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THE INHIBITION OF PHOTOSYNTHETIC ELECTRON TRANSFER IN *RHODOSPIRILLUM RUBRUM* BY *N,N'*-DICYCLOHEXYLCARBODIIMIDE

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Summary

N,N'-Dicyclohexylcarbodiimide (DCCD) at concentrations above 0.1 mM inhibits light-induced generation of a membrane potential in the course of cyclic and non-cyclic electron transfer, as well as light-induced oxygen uptake due to an interaction of photoreduced secondary (loosely bound) ubiquinone with O_2 in *Rhodospirillum rubrum* chromatophores. Similarly to *o*-phenanthroline, DCCD blocks the electron transfer in the chromatophores between the primary (tightly bound) and secondary ubiquinones.

Introduction

N,N'-Dicyclohexylcarbodiimide inhibits synthesis and hydrolysis of ATP in mitochondria [1], chloroplasts [2] and in membranes of phototrophic [3] and organotrophic [4,5] bacteria, as a consequence of binding to a low molecular weight proteolipid which functions as a proton channel to the ATP synthetase complex (see Refs. 6 and 7). The inhibition of the membrane phosphorylation of ADP by DCCD causes an electron transfer retardation which is reversed by adding proton-ionophore uncouplers.

However, the suppression of light-induced electron transfer in chloroplasts is not removed by an uncoupler when DCCD is added at high concentrations [2].

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Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCCD, *N,N'*-dicyclohexylcarbodiimide; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine.

This effect is due to inhibition of plastoquinone protonation and deprotonation reactions, as suggested by Trebst and coworkers [8]. Besides, the proton translocation by mitochondrial cytochrome *c* oxidase is suppressed by DCCD at a concentration of 0.1 mM and over [9].

In this paper, effects of DCCD on the electron transfer and the membrane potential generation in the chromatophores of the non-sulfur purple bacterium *Rhodospirillum rubrum* are studied. The results obtained show that DCCD, similarly to *o*-phenanthroline, blocks the light-induced electron transfer in the chromatophores between the primary (tightly bound) and secondary (loosely bound) ubiquinones.

Methods

Cells of *Rhodospirillum rubrum* (wild-type strain No. 1 MGU) were grown and chromatophores were prepared as described previously [10]. The bacteriochlorophyll content of chromatophores was measured using an extinction coefficient of 75 $mM^{-1} \cdot cm^{-1}$, at 772 nm in vitro [11]. The membrane potential generation in the isolated chromatophores was estimated from the uptake of penetrating tetraphenylborate anions using a phospholipid (azolectin)-impregnated membrane (Teflon) filter as a selective electrode [12] (see also Ref. 10). Oxygen uptake by the chromatophores was measured polarographically with a platinum electrode at a voltage of 0.65 V. Actinic light of saturating intensity ($\lambda > 660$ nm) was employed for the illumination of the chromatophore suspensions. The measurements of the luminescence lifetimes and relative quantum yields were performed with a phase-type fluorimeter [13]. Some experiments were carried out in an anaerobic cuvette: 0.17 mg/ml glucose oxidase (EC 1.1.3.4) and 0.17 mg/ml catalase (EC 1.11.1.6) were added to an incubation mixture containing 30 mM glucose; sunflower oil (6—8 mm thickness) was layered on top of the aqueous phase.

Results and Discussion

DCCD has been reported to inhibit photophosphorylation and ATP-dependent transhydrogenase reaction in *R. rubrum* chromatophores at low concentrations [3]. As seen in Fig. 1, DCCD at a concentration of 20 μM inhibits ATP-dependent uptake and has no appreciable effect on inorganic pyrophosphate-dependent uptake of penetrating tetraphenylborate anions — a process indicating that an electrical potential difference is generated across the chromatophore membrane, with a positive charge inside the vesicles [10]. The tetraphenylborate response induced by inorganic pyrophosphate is suppressed by an uncoupler, CCCP. Inasmuch as the inhibitory effect developed with time, the chromatophores were preincubated with DCCD in subsequent experiments.

Fig. 2 (circles) shows an effect of DCCD on the light-induced membrane potential generation by the chromatophores incubated anaerobically under conditions of the cyclic electron transfer. A progressive inhibition is caused by DCCD at concentrations exceeding 0.1 mM.

