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PHOTOOXIDASE ACTIVITY OF *RHODOSPIRILLUM RUBRUM* CHROMATOPHORES AND REACTION CENTER COMPLEXES. THE ROLE OF NON-CYCLIC ELECTRON TRANSFER IN GENERATION OF THE MEMBRANE POTENTIAL

VALENTIN G. REMENNIKOV and VITALY D. SAMUILOV

Department of Microbiology, Moscow State University, Moscow 117234 (U.S.S.R.)

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Summary

The mechanism of light-induced O_2 uptake by chromatophores and isolated *P*-870 reaction center complexes from *Rhodospirillum rubrum* has been investigated.

The process is inhibited by *o*-phenanthroline and also by an extraction of loosely bound quinones from chromatophores. Vitamin K-3 restored the *o*-phenanthroline-sensitive light-induced O_2 uptake by the extracted chromatophores and stimulated the O_2 uptake by the reaction center complexes. It is believed that photooxidase activity of native chromatophores is due to an interaction of loosely bound photoreduced ubiquinone with O_2. Another component distinguishable from the loosely bound ubiquinone is also oxidized by O_2 upon the addition of detergents (lauryldimethylamine oxide or Triton X-100) to the illuminated reaction center complexes and to the extracted or native chromatophores treated by *o*-phenanthroline. Two types of photooxidase activity are distinguished by their dependence on pH.

The oxidation of chromatophore redox chain components due to photooxidase activity as well as the over-reduction of these components in chromatophores, incubated with 2,3,5,6-tetramethyl-*p*-phenylenediamine ($Me_4Ph(NH_2)_2$) or *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD) (plus ascorbate) in the absence of exogenous electron acceptors, leads to an inhibition of the membrane potential generation, as measured by the light-induced uptake of

Abbreviations: CCCP, carbonylcyanide *m*-chlorophenylhydrazone; $Me_4Ph(NH_2)_2$, 2,3,5,6-tetramethyl-*p*-phenylenediamine; PCB^-, phenyldicarbaundecaborane anions; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine.

penetrating phenyldicarbaundecaborane anions (PCB^-) and tetraphenylborate anions. The inhibition of the penetrating anion responses observed under reducing conditions is removed by oxygen, 1,4-naphthoquinone, fumarate, vitamin K-3 and methylviologen, but not by NAD^+ or benzylviologen. Since methylviologen does not act as an electron acceptor with the extracted chromatophores, it is believed that this compound, together with fumarate and O_2, gains electrons at the level of the loosely bound ubiquinone. Data on the relationship between photooxidase activity and membrane potential generation by the chromatophores show that non-cyclic electron transfer from reduced $Me_4Ph(NH_2)_2$ to the exogenous acceptors is an electrogenic process, whereas non-cyclic electron transfer from reduced TMPD is non-electrogenic.

Being oxidized, $Me_4Ph(NH_2)_2$ and TMPD are capable of the shunting of the cyclic redox chain of the chromatophores. Experiments with extracted chromatophores show that the mechanisms of the shunting by $Me_4Ph(NH_2)_2$ and TMPD are different.

Introduction

Light-induced uptake of oxygen by cell extracts (photooxidase activity) of the non-sulphur purple bacterium *Rhodospirillum rubrum* coupled with the oxidation of ascorbic acid was described by French [1,2]. Isolated chromatophores of *R. rubrum* take up oxygen upon the oxidation of exogenous cytochrome *c*, 2,6-dichlorophenolindophenol [3], *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD) [4] or 2,3,5,6-tetramethyl-*p*-phenylenediamine ($Me_4Ph(NH_2)_2$) [5]. Photophosphorylation in chromatophores incubated with TMPD and ascorbate is observed only in the presence of O_2 or other electron acceptors, such as fumarate and methylviologen [4,6]. The substrates and uncouplers of photophosphorylation stimulate photooxidase activity of *R. rubrum* chromatophores with 2,6-dichlorophenolindophenol or $Me_4Ph(NH_2)_2$ as electron donors [5,7], but not with TMPD [5]. It has been shown that electron transfer from the exogenous donors to O_2 by the illuminated chromatophores of *R. rubrum* is mediated by a part of the cyclic redox chain [4,5,7,8].

In this paper, we report on the mechanism of the light-induced O_2 uptake by *R. rubrum* chromatophores and on the role of non-cyclic electron transfer in the membrane potential generation. Preliminary reports of some of this work have already been published [9,10].

Methods

Cells of *R. rubrum* (wild type strain) were grown in the light under anaerobic conditions in the medium of Bose et al. [11] in the presence of malate and yeast extract. The chromatophores were isolated by ultrasonication and by consecutive centrifugation at 40 000 × *g* for 15 min and at 165 000 × *g* for 60 min as described previously [4,6]. The chromatophores were then washed twice with distilled water, lyophilized and extracted initially [9,10] with isooctane for the removal of quinone, as detailed by Higuti et al. [12]. Since extraction with hydrocarbon solvents does not lead to the removal of loosely

bound quinones in some chromatophore samples [13], extraction was consequently carried out with a mixture of isooctane and acetone (95 : 5, v/v). Lyophilized chromatophores were suspended in 25—30 ml of the cooled isooctane and acetone mixture/μmol of bacteriochlorophyll, stirred magnetically at 2—4°C for 40 min in the dark and then centrifuged at 40 000 × g for 15 min. After three-fold extraction, the chromatophores were dried under vacuum for 6—10 h in the dark to remove the organic solvents. It has shown that such a treatment leads to the removal of rhodoquinone and loosely bound ubiquinone from chromatophores [13].

P-870 reaction center complexes were isolated by treating the chromatophores with lauryldimethylamine oxide [14]. The complexes were then dialyzed and stored at 0°C in the dark in 50 mM sodium phosphate buffer, pH 7.8.

