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Induction of an electrogenic transfer of monovalent cations (K^+, NH_4^+) in thylakoid membranes by *N,N'*-dicyclohexylcarbodiimide

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The effect of *N,N'*-dicyclohexylcarbodiimide (DCCD) on photosynthetic electron transport and light-induced NH_4^+ and K^+ uptake in the presence of ammonium or nigericin was studied. DCCD alone had no effect on either the electron transport or the uptake of protons. The simultaneous action of DCCD and low concentrations of ammonium or nigericin was shown to lead to a significant increase in the electron transport rate and a decrease in the steady-state uptake value of H^+ and NH_4^+ or K^+. The effect of DCCD on these processes was compared with the effect of the ionophore, valinomycin, which transports potassium and ammonium cations through membranes. The conclusion was made that 1.0–1.5 mol DCCD per mol chlorophyll activated the transfer system of monovalent cations (K^+ and NH_4^+) in thylakoid membranes.

N,N'-Dicyclohexylcarbodiimide; Thylakoid membrane; Electron transport; K^+; NH_4^+; Cation transfer

1. INTRODUCTION

N,N'-Dicyclohexylcarbodiimide (DCCD) is often used to modify carboxylic groups of proteins in studies of energy transformation in coupling membranes. DCCD suppresses ATP synthesis and hydrolysis by abolishing the proton transfer via the CF_o of the ATP-synthase at a DCCD-to-chlorophyll ratio near 1:1 [1]. It also inhibits electron transfer in a partial reaction with duroquinone as an electron donor and methyl viologen (MV) as an acceptor [2]. At high concentrations DCCD decreases the electron transfer rate from water to MV [2,3]. It has been reported recently that under flashing light, the extent of both proton release into the lumen by water oxidation and of proton uptake from the medium by reduced quinone is diminished in DCCD-treated membranes [3].

The data reported in the present paper show that in thylakoid membranes DCCD activates a transfer system for monovalent cations, e.g. K^+, NH_4^+, rather than protons.

2. MATERIALS AND METHODS

Chloroplasts were isolated from pea leaves as described [4] and suspended in medium containing 200 mM sucrose, 5 mM $MgCl_2$, 10 mM NaCl and 5 mM Tris-HCl, pH 7.8. The composition of reaction mixtures and chlorophyll content are given in the figure legends. All the reactions were carried out in thermostatic cells at 20°C and pH 7.6–7.8. The oxygen consumption was measured by a platinum Clark-type electrode in the presence of MV. The light-dependent uptake of H^+, K^+ and NH_4^+ cations was determined with ion-selective electrodes. Calibrations were performed with standard solutions of HCl, NH_4Cl and KCl. In experiments where NH_4^+ or K^+ uptake was determined, the actual pH change observed did not contribute more than 2% to the total amount of cation uptake measured. DCCD, valinomycin and nigericin (Sigma) were dissolved in ethanol and added to the reaction mixture 1 min before the light was switched on. The chlorophyll concentration was determined according to Arnon [5].

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3. RESULTS

The results of the simultaneous action of DCCD and either NH_4^+ or nigericin on the electron transfer from water to MV are shown in Fig. 1. DCCD alone did not significantly affect the electron transfer rate (Fig. 1, curve 1). A considerable increase was observed if the reaction medium contained ammonium at low concentration (Fig. 1, curve 3). The latter occurred irrespective of the presence of 10 mM NaCl or KCl. DCCD addition also stimulated electron transfer in the presence of low nigericin concentrations. Such an effect was only observed in the presence of 10 nM nigericin if the reaction mixture contained K^+ ions (Fig. 1, curve 2). The stimulation of electron transport was maximal when the DCCD-to-chlorophyll ratio was 1.2–1.5. The increase in DCCD concentration higher than 2 mol per mol chlorophyll suppressed electron transfer, stimulated by low concentrations of ammonium or nigericin (Fig. 1, curves 2, 3), and uncoupled by 0.1 μM gramicidin D (Fig. 1, curve 4). This might be caused by the direct action of high DCCD concentrations on electron transport carriers [2].

The electron transport acceleration under the simultaneous action of DCCD and either ammonium or nigericin was accompanied by a decrease in light-induced proton uptake (Table I).

