Volume 320, number 2, 118–120 FEBS 12272 April 1993

Interaction of carbonyl cyanide *m*-chlorophenylhydrazone with the photosystem II acceptor side

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Received 11 February 1993

We show that CCCP, known as an uncoupler of photophosphorylation and an ADRY agent, inhibits FeCy photoreduction and coupled O_2 evolution by isolated chloroplasts equally (I_{50} ~2 μM), but is practically without effect on the O_2 evolution coupled with SiMo reduction within the 0.2–10 μM concentration range. CCCP has no effect on the nanosecond chlorophyll fluorescence in chloroplasts incubated at low light intensity, but decreases it at high light intensity. The electron transfer from reduced TMPD or duroquinol to methylviologen is resistant to CCCP. The efficiency of the CCCP inhibitory action on the FeCy photoreduction depends on the rate of electron flow, which is controlled by the light intensity. The data obtained show that CCCP is oxidized by the photosystem II donor side and is reduced by Q_P, competing for electrons with FeCy and the cytochrome *b/f* complex.

Chloroplast; Electron transport; Photosystem II; ADRY agent; CCCP

1. INTRODUCTION

Photosystem II couples the oxidation of water and the reduction of plastoquinone (for review, see [1]):

$$H_2O \rightarrow (Mn)_4 \rightarrow Y_Z \rightarrow P680 \rightarrow Pheo \rightarrow Q_A \rightarrow Q_B \rightarrow Q_p$$

Photosynthetic water oxidation includes four one-electron steps:

$$\begin{array}{l} \quad S_0 \xrightarrow{-e^-} S_1 \xrightarrow{-e^-} S_2 \xrightarrow{-e^-} S_3 \xrightarrow{-e^-} S_4 \\ + 2H_2O \uparrow \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad \downarrow - O_2 \end{array}$$

Symbols S_{0-4} represent states of the Mn cluster of the complex Y, and the figures refer to the number of oxidizing equivalents in the complex stored during stepwise water oxidation. S_2 and S_3 states are unstable and relax in the dark down to S_1 in the tens of seconds [2]. Deactivation of the S_2 and S_3 states is accelerated by compounds known as ADRY agents [3]. ADRY agents are mobile catalysts inducing cyclic electron flow from unidentified endogenous electron donors to S_2 and S_3 [4,5] as well as to Y_Z [6]. In DCMU-poisoned chloroplasts these agents inhibit Q_A reoxidation by recombination with $P680^+$ [4,7,8].

Phenylhydrazone derivatives represent potent ADRY agents [3,7,8]. CCCP is extensively used as an uncoupler of photosynthetic and oxidative phosphorylation. At low concentrations it speeds up electron transfer in energy-transducing membranes, collapsing $\Delta\bar{\mu}H$. With increasing concentrations, electron transfer is suppressed [4,7–9].

The purpose of this work was to study the effect of CCCP as an ADRY agent on the photosystem II acceptor side in chloroplasts.

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Abbreviations: ADRY, acceleration of the deactivation reactions of the water-splitting enzyme system Y; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DNP-INT, 2-iodo-6-isopropyl-3-methyl-2′,4,4′-trinitrodiphenyl ether; FeCy, potassium ferricyanide; HQNO, 2-*n*-heptyl-4-hydroxyquinoline *N*-oxide; $(Mn)_4$, the tetranuclear Mn cluster of water-splitting complex Y; P680, the reaction center of photosystem II; Pheo, the intermediate electron acceptor of photosystem II (pheophytin *a*); Q_A and Q_B, primary and secondary plastoquinones of photosystem II; Q_C and Q_Z, plastoquinone binding sites in the cytochrome *b/f* complex; Q_P, membrane pool of plastoquinone; SiMo, sodium silicomolybdate; TMPD, *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine; Y_Z, tyrosine-161 of the photosystem II D1 polypeptide.

2. MATERIALS AND METHODS

Pea and wheat chloroplasts were isolated as described earlier [9,10]. Medium I containing 0.4 M sucrose, 35 mM NaCl, 50 mM Tricine-KOH (pH 7.8), or medium II containing 0.5 M sucrose, 0.5 M sodium citrate, 50 mM NaH_2PO_4 (pH 7.0), 20 mM KCl and 2 mM $MgCl_2$, were used in experiments. O_2 evolution or uptake by chloroplasts (with a chlorophyll content of 20–30 $\mu g \cdot ml^{-1}$) incubated in medium I was recorded polarographically [9] at a white light intensity of ~0.1 $W \cdot cm^{-2} \cdot s^{-1}$. 2 mM FeCy, 50 μM SiMo + 2 mM FeCy or 0.2 mM methylviologen were employed as electron acceptors. 0.2 mM duroquinol or 5 mM sodium ascorbate + 0.1 mM TMPD were used as electron donors