The bacteriochlorophyll content of chromatophores was estimated using a molar extinction coefficient of 140 $mM^{-1} \cdot cm^{-1}$ at 880 nm [15]. The concentration of *P*-870 in the isolated reaction centers was determined from light-induced absorption changes at 870 nm using an extinction coefficient of 125.7 $mM^{-1} \cdot cm^{-1}$ [16].

Oxygen uptake by the chromatophores and the reaction center complexes was measured polarographically with a platinum electrode at a voltage of 0.65 V. The solution in the experimental cell, of volume 1.5 ml, was mixed by means of a magnetic stirrer.

The uptake of penetrating phenyldicarbaundecaborane anions (PCB^-) and tetraphenylborate anions by chromatophores was monitored using a phospholipid (azolectin) membrane [17] separating two compartments of a Teflon cuvette with the solution.

Actinic light of saturating intensity ($\lambda > 660$ nm) for the illumination of the suspensions of chromatophores and reaction center complexes was provided by a tungsten lamp. Light was passed through a 5 cm thick water filter to prevent heating the electrodes.

Some experiments, in which membrane potential generation by chromatophores was measured, were carried out under anaerobic conditions: 0.17 mg/ml of glucose oxidase (EC 1.1.3.4) and 0.17 mg/ml of catalase (EC 1.11.1.6) were added to both compartments of the experimental cell with a solution containing 30 mM glucose. Paraffin oil (6—8 mm in thickness) was layered on top of the aqueous phase.

Results

Oxygen uptake by *R. rubrum* chromatophores incubated with TMPD and ascorbate is shown in Fig. 1A. The process is light dependent and ceased after switching off the light. Subsequent illumination causes resumption in O_2 uptake, which continues until the oxygen is exhausted in the incubation medium.

We reported previously [10] that the rate of the light-induced O_2 uptake by the chromatophores depends on the nature of the electron donor. High rates are observed with $Me_4Ph(NH_2)_2$ and TMPD. Cytochrome *c* and ferrocyanide are less effective. The rate of O_2 uptake with reduced cytochrome *c* by different

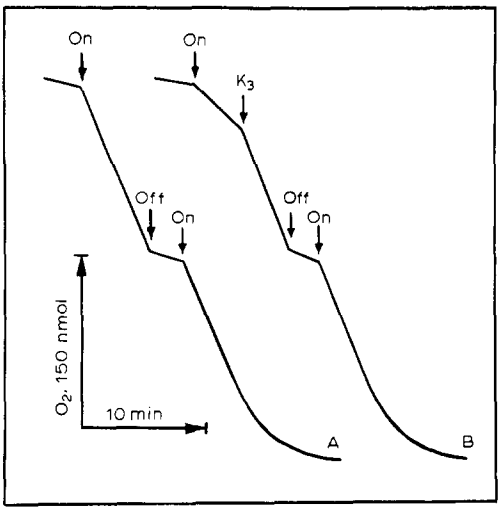

Fig. 1. Photooxidase activity of chromatophores (A) and *P*-870 reaction center complexes (B) from *R. rubrum*. Incubation mixture: 250 mM sucrose, 50 mM Tris-HCl buffer (pH 7.6), 5 mM Tris/ascorbate, 0.1 mM TMPD, chromatophores with bacteriochlorophyll content $1 \cdot 10^{-5}$ M or $0.62 \cdot 10^{-6}$ M reaction centers. Addition: $1 \cdot 10^{-5}$ M vitamin K-3 (K_3). On and Off, switching on or off the light.

chromatophore preparations varies between 15 and 30% of the rates with $Me_4Ph(NH_2)_2$ or TMPD.

Photooxidase activity is unaffected by rotenone, antimycin A and CN^- and is suppressed by *o*-phenanthroline [10], an inhibitor of electron transfer between the primary and secondary acceptors of non-porphyrin nature [18,19]. This indicates that the light-induced O_2 uptake occurs without the participation of the rotenone-sensitive NADH-ubiquinone reductase segment responsible for NAD^+ reduction from a reversal of electron flow [4,6,20], as well as the antimycin-sensitive segment of the cyclic redox chain at the level of cytochrome *b* [21], or CN^--sensitive cytochrome oxidase, which contains cytochrome *o* as shown for *R. rubrum* [22].

The light-induced O_2 uptake is inhibited upon the extraction of quinones from chromatophores and is restored upon the addition of vitamin K-3. Photooxidase activity also recovers upon reconstitution of the extracted chromatophores with an isooctane extract, vitamin K-1, 1,4-naphthoquinone or ubiquinone-6 [9,10]. Methylviologen and benzylviologen added as autoxidizable compounds, instead of quinones, are ineffective (data not shown). Methylviologen has been reported to stimulate photooxidase activity of non-extracted chromatophores [8].

The light-induced O_2 uptake is observed not only with the chromatophores, but with simpler systems, with isolated *P*-870 reaction center complexes. It was determined that the overall composition of the reaction centers is as follows: three protein subunits, four bacteriochlorophylls *a*, two bacteriopheophytins *a*, two ubiquinones, one Fe^{2+}, and one spirilloxanthin [16,23].

Fig. 1B shows that the isolated complexes of *P*-870 reaction centers incubated with TMPD and ascorbate take up oxygen upon illumination. This process is inhibited upon the extraction of the lyophilized complexes with iso-

octane (data not shown) and is stimulated by vitamin K-3 (Fig. 1B). Photooxidase activity of the reaction center complexes incubated with TMPD, ascorbate and vitamin K-3 is about 30 μmol of O_2/μmol of *P*-870 per min. Inasmuch as a molar ratio between the *P*-870 reaction centers and the bulk of light-harvesting bacteriochlorophyll accounts for 1 : 30–50, the rates of O_2 uptake on 1 μmol of *P*-870 basis are distinguished slightly in the native chromatophores and in the isolated reaction centers. Earlier reports have stated that the reaction center plus light-harvesting antenna complexes isolated from *R. rubrum* chromatophores also show photooxidase activity [9,10].

