Specific binding of DCCD to reaction centers of the photosynthetic bacterium *Rhodospirillum rubrum* and its effect of certain photosynthetic reactions

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DCCD binds strongly to chromatophores and reaction centers of the photosynthetic bacterium *Rhodospirillum rubrum*. Analysis by gel electrophoresis reveals a highly specific binding to subunit M of the reaction center. The binding of the hydrophilic carbodiimide EDC to reaction centers or membranes is weak. Binding of up to 4 DCCD molecules/M subunit has no effect on primary electron transport. With membrane levels of $500 \mu M$ DCCD, ATP synthesis was inhibited to 50% but had no effect on the function of the reaction center, the electron transport or the formation of the Δ pH and $\Delta\Psi$.

DCCD binding	*Reaction center*	*Chromatophore*	*Photosynthetic bacterium*
Rhodospirillum rubrum	*Protonmotive force*	*Electron transport*	

1. INTRODUCTION

DCCD is known to inhibit the synthesis as well as the hydrolysis of ATP in energy-transducing organelles such as mitochondria [1,2], chloroplasts [3] and in membranes of bacteria [4–6] by binding to a low molecular subunit of the F_o-F_1 coupling factor. It is assumed that this subunit, the DCCD-binding protein, acts as a proton channel in the ATPase complex. DCCD also binds to other membrane components, e.g., to the cytochrome oxidase [7,8] and in phototrophic bacteria to the bound quinones. In the latter case it binds to the acceptor site of the photosynthetic reaction center thereby inhibiting photosynthetic electron transport [9,10]. The electron transport of chloroplasts is inhibited by DCCD [11]. Furthermore, certain carbodiimides also act on the proton pump of *Halobacterium* [12,13] or on the proton/sugar symport of *Rhodotorula* [14]. In all these cases it has been suggested that the inactivation is related

to proton uptake or proton translocation.

Incubation of chromatophores of *Rhodospirillum rubrum* with low [DCCD] results in a strong binding of the reagent with compounds of the membrane. Besides, the well known DCCD-binding protein analysis of the protein pattern reveals a preferential labelling of a polypeptide of M_r 20000–25000, in the region where the subunits of the bacterial reaction center are found [15].

This paper gives evidence that only 1 of the 3 subunits of the reaction center, subunit M, reacts with the hydrophobic marker DCCD, DCCD thus being a specific marker for this polypeptide. No effect of DCCD on the bleaching of P870 was observed, the DCCD-binding to subunit M has thus no effect on the electron transport within the reaction center. In contrast to other DCCD-binding membrane proteins no relation to transport of protons was found.

2. MATERIALS AND METHODS

Chromatophores and reaction centers were prepared from cells of the carotenoidless mutant G-9 of *R. rubrum* [16]. DCCD (stock solution

1.4 mg/ml in ethanol) was added to chromatophores or reaction centers (chromatophores 18 mg/ml, reaction centers 7 mg protein/ml in 10 mM Tris buffer, pH 8) which were then incubated for 12 h at 4°C. For the analysis of binding the incubation was done with [14C]DCCD (Amersham or CEA). After incubation the membranes or proteins were separated on Sephadex G-25 from the incubation medium containing the unbound DCCD. The amount of marker bound, and its localisation on the polypeptides were determined in the chromatophore membrane fraction and reaction centers, respectively, by the use of SDS–PAGE. Gradient gels were done as in [17] and stained with Coomassie Blue. Single tracks were cut out and sliced into 2-mm pieces. The radioactivity in the samples was determined after digestion in hydrogen peroxide at 50°C. For the studies of functional changes due to modification with DCCD, incubations were run with unlabelled DCCD. The following parameters have thereby been tested.

2.1. Photophosphorylation

Chromatophores equal to 150 μg bacteriochlorophyll, Tris–HCl (pH 8) 50 mM; NaCl 10 mM; MgCl$_2$ 10 mM; ADP 5 mM; PMS 50 μM; Na-ascorbate 1 mM; [32P]phosphate 5 mM; total volume of 2 ml was incubated for 1 min at 25°C in white light at a light intensity of 20 mW/cm². The reaction was terminated by addition of perchloric acid, then the esterified phosphate was determined as in [18]. Bacteriochlorophyll was measured as in [19].