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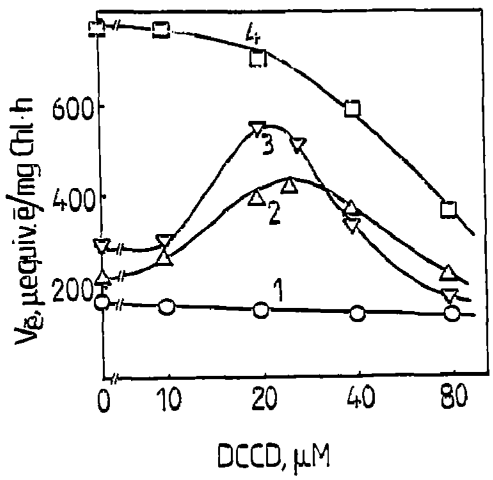

Fig. 1. Effect of DCCD on electron transport rate in the absence (1) and presence of 10 nM nigericin (2), 0.2 mM NH_4Cl (3), or 0.1 μM gramicidin D (4). The reaction mixture contained 200 mM sucrose, 5 mM $MgCl_2$, 10 mM KCl, 0.1 mM MV, 5 mM Tris-HCl (pH 7.8) and 20 μg chl/ml.

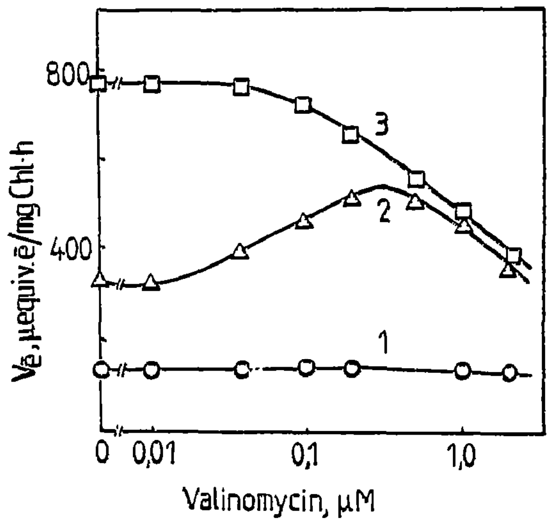

Fig. 2. Effect of valinomycin on electron transport rate in the absence (1) and presence of 0.4 mM NH_4Cl (2) or 0.1 μM gramicidin D (3). The reaction mixture is as in Fig. 1.

Similar results were obtained when studying the simultaneous action of ammonium and valinomycin. Curve 1 in Fig. 2 shows that valinomycin at a concentration up to 1 μM had no effect on the basal electron transport rate, but it increased the electron transfer rate in the presence of small quantities of ammonium ions (Fig. 2, curve 2). At high concentrations (above 0.5 μM) valinomycin inhibited the ammonium-stimulated (Fig. 2, curve 2) and gramicidin D-uncoupled transport (Fig. 2, curve 3). The electron transport acceleration in the presence of low concentrations of ammonium or nigericin was also observed after addition of 0.1 nM gramicidin D [6,7], which at this concentration induces transmembrane fluxes of K^+, Na^+ and NH_4^+ ions.

Using an NH_4^+-sensitive electrode to measure ammonium uptake we showed that in the light, chloroplasts took up about 0.7 equiv. NH_4^+ per mol chlorophyll (Fig. 3, curve a). With choline chloride instead of sucrose it was possible to considerably increase the ΔNH_4^+ value (Fig. 3, curve b, c). The addition of 1.0–1.5 mol of DCCD per mol of chlorophyll or 0.2–0.4 μM valinomycin to the incubation medium significantly decreased the ΔNH_4^+ value (Fig. 3, curve a, b, c), which was followed by increases in the electron transport rate (Figs. 1 and 2).

As seen from Fig. 4 the light-induced K^+ uptake without addition of nigericin was minor, however, K^+ uptake increased to 0.8 equiv. K^+ per mol chlorophyll following addition of nigericin (Fig. 4). One should take into account that to induce the light-dependent K^+ uptake, the nigericin concentration needed to be near 0.1 μM rather than the 0.01 μM used in Fig. 1. This is understandable owing to the requirement that KCl concentration was no higher than 0.5–1.0 mM in the experiments with a K^+-selective electrode. The addition of DCCD decreases the light-induced K^+ uptake in the presence of nigericin (Fig. 4). Valinomycin acted similarly (data not shown).