Quinone-dependent light-induced O_2 uptake by the isolated reaction centers as well as by the native and extracted chromatophores is inhibited by *o*-phenanthroline (Fig. 2). O_2 uptake is renewed upon the addition of lauryldimethylamine oxide (Fig. 2A). *o*-Phenanthroline-insensitive photooxidase activity, appearing on the addition of detergent, is also found in the extracted (Fig. 2B and 2C) and native (Fig. 2D) chromatophores. Triton X-100 (Fig. 2B and 2C) is effective along with lauryldimethylamine oxide, but not sodium cholate. O_2 uptake by illuminated chromatophores or reaction center complexes in the presence of detergents occurs with similar rates both with ascorbate and with TMPD plus ascorbate (data not shown).

Thus, two types of photooxidase activity are found in chromatophores and reaction center complexes from *R. rubrum:* (1) *o*-phenanthroline-sensitive activity and (2) *o*-phenanthroline-resistent activity, occurring only upon the addition of detergent.

Consideration of the data in Figs. 1 and 2 shows that these activities are distinguished by their affinity for oxygen. The rate of quinone-dependent O_2 uptake decreases as the O_2 concentration in the incubation medium is lowered (Fig. 1), whereas the rate of *o*-phenanthroline-insensitive O_2 uptake remains almost unaltered down to O_2 exhaustion of the medium (Fig. 2).

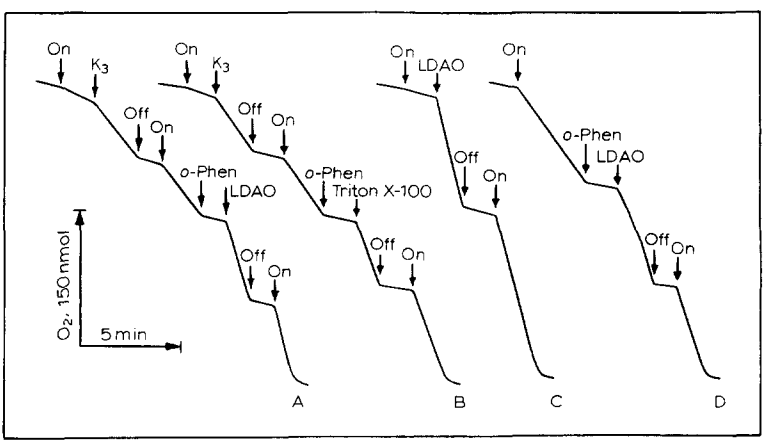

Fig. 2. *o*-Phenanthroline-sensitive and *o*-phenanthroline-resistant photooxidase activity of *P*-870 reaction center complexes (A), extracted (B, C) and non-extracted (D) chromatophores of *R. rubrum*. Incubation mixture: 250 mM sucrose, 50 mM Tris-HCl buffer (pH 7.6), 5 mM Tris/ascorbate, 0.1 mM TMPD, $0.62 \cdot 10^{-6}$ M reaction centers or chromatophores with bacteriochlorophyll content $1 \cdot 10^{-5}$ M. Additions: $1 \cdot 10^{-5}$ M vitamin K-3 (K_3), 5 mM *o*-phenanthroline (*o*-Phen), 0.1% (A) or 0.3% (C and D) lauryldimethylamine oxide (LDAO), 0.5% Triton X-100.

Fig. 3 illustrates that *o*-phenanthroline-sensitive photooxidase activity increases, whereas *o*-phenanthroline-resistant activity of chromatophores decreases with an increase in pH of the incubation medium from 5 to 9. Data on autoxidizability of quinones are also presented in Fig. 3. Addition of ubiquinone-1 to aqueous solution of ascorbate causes an uptake of O_2. The rate of the ubiquinone-induced O_2 disappearance is significantly increased with the increase in pH. Character of the curve depends on series of conditions. In particular, a plateau is observed at pH values from 8.5 to 9 in the presence of bovine serum albumin (data not shown). The activity of different water-soluble quinones decreases in a sequence: 1,4-naphthoquinone, ubiquinone-1, vitamin K-3, duroquinone. Free *p*-benzoquinone as well as membrane-bound quinones are inactive. The lack of activity of the membrane-bound quinones may be caused by the loss of the availability of quinones for ascorbate. Besides, polarographic studies [24] show that half-wave potentials for quinones bound with liposomes are significantly more negative than those for free quinones.

On 1 ml of reaction mixture/min basis, the rates of the light-induced O_2 uptake by the native chromatophores and the rates of the ubiquinone-induced O_2 uptake are 19 and 20 nmol, respectively, at pH 7.0 or 26 and 44 nmol at pH 8.0, as seen from Fig. 3.

Results obtained show that quinones play an important role in the light-induced O_2 uptake by the chromatophores. Most apparently, the photooxidase activity of the chromatophores is due to *P*-870 reaction center-dependent non-

