster than that of DCPIP/asc with P870^+). The extent to which this cyclic reaction occurs is not possible to determine from the present experiments. Direct accumulation of reduced methyl viologen, although in small quantities, was inferred from an increase in optical density at 390 nm under anaerobic conditions (see Fig. 4).

The effect of viologens on the ΔA_{870} is seen (Fig. 5) even in the presence of 1 mM *o*-phenanthroline (an inhibitor of electron transport between the primary and secondary electron acceptors; see Clayton *et al.*, 1972). This further substantiates our argument for a non-cyclic electron flow from P870 to the viologen dyes.

The increased steady-state level of the change in optical density at 870 nm, in the presence of the viologens (cf. Fig. 2B-2 with 2A-2) and the DCPIP/asc donor, implies an interaction between the dyes and a primary acceptor X , with a redox potential close to that of the dyes. Two possibilities to explain our data are: (a) If enough X (with an E_0' of -0.15 V) can accumulate in the reduced state, viologen dyes (with E_0' of -0.4 V) could conceivably interact with X^- (Clayton, 1973) or (b) X , indeed, has E_0' as negative as -0.4 V. As noted earlier, Loach (1973) has proposed that titrations of the primary electron acceptor made thus far represent that of the secondary rather than of the primary electron acceptor (X). From observations on flash-induced paramagnetic resonance (EPR) signals in

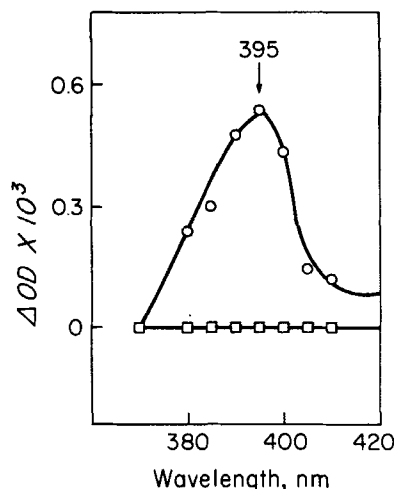


Figure 4. Light-minus-dark difference spectrum of absorbance changes measured in chromatophore fractions from *R. rubrum* in DCPIP/ascorbate system; (○) in the presence of 0.4 mM methyl viologen; (□) no methyl viologen. The intensity of the actinic light at 880 nm was 5.4 einsteins $\text{cm}^{-2}\text{s}^{-1}$. 24.3 μg Bchl/ml reaction mixture; 10 mM glucose and 15 units/ml glucose oxidase (Sigma) were added to achieve anaerobic conditions.

the presence of various redox buffers, Loach (1973) has suggested that the E_0' of the primary electron acceptor is -0.337 V in *R. rubrum* and *R. spheroides*. Our results are not inconsistent with this finding.

Oxygen uptake. The reduced viologens are highly auto-oxidizable; thus, if the chromatophores are

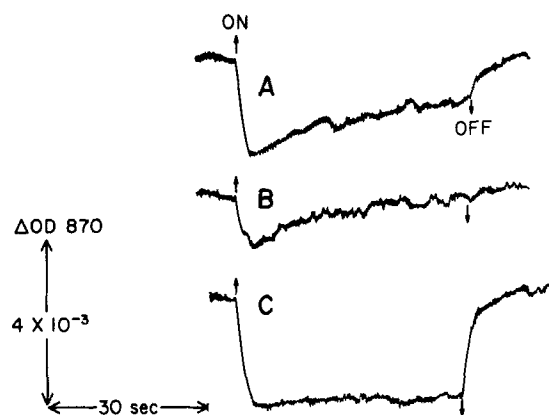


Figure 5. Time course of light-induced change in absorption at 870 nm in chromatophore fractions of *R. rubrum* in DCPIP/ascorbate system. (A), no additions; (B), plus 1 mM *o*-phenanthroline; (C), plus 1 mM *o*-phenanthroline and 0.1 mM benzyl viologen (V320). The reaction mixture was left in dark for about 5 min prior to each measurement. Otherwise, as in Fig. 1.

capable of reducing the viologens, one could conceivably measure a light-induced O_2 uptake from the reaction mixture. Figure 6 shows the rate of O_2 uptake in the presence of 0.4 mM methyl viologen (V440), as a function of light intensity, by a chromatophore preparation, when either succinate (3.2 mM) or 2,6-dichlorophenol indophenol (DCPIP, 70 μM , with excess ascorbate (asc, 14 mM) was used as electron donor. In these experiments,