The membrane potential generation in the chromatophores is observed in the course of both the light-induced cyclic and the non-cyclic electron transfer

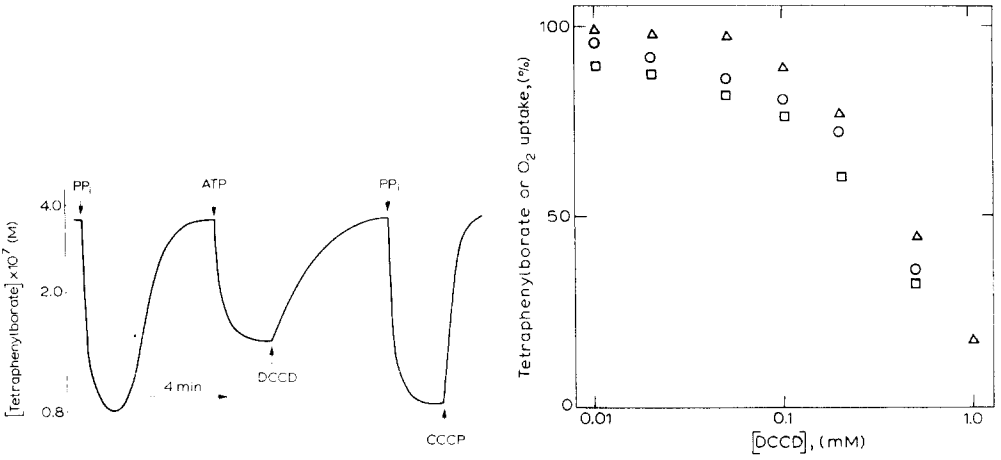

Fig. 1. Inorganic pyrophosphate (PP_i)- and ATP-induced uptake of tetraphenylborate anions by *R. rubrum* chromatophores. Incubation mixture: 50 mM Tris-HCl buffer (pH 7.6), 5 mM $MgSO_4$ and chromatophores (6.4 μM bacteriochlorophyll). Aerobic conditions. Additions: 10 μM PP_i, 0.1 mM ATP, 20 μM DCCD, 50 μM PP_i, 2 μM CCCP.

Fig. 2. Effect of DCCD on light-induced uptake of tetraphenylborate anions (○,□) and light-induced O_2 uptake (△) by *R. rubrum* chromatophores. Incubation mixture: 50 mM Tris-HCl buffer (pH 7.6). Conditions: ○, anaerobiosis, 5 mM Tris succinate, chromatophores (14.3 μM bacteriochlorophyll), 100% of tetraphenylborate uptake level corresponds to an electrical potential difference of 94 mV across the phospholipid membrane; □, aerobiosis, 50 mM Tris ascorbate, 2 mM methylviologen, 0.1 mM diaminodurene, chromatophores (14.3 μM bacteriochlorophyll), 100% of tetraphenylborate uptake level corresponds to 84 mV; △, aerobiosis, 10 mM Tris ascorbate, 0.1 mM diaminodurene, chromatophores (14.3 μM bacteriochlorophyll), 100% of photooxidase activity corresponds to 2200 nmol of O_2 consumed per μmol bacteriochlorophyll per min minus dark O_2 uptake. Chromatophores were preincubated with DCCD for 10 min.

[14]. DCCD inhibits the tetraphenylborate responses in the chromatophores incubated aerobically with ascorbate, diaminodurene (2,3,5,6-tetramethyl-*p*-phenylenediamine) and methylviologen (Fig. 2, squares). It was shown that the non-cyclic electron transfer from diaminodurene to methylviologen and O_2 in *R. rubrum* chromatophores proceeds without cytochrome participation [14].

Thus, the membrane potential generation in the chromatophores under conditions of the cyclic and non-cyclic electron transfer is inhibited by DCCD at concentrations above 0.1 mM.