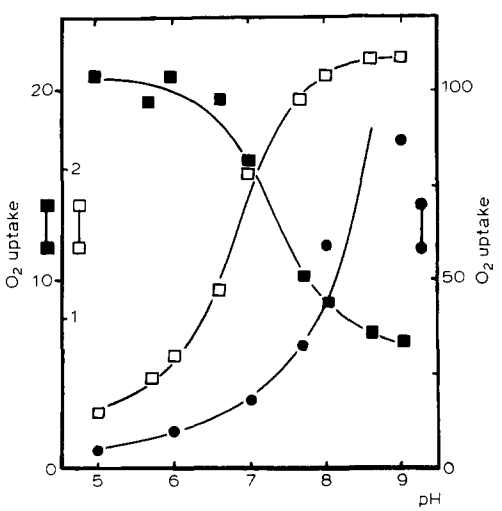

Fig. 3. Photooxidase activity of *R. rubrum* chromatophores and ubiquinone-1-induced oxidation of ascorbate by O_2 as functions of pH. □———□, photooxidase activity of native chromatophores; ■———■, photooxidase activity of chromatophores treated with 0.3% lauryldimethylamine oxide (LDAO); ●———●, ubiquinone-induced O_2 uptake. Incubation mixture for the measurement of photooxidase activity: 100 mM potassium phosphate buffer, 0.2 mM EDTA, 5 mM Tris/ascorbate, 0.1 mM TMPD, chromatophores with bacteriochlorophyll content $1 \cdot 10^{-5}$ M; in the experiment with lauryldimethylamine oxide (LDAO), incubation mixture also contained 5 mM *o*-phenanthroline; data are expressed as μmol of O_2 consumed/μmol bacteriochlorophyll per min minus dark O_2 uptake. Incubation mixture for the measurement of ubiquinone-induced O_2 uptake: 50 mM potassium phosphate buffer, 0.2 mM EDTA, 5 mM Tris/ascorbate, $5 \cdot 10^{-5}$ M ubiquinone-1; data are expressed as nmol of O_2 consumed/ml of reaction mixture per min minus O_2 uptake due to ascorbate autoxidation.

cyclic electron transfer from the exogenous donor to ubiquinone and to the subsequent interaction of photoreduced ubiquinone with O_2.

In a second series of experiments, we studied the role of non-cyclic electron transfer from different donors to oxygen and other acceptors in membrane potential generation measured by uptake of the penetrating PCB^- and tetraphenylborate anions by *R. rubrum* chromatophores. Most of these experiments were conducted with $Me_4Ph(NH_2)_2$, a carrier of H-atoms across the membranes, and of TMPD known as a carrier of electrons [25].

The illumination of chromatophores causes an uptake of PCB^- against the concentration gradient (Fig. 4), which indicates the generation of electric potential difference across the chromatophore membrane with a positive charge inside the vesicles [6]. A marked decrease in the PCB^- response of the illuminated chromatophores, when incubated aerobically in the absence of exogenous electron donors, may be attributed to photooxidase activity, causing an oxidation of cyclic redox chain components. A further decline in the membrane potential is observed upon the repeatedly switching on the light. PCB^- uptake by the illuminated chromatophores is restored to its original level under anaerobic conditions. Such an effect may be associated with the anaerobic

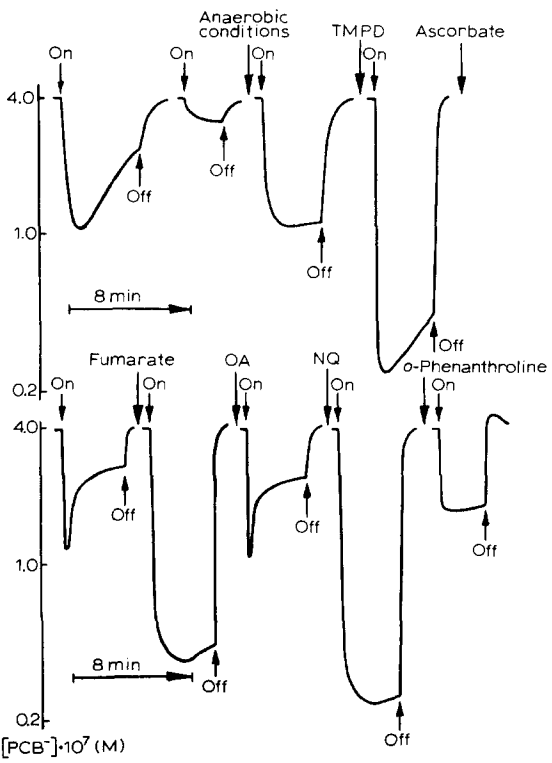

Fig. 4. Light-induced uptake of PCB^- by *R. rubrum* chromatophores under conditions of cyclic and non-cyclic electron transfer. Incubation mixture: 220 mM sucrose, 30 mM glucose, 50 mM Tris-HCl buffer (pH 7.6) and chromatophores (A_{880nm} = 2.3). Additions: 0.17 mg/ml catalase, 0.17 mg/ml glucose oxidase and paraffin oil for the establishment of anaerobiosis (see Methods), 0.1 mM TMPD, 5 mM Tris/ascorbate, 5 mM Tris/fumarate, 1 mM Tris/oxaloacetate (OA), 0.1 mM 1,4-naphthoquinone (NQ), 1 mM *o*-phenanthroline.

reduction of cyclic redox chain components by endogenous substrates. The aerobic decline of PCB^- response is also prevented upon the addition of TMPD and $Me_4Ph(NH_2)_2$ with or without ascorbate (not shown). PCB^- uptake by the anaerobic chromatophores is stimulated greatly by TMPD; PCB^- response tends to cause a decrease under these conditions. An inhibition of the PCB^- response occurs upon the subsequent addition of ascorbate. It is probable that this effect observed in the absence of exogenous electron acceptors is due to an over-reduction of cyclic redox chain components [26,27]. It should be noted that the oxidized TMPD is reduced by glucose with the help of glucose oxidase used in the experiments for the establishment of anaerobiosis. The rate of TMPD reduction by glucose is significantly below that with ascorbate.

TMPD and ascorbate inhibition of PCB^- uptake is not observed upon the addition of fumarate, an electron acceptor at the ubiquinone level. The fumarate action is removed by oxaloacetate, a competitive inhibitor of succinate dehydrogenase. The subsequent addition of another electron acceptor, 1,4-naphthoquinone, causes the maximal light-induced PCB^- response, which is inhibited by *o*-phenanthroline.

Of particular interest is a study of other electron acceptors with more negative redox potentials. It was previously shown that photophosphorylation in *R. rubrum* chromatophores incubated with TMPD and ascorbate is dependent upon exogenous electron acceptors such as oxygen, fumarate or methylviologen [4,6]. The stimulatory action of methylviologen is found also in experiments with the penetrating anions.