2.2. Light-induced absorption changes in the reaction center

Chromatophores were equal to 20 μg bacteriochlorophyll in tricine (pH 8) 50 mM; MgCl$_2$ 3 mM. Light-induced absorption changes were recorded at 600–650 nm on a DW-2 equipped with side illumination (red light of wavelength higher than 720 nm, light intensity 1.5 mW/cm²).

2.3. Light-induced changes in the ΔpH by the 9-amino acridine method [20]

Chromatophores were equal to 10 μg bacteriochlorophyll; tricine buffer (pH 8) 50 mM; PMS 50 μM; MgCl$_2$ 3 mM; Na-succinate 100 μM; 9-amino acridine 3.3 μM; total volume 3 ml. The

light-induced quenching of the fluorescence of 9-amino acridine was measured with a Hitachi/Perkin Elmer fluorimeter 203, equipped with side illumination through a glass fiber light conductor. The wavelength of the actinic light for the photosynthetic reaction was greater than 600 nm (light intensity 3 mW/cm²), the fluorescence was induced with 390 nm actinic light and measured at 450 nm using an appropriate filter (Balzers K 5) to eliminate scattering.

2.4. Light-induced changes in the $\Delta\psi$ by the carbocyanine method [20]

Chromatophores were equal to 200 μg bacteriochlorophyll; tricine buffer (pH 8) 50 mM; MgCl$_2$ 3 mM; Na-succinate 1 mM; PMS 50 μM; DiS-C$_3$ [5] 2 μM. The reaction was followed by measuring the absorption changes at 670–630 nm on a DW-2 equipped with side illumination (conditions as in [2]).

3. RESULTS AND DISCUSSION

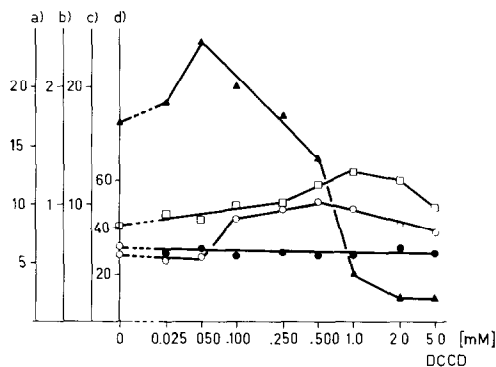Fig.1 shows the effect of DCCD on photo-

Fig.1. Effect of DCCD on various photosynthetic reactions of chromatophores of *R. rubrum*. All experiments have been done after 12 h incubation of chromatophores (18 mg protein/ml) with DCCD (concentration given on abscissa). The chromatophore concentration for the different experiments is given in section 2: (a) light-induced bleaching of P870 given as absorption changes (600–650 nm) × 10⁻³; (b) photophosphorylation, given as μmol ATP formed.mg bacteriochlorophyll⁻¹.min⁻¹; (c) light-induced changes in membrane potential ($\Delta\psi$) given as absorption changes of carbocyanine at 670–630 nm × 10⁻³; (d) light-induced changes in Δ pH-given fluorescence quenching of 9-aminoacridine (in % of dark value).

phosphorylation and on the formation of a protonmotive force; i.e., on ΔpH and $\Delta\psi$. With well-washed chromatophores at high concentrations of protein (18 mg/ml) low levels of DCCD exhibit a significant stimulation of phosphorylation, possibly by lowering the permeability of the membrane to protons [21,22]. The relative value of the ΔpH, unchanged at low concentrations of DCCD (0–0.1 mM), increases with increasing concentrations (0.1–1 mM DCCD). At higher concentrations the ΔpH is again reduced. The dependence of the $\Delta\psi$ on DCCD is similar to the one for the ΔpH. The decrease in both ΔpH and $\Delta\psi$, parallel to a rapid decrease in the phosphorylation between 1–2.5 mM DCCD further suggests an inhibition of the electron transport [9,10]. The primary photochemical act, the bleaching of the P865 in the light, is not influenced at all by DCCD. In contrast, when DCCD is added to diluted chromatophores (concentrations as in the assay systems) ΔpH and $\Delta\psi$ are abolished up to 85% at

Fig.2. Binding of [^{14}C]DCCD to chromatophores and reaction centers of *R. rubrum*. Chromatophores or reaction centers (protein concentration 18 mg/ml and 7 mg/ml, respectively) were incubated with [^{14}C]DCCD (final concentration indicated on abscissa) for 12 h, the proteins separated from the medium by gel filtration and aliquots of the membrane fraction used for the determination of the radioactivity and the protein concentration.