The decay kinetics of the chlorophyll fluorescence from chloroplasts (with a chlorophyll content of 200–300 $\mu g \cdot ml^{-1}$) incubated in medium

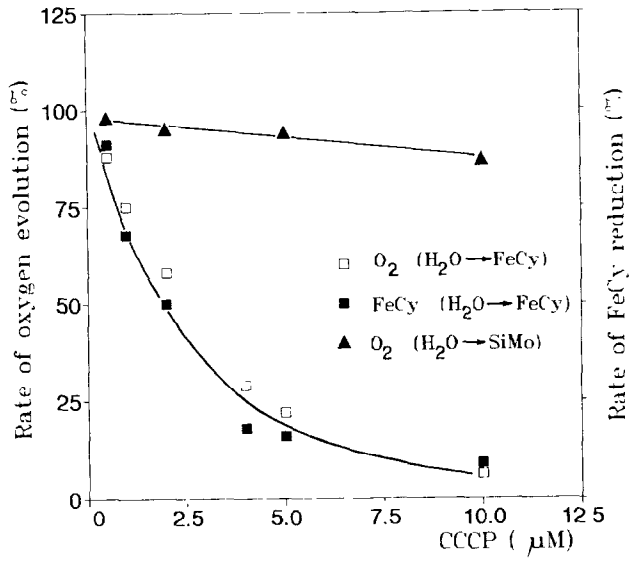

Fig. 1. Effect of CCCP on light-induced O_2 evolution coupled with reduction of FeCy and SiMo and on photoreduction of FeCy in pea chloroplasts incubated with 4 μM gramicidin D. The 100% rates of O_2 evolution and FeCy reduction were equal to 100–120 and 375–400 μmol · mg^{-1} Chl per h, respectively.

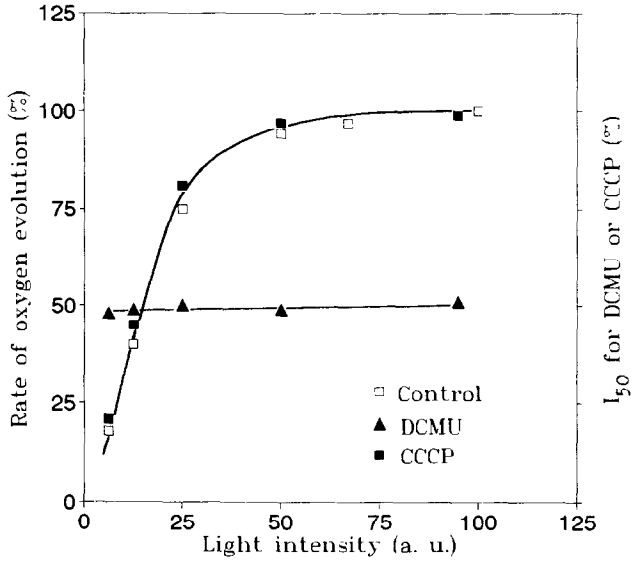

Fig. 2. Effect of light intensity on O_2 evolution coupled with FeCy reduction and on I_{50} values for DCMU and CCCP in pea chloroplasts incubated with 5 μM gramicidin D. The 100% rates of O_2 evolution were 100–120 μmol · mg^{-1} Chl per h. I_{50} for DCMU and CCCP at maximum light intensity equalled 20 nM and 2 μM, respectively.

II were measured with a laser picosecond fluorometer [10]. The kinetics were fitted by a sum of two exponential components with lifetimes (τ) of 300 ps and 1.2 ns. The relative amplitude of the 1.2 ns fluorescence component was measured in the experiments. The chloroplasts were exposed to constant white light of either low (0.1–0.2 mW · cm^{-2} · s^{-1}) or high (saturating) intensity (0.1 W · cm^{-2} · s^{-1}).

3. RESULTS AND DISCUSSION

CCCP caused an inhibitory effect on O_2 evolution by chloroplasts incubated with gramicidin D and FeCy as the electron acceptor (Fig. 1). This inhibition can be explained on the basis of data on ADRY agents [3–8], which serve as artificial electron donors, accelerate the deactivation of the S_2 and S_3 states of the O_2-evolving complex and thereby decelerate H_2O oxidation. FeCy photoreduction was equally suppressed by CCCP: the concentration causing 50% inhibition (I_{50}) was about 2 μM in both cases. Nevertheless, CCCP was practically without effect, within the concentration range tested, on light-induced O_2 evolution with SiMo as an electron acceptor. Photoreduction of SiMo was resistant to DCMU: SiMo displaces DCMU from its binding site [11]. DCMU inhibits electron transfer between plastoquinones Q_A and Q_B by competing with plastoquinone Q_B [12].