Fig. 5 shows that the PCB^- responses of chromatophores incubated with

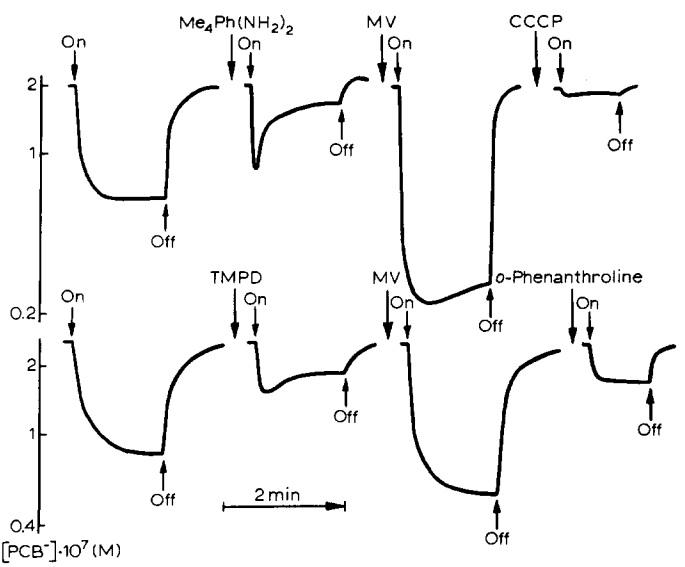

Fig. 5. Effect of methylviologen on light-induced uptake of PCB^- by *R. rubrum* chromatophores under anaerobic conditions. Incubation mixture: 220 mM sucrose, 30 mM glucose, 50 mM Tris-HCl buffer (pH 7.6), 5 mM Tris/ascorbate, chromatophores (A_{880nm} = 1.5), catalase and glucose oxidase. Additions: 0.1 mM $Me_4Ph(NH_2)_2$, 0.1 mM TMPD, 1 mM methylviologen (MV), $5 \cdot 10^{-6}$ M CCCP, 2 mM *o*-phenanthroline.

$Me_4Ph(NH_2)_2$ or TMPD in combination with ascorbate are inhibited significantly in the absence of oxygen. Under these conditions, the addition of methylviologen ($E_m = -440$ mV) enhances the PCB^- response, which is inhibited by carbonylcyanide *m*-chlorophenylhydrazone (CCCP) or *o*-phenanthroline, but not rotenone (data obtained with rotenone not shown).

Unlike methylviologen, NAD^+ and benzylviologen ($E_m = -320$ mV) do not remove the inhibitory action of TMPD and ascorbate upon the anaerobic generation of membrane potential. Vitamin K-3 and 1,4-naphthoquinone added in the presence of benzylviologen are also ineffective. Benzylviologen inhibits the light-induced tetraphenylborate anions response by a factor from 3 to 3.5 in chromatophores incubated aerobically with $Me_4Ph(NH_2)_2$ or anaerobically without electron donors (data not shown).

Thus, the membrane potential generation by anaerobic chromatophores is inhibited upon addition of permeable electron donors, such as $Me_4Ph(NH_2)_2$ or TMPD, in combination with ascorbate. The inhibitory effect is removed by oxygen, fumarate, 1,4-naphthoquinone, vitamin K-3, methylviologen, but not by NAD^+ or benzylviologen. A close similarity in the effects of $Me_4Ph(NH_2)_2$ (a carrier of H-atoms) and TMPD (a carrier of electrons) is found in the systems studied.

The illumination of *R. rubrum* chromatophores in the presence of 2,6-dichlorophenolindophenol and ascorbate has been reported to lead to the oxidation of the dye, whose content decreases upon the increase in the ascorbate concentration [28]. It is believed that $Me_4Ph(NH_2)_2$ and TMPD exhibit the same properties. The oxidized dye is likely to be bound to the membranes and/or located in the intrachromatophore volume.

Fig. 6 illustrates the light-induced uptake of tetraphenylborate anions by chromatophores, incubated aerobically with methylviologen and $Me_4Ph(NH_2)_2$ or TMPD, versus ascorbate concentration. An increase in ascorbate concentration from 0.5 mM to 50 mM has no significant effect upon aerobic tetraphenylborate anions uptake with $Me_4Ph(NH_2)_2$, but leads to inhibition of the tetra-

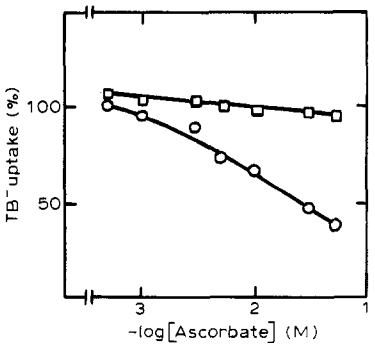

Fig. 6. Light-induced uptake of tetraphenylborate anions by *R. rubrum* chromatophores incubated aerobically with methylviologen and $Me_4Ph(NH_2)_2$ (□———□) or TMPD (○———○) versus ascorbate concentration. Incubation mixture: 250 mM sucrose, 50 mM Tris-HCl buffer (pH 7.6), 2 mM methylviologen, 0.1 mM $Me_4Ph(NH_2)_2$ or 0.1 mM TMPD, chromatophores ($A_{880nm} = 2.1$). 100% of tetraphenylborate anions uptake level corresponds to electric potential difference across the measuring phospholipid membrane of 80 mV in experiment with $Me_4Ph(NH_2)_2$ and of 84 mV in experiment with TMPD.

phenylborate anions response with TMPD. The increase in $Me_4Ph(NH_2)_2$ or TMPD concentration from $1 \cdot 10^{-6}$ M to 0.2—0.5 mM in the presence of 50 mM of ascorbate causes an increase in photooxidase activity (Fig. 7A) and inhibition of anaerobic tetraphenylborate anions uptake (Fig. 7B). However, tetraphenylborate anions uptake by chromatophores, under non-cyclic electron transfer conditions to methylviologen and O_2, is stimulated, as the $Me_4Ph(NH_2)_2$ concentration increases, and is significantly inhibited, as TMPD concentration increases (Fig. 7C). Thus, the increase of the rate of non-cyclic electron transfer leads to inhibition of membrane potential generation with TMPD and to its stimulation with $Me_4Ph(NH_2)_2$. Together with the data on phosphorylation [5], these results show that non-cyclic electron transfer from $Me_4Ph(NH_2)_2$ to O_2 is an electrogenic process, whereas non-cyclic electron transfer from TMPD to O_2 is non-electrogenic.