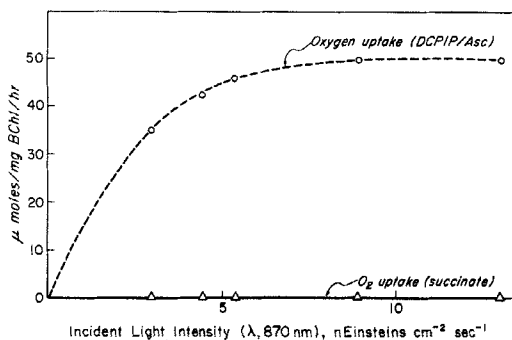


Figure 6. Rate of light-induced oxygen uptake by chromatophore fractions from *R. rubrum* as a function of incident light intensity (in the two donor systems—DCPIP/ascorbate and succinate) in presence of 0.4 mM methyl viologen (V440). Bacteriochlorophyll (Bchl), 21.4 $\mu g/ml$ reaction mixture; succinate, 3.2 mM; DCPIP, 70 μM ; ascorbate, 14 mM.

0.2 M dithiothreitol was present at a concentration of 1 l/g Bchl. No oxygen uptake could be observed in our experiments with any of the viologens when succinate was the electron donor (triangles). This result is in apparent contradiction to those of Feldman and Gromet-Elhanan (1972), who observed a small (8–14 $\mu mol/mg$ Bchl/h) O_2 uptake in such a system. In view of the smallness of O_2 uptake, it is not surprising that we did not observe it in our preparations that were under different buffer and donor concentrations. The DCPIP/asc system, however, catalyzed light-induced O_2 uptake (dots). Benzyl viologen (V320) at the same or lower concentration gave similar results.

In confirmation of Feldman and Gromet-Elhanan (1972), we also observed a light-induced oxygen uptake in the absence of viologen dyes, when DCPIP/asc was the donor system. But, unlike the above authors, we observed a slight (~ 8 –10 $\mu mol/mg$ Bchl/h) enhancement of O_2 uptake by viologens (see Table 1); different experimental conditions may be responsible for this difference. The occurrence of a light-induced oxygen uptake in the DCPIP/asc system, even in the absence of the viologen dyes, implies a photoproduct

Table 1. Effect of HOQNO and methyl viologen on the light (870 nm)-induced oxygen uptake by *R. rubrum* chromatophores with DCPIP/asc as the electron donor. 5 μg Bchl/ml reaction mixture

Additions	$\mu mol O_2$ Uptake/mg Bchl/h
None	43
4 mM Methyl viologen	51
2 μM HOQNO	66
4 μM HOQNO	75
4 μM HOQNO + 4 mM methyl viologen	93

(No DTT was added to the reaction mixture.)

that is autooxidizable. Methyl viologen has only a very small effect because Y is in the oxidized state (because of the presence of O_2 which was necessary in order to study the oxygen uptake), and, as stated before, the transfer of electrons from X^- to the natural acceptor (Y) is perhaps very fast. Addition of an electron transport inhibitor HOQNO (see Nishimura, 1963) caused a significant stimulation of O_2 uptake, presumably by blocking electron transfer from X^- to Y. Addition of methyl viologen, in the presence of HOQNO, increases significantly (~ 18 –20 $\mu mol/mg$ Bchl/h) the rate of O_2 uptake (see Table 1). When electron transfer from X^- to Y is blocked, methyl viologen can interact with X^- and thereby enhance the rate of oxygen uptake. Thus, for the interaction of viologen dyes with X^- , the necessary condition is either the existence of Y in the reduced state, or a block of electron flow from X^- to Y. (See note added in proof.)