10^{-5} M DCCD. Under these conditions a complete inhibition is also seen for photophosphorylation, due to an inhibitory effect on electron transport and ATP synthesis. However, the bleaching of the P865 in the light is not changed.

Fig.2 demonstrates the binding of radioactive DCCD to chromatophores and isolated reaction centers. On a protein basis the binding to reaction centers is about 1.5-times higher than to chromatophores. When the reaction centers or chromatophores are separated from the incubation medium on Sephadex, about 1/3 of the DCCD is found to be bound to the membrane fraction eluted with the void volume. The rest appears later after the elution of the chromatophore membranes or reaction center proteins. Gels of chromatophores and reaction centers labelled with low concentrations of DCCD clearly show most of the label in the subunit M of the reaction center. In chromatophores about 2/3 of the label is found in the subunit M of the reaction center while the rest is bound to low-M_r proteins, possibly the DCCD-binding proteolipid of the F_0 part of the ATPase (fig.3a,b).

The water-soluble EDC shows a weaker affinity to the chromatophore proteins. At up to 50 mM EDC, only 1–2% of the reagent is bound to the protein fraction. A specific binding of EDC to reaction center peptides under the experimental conditions described is hardly evident. This indicates that whole membranes contain more accessible carboxyl groups of amino acids in the hydrophilic region compared to reaction center proteins. In chromatophores, EDC is bound to proteins of M_r 10000–20000 and of $M_r > 30000$. In isolated reaction centers some radioactivity is found in the region of M_r-values greater than the reaction center subunits. Carbodiimides react preferentially with accessible carboxylic groups [23]. Reaction centers of *R. rubrum* contain about 7 mol% of glutamic and aspartic acid. In *Rhodopseudomonas spheroides* all 3 subunits contain these two amino acids in roughly equal amounts [15]. So far only partial information is available on the amino acid sequence of the 3 reaction center polypeptides of any photosynthetic bacterium. In *R. spheroides* glutamic and/or aspartic acid are present among the first 25 amino acids from the N-terminus of all 3 subunits [24]. The orientation of the N-termini in relation to the