It was shown that $Me_4Ph(NH_2)_2$ and TMPD, by shunting of the cyclic chain, can also accelerate electron transfer and thereby stimulate the membrane

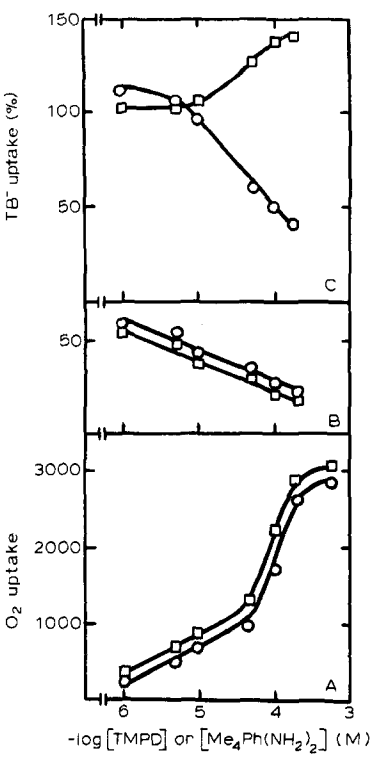

Fig. 7. Photooxidase activity (A) and light-induced anaerobic (B) and aerobic (C) uptake of tetraphenylborate anions by *R. rubrum* chromatophores versus $Me_4Ph(NH_2)_2$ (□———□) or TMPD (○———○) concentration. Incubation mixture: 220 mM sucrose, 30 mM glucose, 50 mM Tris-HCl buffer (pH 7.6), 5 mM (in experiment B) and 50 mM (in experiments A and C) Tris/ascorbate, chromatophores (A_{880nm} = 1.4 in A and 2.1 in B and C). Methylviologen (2 mM) was contained in experiment C. Photooxidase activity is expressed as nmol of O_2 consumed/μmol bacteriochlorophyll per min minus dark O_2 uptake. 100% of tetraphenylborate anions uptake level corresponds to electric potential difference across the measuring phospholipid membrane of 74 mV in experiment B, of 70 mV in experiment C with $Me_4Ph(NH_2)_2$ and of 66 mV in experiment C with TMPD.

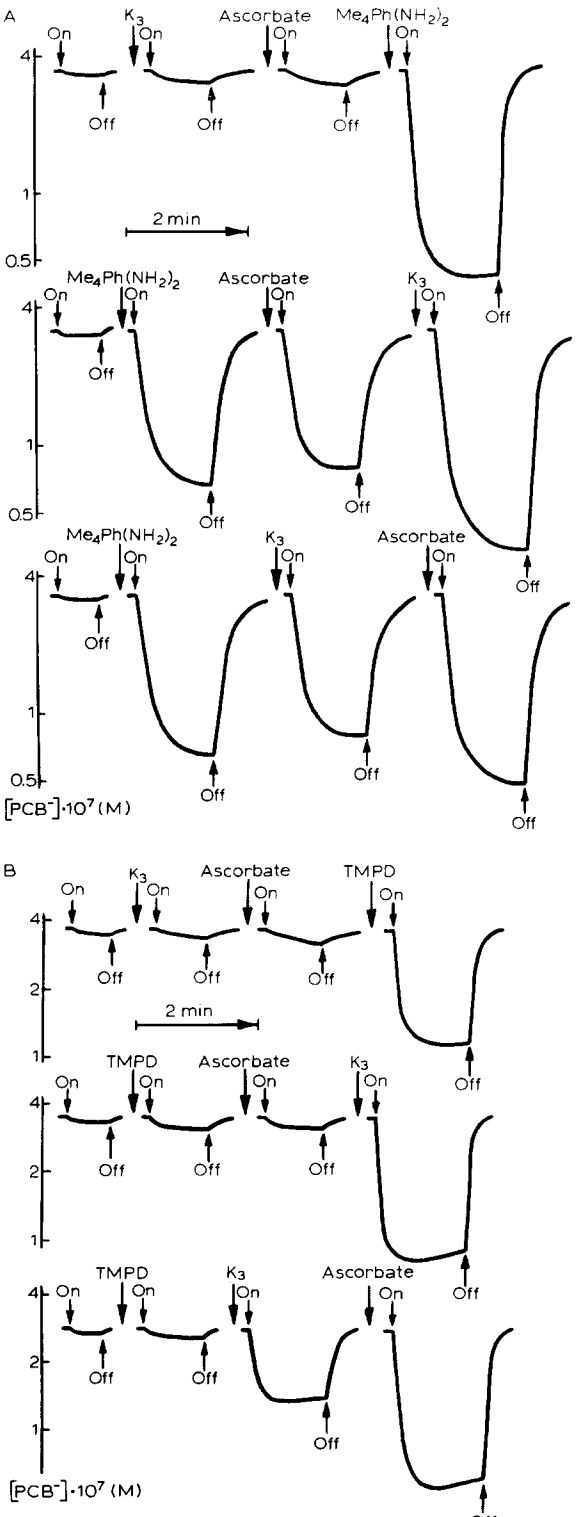

Fig. 8. Light-induced uptake of PCB^- by extracted *R. rubrum* chromatophores under aerobic conditions. Incubation mixture: 250 mM sucrose, 50 mM Tris-HCl buffer (pH 7.6), chromatophores (A_{880nm} = 1.4). Additions: 0.1 mM vitamin K-3 (K_3), 5 mM Tris/ascorbate, 0.1 mM $Me_4Ph(NH_2)_2$, 0.1 mM TMPD.