Reaction center preparation

P870 Change. Viologen dyes had a similar effect, as shown for chromatophores in Fig. 2, on the light-induced change in absorbance at 865 nm when large reaction center preparations from *R. rubrum* were used. Figure 7A shows the time course of the bleaching at 865 nm in the presence of $\sim 1 mM$ ascorbate. The same results were obtained with DCPIP/asc system. The magnitude of the bleaching decreased during the light exposure, indicating the filling up of the electron acceptor pools (see Clayton and Yau, 1972). In the presence of both V320 and ascorbate (Fig. 7B) the decrease in the magnitude of ΔA_{865} could not be observed during illumination. It seems that V320 is capable of interacting with an electron acceptor coupled to P870. (It may be noted here that the effect of the viologen dyes on ΔA_{865} with the reaction-center preparations was best seen with old preparations that did not show the

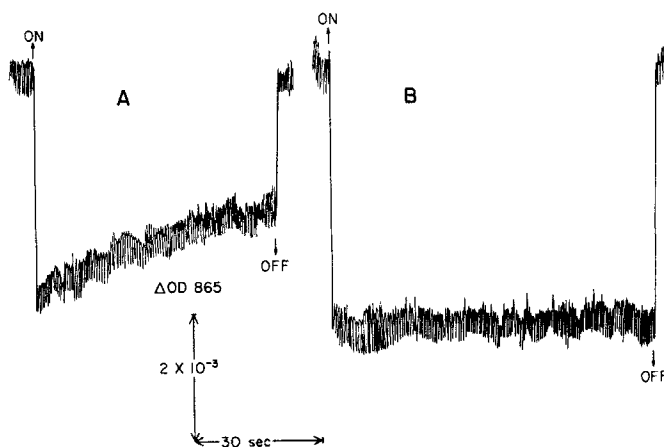


Figure 7. Time course of light-induced change in absorption at 865 nm in large reaction-center preparation of *R. rubrum* in 50 mM Tris buffer (pH 8.0). (A), plus 1 mM sodium ascorbate; (B), plus 1 mM sodium ascorbate and 0.4 mM benzyl viologen (V320). Intensity of actinic light at 800 nm was 1.6 n Einsteins $\text{cm}^{-2}\text{s}^{-1}$. The absorbance of the sample in a 1-cm cuvette at 590 nm was 0.035.

light-induced cytochrome reactions. Likewise the chromatophore preparations did not show any light-induced cytochrome reactions.)

A light-minus-dark difference absorption spectrum of the reaction-center preparation in the presence of benzyl viologen and ascorbate showed the characteristic spectrum of P870 with a blue shift of the 800 nm absorption band and a bleaching around 865 nm (Fig. 8). No increase around 900 nm was observed in contrast with the observation with chromatophore preparations. (The ΔA values were obtained with a time regimen represented by Fig. 2B-2). The increase in absorption at 900 nm in chromatophores could then be due to a light-induced red shift of the light-harvesting bacteriochlorophyll (Vredenberg and Ames, 1966; Cusanovich *et al.*, 1968).

Oxygen uptake. A light-induced oxygen uptake was also observed with the large reaction-center preparations from *R. rubrum* in the presence of ascorbate, confirming the data with chromatophores.

Working hypothesis

A schematic representation of our working model is illustrated in Fig. 9. In this model the reaction center bacteriochlorophyll, P870, upon excitation, donates electrons to the primary acceptor X. A secondary acceptor Y then accepts the electrons and transfers them back to P870, thus mediating a cyclic electron transport (Fig. 9A). Such conditions prevail in our succinate-containing medium. During the cyclic electron transport a 'high-energy inter-

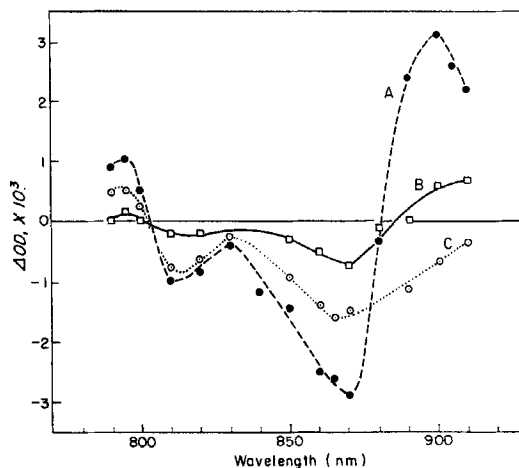


Figure 8. Light-minus-dark difference spectra of absorbance changes. (A), chromatophore fractions from *R. rubrum* in DCPIP/ascorbate system with benzyl viologen (V320), (B) without V320; intensity of actinic light at 586 nm, 1.2 n Einsteins $\text{cm}^{-2}\text{s}^{-1}$; 32 μg Bchl/ml reaction mixture; measurements made with a time regimen comparable to that in Figs. 2A-2 and 2B-2; (C), large reaction-center preparation of *R. rubrum* in 50 mM Tris buffer (pH 8.0) in presence of 1 mM ascorbate and 0.4 mM V320; intensity of actinic light in 586 nm, 2.4 N Einsteins $\text{cm}^{-2}\text{s}^{-1}$; absorbance of sample in a 1-cm cuvette at 590 nm, 0.035.