Nanosecond fluorescence of chlorophyll in chloroplasts is a sensitive probe for electron transfer disruption in photosystem II [10]. Table I shows that the relative amplitude of the nanosecond fluorescence at low light intensity markedly increased in response to the addition of DCMU, HQNO (inhibiting Q_B oxidation by Q_p [13] as well as Q_C reduction by cytochrome *b* [14,15]),

Table I

Effect of CCCP and other agents on the component of chlorophyll fluorescence with τ = 1.2 ns in wheat chloroplasts

Agent	Concentration (μM)	Amplitude of fluorescence at low light intensity*	Concentration (μM)	Amplitude of fluorescence at high light intensity*
Control	–	3	–	4
DCMU	25	100	25	100
HQNO	25	79	30	94
DBMIB	5	3	10	94
DNP-INT	5	3	20	100
CCCP	20	3	20	52

*Values are given as arbitrary units.

Table II

Effect of CCCP and other agents on photosynthetic electron transport in pea chloroplasts

Agent	Concentration (μM)	Rate of electron transport (%)			
		O_2 evolution H_2O ↓ SiMo	O_2 uptake TMPD + asc. ↓ methylviologen (+ DCMU)	O_2 uptake duroquinol ↓ methylviologen (+ DCMU)	O_2 evolution H_2O ↓ FeCy
DBMIB	1	96	98	5	45
DNP-INT	10	98	100	3	40
HQNO	10	96	95	90	10
CCCP	10	98	104	80	15

Gramicidin D and DCMU concentrations were 4 and 1 μM, respectively. The 100% rate of electron transport in each column, starting from the left, equalled 100, 85, 75, and 115 μmol $O_2 \cdot mg^{-1}$ Chl per h, respectively.

but not DBMIB or DNP-INT (inhibiting Q_Z oxidation in cytochrome *b/f* complex [14–16]) as well as CCCP. It follows from these data that the CCCP site of action on the acceptor side of photosystem II is located posterior to Q_B. High intensity light acted in a DCMU-similar fashion: it increased the nanosecond fluorescence amplitude. Under these conditions, DCMU, HQNO, DBMIB and DNP-INT had no appreciable effect, whereas CCCP significantly decreased the fluorescence amplitude: this is an effect to be expected with an ADRY agent which, being an electron donor, causes $P680^+$ to reduce and in so doing lowers the yield of chlorophyll fluorescence due to recombination of the primarily separated charges.

Table II shows that DBMIB, DNP-INT, HQNO and CCCP do not considerably influence the electron transfer from H_2O to SiMo (involving photosystem II), nor that from reduced TMPD to methylviologen (involving photosystem I). Electron transfer from duroquinol reducing Q_Z [15,17] to methylviologen with participation of photosystem I was almost completely inhibited by DBMIB and DNP-INT, but only slightly sensitive to CCCP. Electron transfer from H_2O to FeCy was over 50% decelerated by DBMIB and DNP-INT (thus indicating that FeCy is reducible both by photosystems II and I) and drastically inhibited by HQNO and CCCP. It follows from the above data that FeCy predominantly interacts as an electron acceptor with a component arranged between Q_B and Q_Z, most probably with Q_p. CCCP oxidized on the donor side of photosystem II [4–6] is reduced by Q_p, competing for electrons with FeCy and the *b/f* complex. As a result, non-cyclic electron transfer is suppressed, and an artificial cyclic system is established. Although duroquinol is a very poor direct donor to the plastoquinone pool [15], a part of Q_p can nevertheless be reduced. This probably accounts for the slight deceleration of the duroquinol → methylviologen reaction by CCCP.

If CCCP really induces cyclic electron transfer, the CCCP inhibitory action should depend on the electron transfer rate. Fig. 2 shows that the I_{50} for DCMU was equal to 20 nM and independent of the light intensity. In contrast, I_{50} for CCCP increased with a rise in the electron transfer rate and generally matched the profile of the light saturation curve. The effect observed is likely to be a distinctive property of ADRY agents and represents a test upon which their selection can be based.

Acknowledgements: We are grateful to Professor V.Z. Paschenko and Dr. S.S. Vasil'ev for the nanosecond fluorescence measurements, and to Dr. A.V. Oleskin for correction of the English version of the manuscript.

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