potential generation and photophosphorylation [6,26,27]. In order to ascertain the mechanism of cyclic redox chain shunting by $Me_4Ph(NH_2)_2$ and TMPD, experiments were conducted with chromatophores extracted with isooctane, containing 5% acetone, to remove loosely bound quinones. As seen from Fig. 8A and 8B, the extracted chromatophores generate the membrane potential upon the addition of $Me_4Ph(NH_2)_2$, but not vitamin K-3 or TMPD. The light-induced PCB^- uptake is observed also in the presence of TMPD and vitamin K-3 combination; its level is below that with $Me_4Ph(NH_2)_2$. The highest PCB^- responses are exhibited upon the addition of three components: $Me_4Ph(NH_2)_2$ plus vitamin K-3 plus ascorbate or TMPD plus vitamin K-3 plus ascorbate. Data on membrane potential generation by the extracted chromatophores show that the mechanisms of the shunting of the cyclic redox chain by $Me_4Ph(NH_2)_2$ and TMPD are different.

In the last series of experiments, we studied the effect of methylviologen upon membrane potential generation by the extracted chromatophores. Fig. 9 (upper trace) shows that the addition of methylviologen does not lead to a light-induced PCB^- uptake by the extracted chromatophores incubated aerobically with TMPD and ascorbate. The PCB^- response is observed upon the subsequent addition of 1,4-naphthoquinone. Fig. 9 (lower trace) shows that $Me_4Ph(NH_2)_2$-dependent PCB^- uptake by the extracted chromatophores, devoid of photooxidase activity, is inhibited by ascorbate when added at a concentration of 50 mM. The inhibitory effect of ascorbate is relieved by

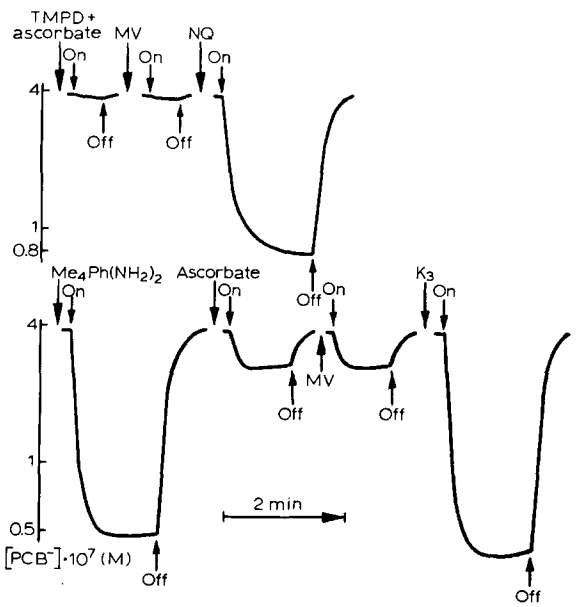

Fig. 9. Effect of methylviologen on light-induced uptake of PCB^- by extracted *R. rubrum* chromatophores under aerobic conditions. Incubation mixture: 250 mM sucrose, 50 mM Tris-HCl buffer (pH 7.6), chromatophores (A_{880nm} = 1.4). Additions: (upper trace) 0.1 mM TMPD, 5 mM Tris/ascorbate, 1 mM methylviologen (MV), 0.1 mM 1,4-naphthoquinone (NQ); (lower trace) 0.1 mM $Me_4Ph(NH_2)_2$, 50 mM Tris/ascorbate, 1 mM methylviologen (MV), 0.1 mM vitamin K-3 (K_3).

vitamin K-3, but not by methylviologen (Fig. 9, lower trace), and decreases as the ascorbate concentration is reduced to 5 mM (Fig. 8A). It is likely that methylviologen does not exert its properties as an electron acceptor in the extracted chromatophores.

Discussion

O_2 uptake by the illuminated chromatophores is sensitive to *o*-phenanthroline, and is inhibited upon the extraction of quinones from the membranes. The inhibitory effect of quinone extraction is relieved by vitamin K-3, but not methyl- and benzylviologen. The isolated *P*-870 reaction center complexes also show photooxidase activity, which is stimulated by vitamin K-3 and inhibited by *o*-phenanthroline.

In both chromatophores [13,29] and isolated reaction centers [29,30], ubiquinone was found to exist in at least two pools: a tightly bound pool and a loosely bound pool (the primary and secondary ubiquinones). It is assumed that electron transfer between the primary and secondary ubiquinones is mediated by non-heme iron and inhibited by *o*-phenanthroline [23,30].

The photooxidase activity which is sensitive to *o*-phenanthroline is most likely due to the interaction of the loosely bound photoreduced ubiquinone with O_2. Based on the data of Fig. 3, the protonation of the secondary quinone leads to the inhibition of its oxidation by O_2. The light-induced O_2 uptake, observed upon the addition of detergents and insensitive to *o*-phenanthroline, may be due to the interaction of O_2 with a photoreduced component distinct from the loosely bound ubiquinone. Two types of photooxidase activity are distinguished by their dependence on pH. Compared to the secondary ubiquinone, the non-identified component exhibits a higher affinity for O_2. It is probable that this component is identical with the tightly bound ubiquinone.

Superoxide radical anion (O_2^-) may be the product of the photooxidase reaction. The formation of O_2^- insensitive to *o*-phenanthroline was shown with illuminated reaction centers from *R. rubrum* [31]. The formation of O_2^-, taking place at a low rate, was observed also in illuminated *Chromatium vinosum* chromatophores [32]. The ubiquinone-dependent formation of O_2^- was also found with beef heart mitochondria and submitochondrial particles which are incubated with antimycin A and succinate or NADH [33].