In further experiments, we studied an effect of DCCD on photooxidase activity of the chromatophores — the light-induced electron transfer from an exogenous donor to oxygen. As seen in Fig. 2 (triangles), the photooxidase reaction of the chromatophores with diaminodurene (plus ascorbate) as electron donor, along with the responses of the penetrating tetraphenylborate anions, is suppressed by DCCD. The pattern of DCCD action on the photooxidase reaction is unaltered upon the addition of an uncoupler, CCCP (data not shown).

The light-induced O_2 uptake by the native chromatophores incubated with diaminodurene or *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPD) was demonstrated to be due to the interaction of photoreduced secondary (loosely

bound) ubiquinone with oxygen [14]. Consequently, DCCD suppresses membrane potential generation by blocking the electron transfer. As shown previously [14], the same effect is exhibited by *o*-phenanthroline, which is known to act as an inhibitor of the electron transfer between primary and secondary non-porphyrin acceptors [15,16]. These acceptors are the tightly bound and loosely bound quinone, respectively; the electron transfer between the primary and secondary quinones is mediated by non-heme iron (see Refs. 17 and 18).

With the primary quinone reduced, a short-lived afterglow, arising from the recombination of oxidized bacteriochlorophyll dimer $P\text{-}870^+$ and reduced intermediate acceptor I^- (bacteriopheophytin or its complex with *P*-800), has been reported to occur along with bacteriochlorophyll prompt fluorescence in the chromatophores of purple bacteria [19,13]. The nanosecond recombination luminescence appears, if the primary quinone is reduced either chemically, by dithionite [13], or photochemically, upon the addition of *o*-phenanthroline in combination with ascorbate [20]. The total chromatophore emission observed under reducing conditions is characterized by the increased lifetime (τ) and the decreased quantum yield (φ) as compared to the prompt fluorescence measured in the light of saturating intensity [13].

In agreement with data [13,20], Table I shows that the addition of dithionite or *o*-phenanthroline in combination with ascorbate and TMPD to the chromatophores incubated with CCCP causes an increase in τ from 0.2 to 2.6—2.7 ns and a decrease in the relative value of φ from 3.1 to 1.8. The same effect is produced by DCCD. The increase in τ and the decrease in φ are also observed upon the addition of bathophenanthroline, but not antimycin A.

Emergence of nanosecond bacteriochlorophyll luminescence in aerobic chromatophores treated with DCCD demonstrates that DCCD inhibits electron transfer between the primary and secondary ubiquinones, inasmuch as the light-induced accumulation of reaction centers in the state with reduced primary quinone under aerobic conditions is possible in this case only. If the inhibition took place after the secondary quinone, electrons from the reduced

TABLE I

LUMINESCENCE LIFETIMES (τ) and QUANTUM YIELDS (φ) OF *R. RUBRUM* CHROMATOPHORES

Incubation mixture: 220 mM sucrose, 50 mM Tris-HCl buffer (pH 7.6), 2 mM $MgSO_4$, 1 μM CCCP and chromatophores (7.2 μM bacteriochlorophyll). Aerobic conditions. Additions: 1 mg/ml sodium dithionite, 5 mM sodium ascorbate, 0.1 mM TMPD, 2 mM *o*-phenanthroline, 0.5 mM DCCD, 30 μM bathophenanthroline, 1 or 2 μM antimycin A. Chromatophores were preincubated with *o*-phenanthroline, DCCD or bathophenanthroline for 10 min.

Conditions	τ(ns)	φ(relative units)
1 Without additions	0.20	3.1
2 Dithionite	2.71	1.8
3 TMPD ascorbate	0.75	2.1
4 (3) + *o*-phenanthroline	2.60	1.8
5 (3) + DCCD	2.57	1.8
6 (3) + bathophenanthroline	2.05	1.6
7 TMPD, ascorbate	0.78	2.1
8 (7) + antimycin A	0.78	2.1
9 (8) + *o*-phenanthroline	2.54	1.7

primary quinone would be transferred to oxygen via the secondary quinone. In particular, the short-lived afterglow does not arise upon addition of antimycin A which inhibits electron transfer in the *b* type cytochrome region.

Thus, DCCD at low concentrations inhibits the synthesis and the hydrolysis of ATP as does oligomycin, and at greater concentrations blocks the electron transfer between the primary and secondary electron acceptors in the chromatophores, similarly to *o*-phenanthroline.

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