mediate' is generated, which can mediate a thermodynamically reversed flow of electrons from succinate to NAD^+ (R. Govindjee and Sybesma, 1972). The viologens do not react because the transfer of electrons is very rapid from the primary acceptor X to the natural secondary acceptor Y. In

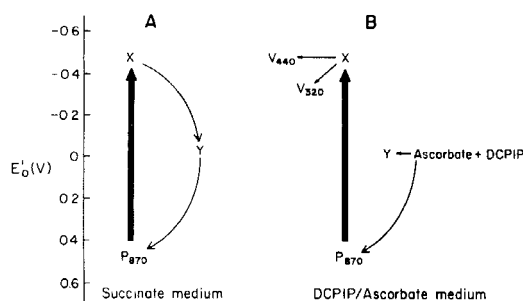


Figure 9. A working model. P870, X, Y, and E'_0 are reaction-center bacteriochlorophyll, primary electron acceptor, secondary electron acceptor, and redox potential, respectively.

the presence of DCPIP/asc (Fig. 9B), the secondary acceptor, Y, is kept in the reduced state by the excess ascorbate. This prevents the electrons from falling back to Y, thus allowing interaction between the viologens and the reduced primary acceptor X. Under the latter conditions a direct light-induced reduction of pyridine nucleotide (R. Govindjee and Sybesma, 1970, 1972) would then be possible. Hence, necessary prerequisite of this type of electron transport is the reduced state of Y. This model can explain the present as well as our earlier results (R. Govindjee and Sybesma, 1970, 1972). This model seems attractive also because of its analogy with photosystem I of green plants.

CONCLUDING REMARKS

(1) Viologen dyes with midpoint redox potentials (E'_0) between -0.320 V (to be referred to as V320) and -0.548 V increase the absorbance change at 870 nm (ΔA_{870}) in chromatophore preparations from *Rhodospirillum rubrum* when dichlorophenol indophenol and a high concentration of ascorbate (DCPIP + asc) were present. $5 \mu\text{M}$ V320 was enough to cause 50% stimulation; however, 0.05 mM V440 and 0.5 – 1 mM V540 were required for the same effect. 1 mM *o*-phenanthroline (an inhibitor of electron flow from primary to secondary acceptor) did not affect this interaction with viologen dyes. Dyes with still lower midpoint redox potentials showed no effect even at 5 mM. There was no effect of the viologens on the ΔA_{870} with succinate as electron donor. (2) An increase in ΔA_{870} was also observed in large reaction-center preparations from *R. rubrum* with V320 and V440 in the presence of ascorbate. (3) In the presence of DCPIP + asc, and under anaerobic conditions, direct accumulation of reduced methyl viologen, although in small quantities, was inferred from an

increase in optical density at 390 nm in chromatophore preparations. (4) Light-induced O_2 uptake was observed with chromatophore preparations in the presence of DCPIP + asc, but not in the presence of succinate, confirming the work of Feldman and Gromet-Elhanan (1972). (O_2 uptake in the presence of DCPIP + asc was, however, slightly stimulated when methyl or benzyl viologen was also added.) This O_2 uptake was not inhibited by 2 – $4 \mu\text{M}$ 2-heptyl-4-hydroxyquinoline-*N*-oxide (HOQNO). In our experimental conditions, O_2 uptake in the presence of the viologens could not be observed when succinate was used as the electron donor. (5) Our experimental results indicate that the E'_0 of the primary electron acceptor X may be as low as that suggested by Loach (1973).

Note added in proof

If HOQNO inhibits the electron flow between X and Y, as suggested in this paper, then one might expect a significant stimulation of O_2 uptake with succinate as the electron donor in the presence of HOQNO and viologen dyes. However, no oxygen uptake was observed by us under such conditions suggesting that succinate is unable to donate electrons to P870.

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