The light-induced O_2 uptake by *Chr. vinosum* chromatophores has been reported to partly inhibit upon the addition of superoxide dismutase or of its combination with catalase [32]. In our experiments, *o*-phenanthroline-sensitive and *o*-phenanthroline-resistant (plus lauryldimethylamine oxide) photooxidase activity of *R. rubrum* chromatophores was lowered to 67 and 50% upon the addition of catalase, and to 40 and 25% upon the subsequent addition of superoxide dismutase isolated from *Neurospora crassa.*

An inhibition of membrane potential generation is observed when illuminated chromatophores are incubated aerobically in the absence of exogenous electron donors. This effect, which is removed by $Me_4Ph(NH_2)_2$ or TMPD (with or without ascorbate) and prevented under anaerobic conditions, may be due to the oxidation of cyclic redox chain components as a consequence of photooxidase activity.

The light-induced uptake of the penetrating PCB^- and tetraphenylborate anions by chromatophores is inhibited by $Me_4Ph(NH_2)_2$ or TMPD in combination with ascorbate under anaerobic conditions. This inhibitory effect is removed by fumarate, 1,4-naphthoquinone, vitamin K-3 or methylviologen, but not by NAD^+ or benzylviologen.

It has been shown that the bacterial membranes are impermeable to the mono- and divalent cations of methylviologen as well as to the divalent cations of benzylviologen, but are permeable to the monovalent cations of benzylviologen [34]. Inasmuch as methylviologen does not exert its properties as an electron acceptor in the extracted chromatophores, it is believed that methylviologen, like O_2, gains electrons from the loosely bound (secondary) ubiquinone (UQ) near the outside of the chromatophore membrane. From the data of Loach and coworkers [13,35], the E_m value for the UQ^-/UQ^{2-} couple apparently is in the neighbourhood of −400 mV in *R. rubrum* chromatophores.

The inhibitory effect of benzylviologen on the membrane potential generation may be due to the permeability of the chromatophore membranes to the monovalent cations of this dye. It is probable that benzylviologen as an electron acceptor competes efficiently with the secondary ubiquinone or with exogenous vitamin K-3. Being reduced by the primary ubiquinone, benzylviologen (in the form of monovalent cation) may act as a transmembrane electron carrier, which shunts the cyclic redox chain generating the membrane potential.

Probable mechanisms of $Me_4Ph(NH_2)_2$ and TMPD action in the cyclic and non-cyclic electron transfer chains are illustrated in Fig. 10. A tentative scheme presented in Fig. 10B implies that non-cyclic electron transfer from $Me_4Ph(NH_2)_2$ to exogenous acceptors is an electrogenic process, whereas the non-cyclic electron transfer from TMPD is non-electrogenic.

A role of exogenous electron acceptors in the system with TMPD and ascorbate could be associated with the maintenance of a suitable redox state of the cyclic photosynthetic chain, generating the membrane potential. This concept of the regulation of the cyclic electron transfer by the redox balance was made by Gest and Bose [26,27].

The data obtained with the extracted chromatophores devoid of the secondary ubiquinone show that the mechanisms of $Me_4Ph(NH_2)_2$ and TMPD action in the cyclic photosynthetic chain are different (Fig. 10A). Most probably, $Me_4Ph(NH_2)_2$ functions as a transmembrane H-atom carrier mediating cyclic electron transfer between the primary ubiquinone and the *P*-870 bacteriochlorophyll. As for TMPD, its effect with the extracted chromatophores occurs only in the presence of a quinone (vitamin K-3). Under these conditions, the reduction of exogenous vitamin K-3 by the primary ubiquinone and its protonation on the external side of the chromatophore membrane, its subsequent transmembrane movement, and its reoxidation by the *P*-870 bacteriochlorophyll on the internal side of the membrane with the help of TMPD, are likely to be the cause of the generation of the transmembrane proton gradient. This assumption is in agreement with data obtained on the proteoliposomes containing reaction center complexes of *R. rubrum* [36]. It was shown that the membrane potential generation in the proteoliposomes

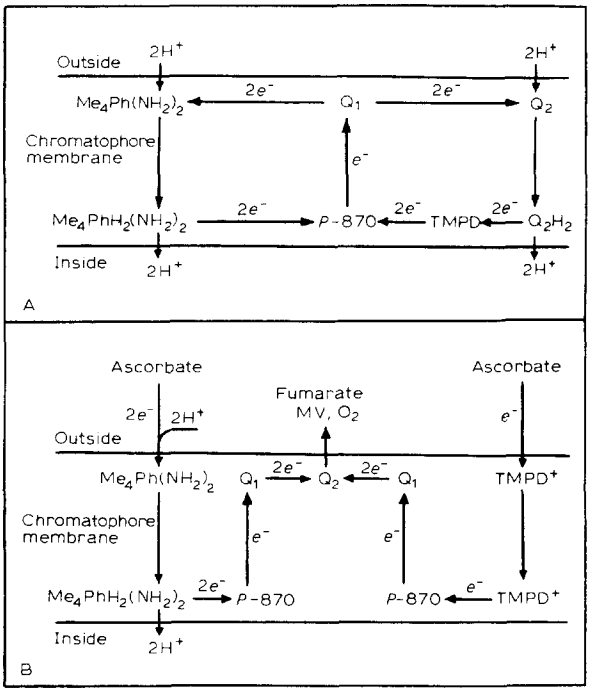

Fig. 10. Scheme of $Me_4Ph(NH_2)_2$ and TMPD action in cyclic (A) and non-cyclic (B) photosynthetic redox chain in *R. rubrum* chromatophores. *P*-870, bacteriochlorophyll reaction center; Q_1, primary ubiquinone; Q_2, secondary ubiquinone or exogenous quinone; MV, methylviologen.

('minus' inside) is dependent upon quinones (ubiquinone-6 or vitamin K-3) and TMPD (or mammalian cytochrome *c*).

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