4. DISCUSSION

It is well known that electron transport from water to photosystem I acceptors is accelerated when the H^+ conductivity of the thylakoid membrane increases. In our experiments DCCD per se did not increase the H^+ conductivity. The addition of both DCCD and either ammonium or nigericin in the presence of K^+ significantly increased the electron transport rate (Fig. 1) and decreased the extent of H^+ uptake (Table I), which suggests an additional H^+ leak across the thylakoid membrane. Either ammonium alone or K^+ plus nigericin are known to show large cation accumulation in thylakoids energized by a ΔpH [8,9], as shown also in this work (Figs. 3 and 4). The similarity of DCCD and valinomycin action shows that DCCD can cause NH_4^+ or K^+ leak, thus decreasing the steady-state value of ΔNH_4^+ (Fig. 3) or ΔK^+ (Fig. 4), and consequently ΔpH, which correspondingly accelerates the electron transport (Fig. 1). In

Table I

Effect of DCCD on light-induced H^+ uptake by chloroplasts*

Addition	H^+ uptake (equiv./mol chl.)	
	Control	+60 μM DCCD
None	0.23	0.28
0.2 mM NH_4Cl	0.18	0.09
10 nM nigericin	0.21	0.15

*The reaction medium contained 0.5 mM Tris-HCl, pH 7.6, 50 μg chl/ml, and other chemicals as in Fig. 1.

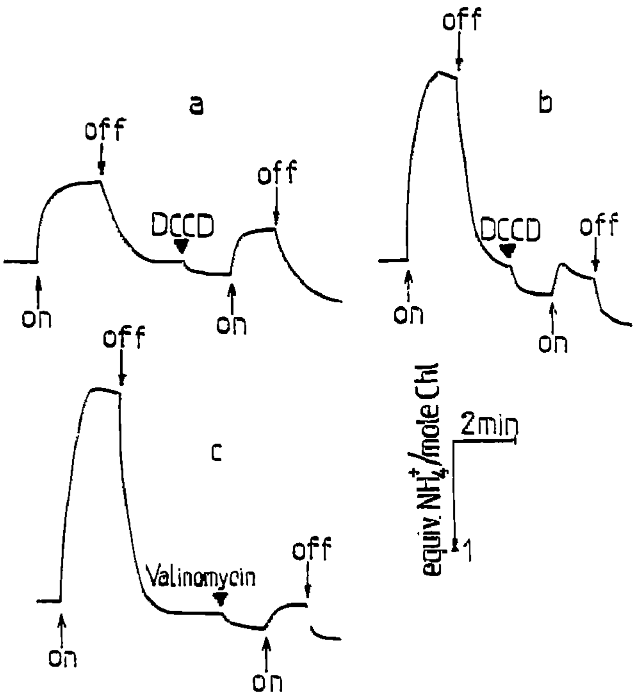

Fig. 3. Effect of 60 μM DCCD (a,b) and 0.4 μM valinomycin (c) on light-dependent ammonium uptake. The reaction medium contained 5 mM $MgCl_2$, 0.2 mM NH_4Cl, 0.1 mM MV, 5 mM Tris-HCl (pH 7.8), 200 mM sucrose (a) or 100 mM choline chloride (b,c) and 50 μg chl/ml.

spite of the similarity in DCCD and valinomycin action it is hardly possible that they transfer cations by a similar molecular mechanism. It seems more likely that DCCD activates an endogenous monovalent cation transport system in thylakoid membranes.

It remains to be shown whether DCCD activates this putative cation transport system through effects on the known targets of DCCD binding or on a separate thylakoid protein, possibly associated with cation transport. It would be tempting to assume that the DCCD-activated cation transport system includes the DCCD-binding photosystem II proteins [3] or subunit III of CF_o of ATP-synthase capable of transferring monovalent cations (K^+ and Na^+) under certain conditions [10].

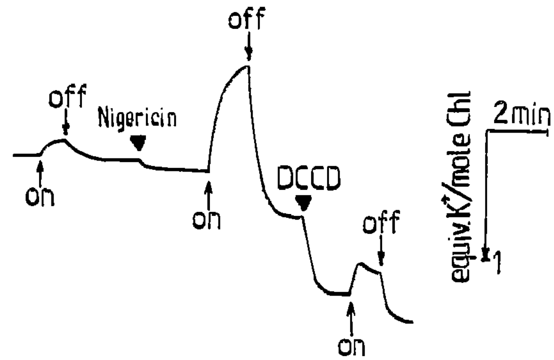

Fig. 4. Effect of 60 μM DCCD on light-dependent K^+ uptake. The reaction medium contained 100 mM choline chloride, 1 mM KCl, 5 mM $MgCl_2$, 0.1 μM nigericin, 0.1 mM MV, 5 mM Tris-HCl (pH 7.8) and 50 μg chl/ml.

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