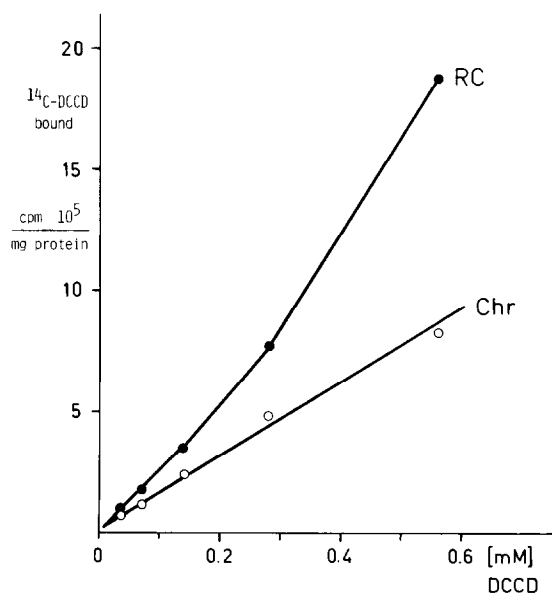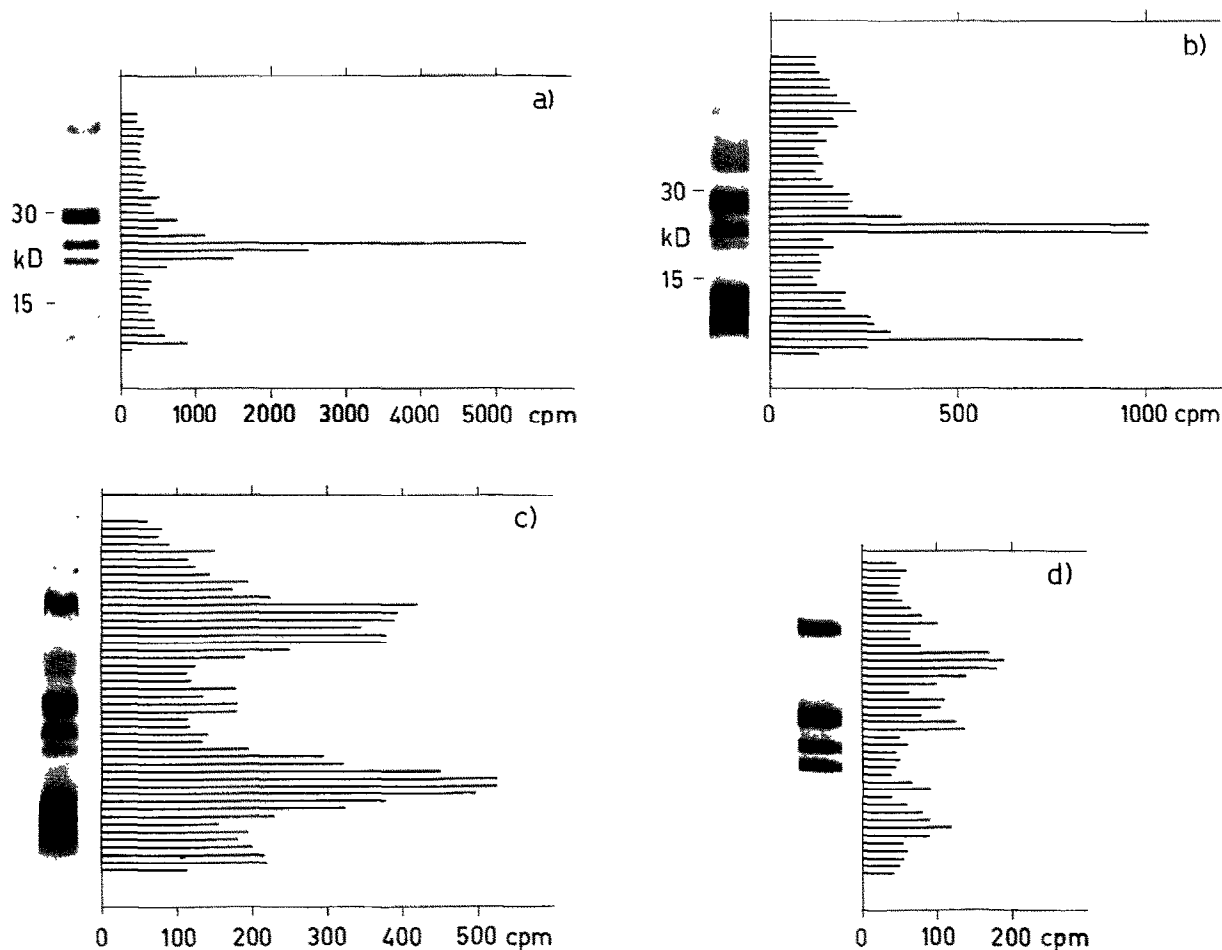
Fig.3. Analysis of the binding of DCCD (a,b) and EDC (c,d) to chromatophores (a,c) and isolated reaction centers (b,d) with gel electrophoresis. Incubation conditions as in fig.2: 20–50 μl were used for gel electrophoresis (gradient gel, 8–16% acrylamide).

2 membrane surfaces is not yet known. Since EDC hardly reacts with the 3 subunits, the N-terminal side chains do not seem to be accessible to modification. In contrast, the DCCD-modified carboxylic group(s) of subunit M are probably situated more in the hydrophobic part of the protein.

From the data in fig.2 it is possible to calculate the stoichiometry of the DCCD bound to the reaction center. The reaction is proportional to the concentration. No saturation is seen in the concentration range investigated. At the highest concentration (550 μM) more than 3 DCCD are bound to 1 molecule of subunit M. It will be of great interest to find out where these possible glutamic acid

residues of the estimated total 20 Glu residues of subunit M are located within the polypeptide. Thus DCCD seems to be a specific marker for the hydrophobic subunit M, no other marker tested so far reacted only with one single protein of the photosynthetic reaction center [25–28].

Already at 500 μM DCCD, ATP synthesis in chromatophores is reduced to 50%. This demonstrates that the binding of the inhibitor to the F_0 part results in an immediate decrease of the ATP synthetase activity whereas the simultaneous binding to the reaction center has no effect, neither on its function nor on the subsequent electron transport and the formation of the $\Delta\psi$ and ΔpH.

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