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Chloramphenicol is an inhibitor of photosynthesis

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Chloramphenicol inhibited significantly but incompletely photosynthesis in leaf segments of rice. Fluorescence and polarographic experiments indicated that chloramphenicol competes with the CO_2 reducing cycle for electrons from photosystem I because it serves as an electron acceptor of photosystem I and its reduction intermediate transfers its electron to molecular oxygen.

Chloramphenicol; Inhibitor; Photosynthesis; Electron acceptor; Photosystem I

1. INTRODUCTION

Photosynthesis involves a number of chlorophyll-carrying proteins, electron carrier proteins and enzymes for ATP synthesis and CO_2 reduction which are present in chloroplasts. A proportion of the functional proteins of photosynthesis are encoded by chloroplast DNA and synthesized by 70 S ribosomes in the plastids, while the rest are encoded by nuclear DNA, synthesized by 80 S ribosomes in the cytoplasm and transported into chloroplasts [1].

Chloramphenicol (CAP) is an antibiotic substance which specifically inhibits protein synthesis of the prokaryote-type. The inhibitor binds to the large subunit of 70 S ribosomes and blocks its peptidyl transferase activity [2] but does not interfere with synthesis of proteins by 80 S ribosomes. CAP has therefore been widely used to selectively study inhibition of protein synthesis within chloroplasts. In particular, CAP has been repeatedly employed to examine if the de novo synthesis of proteins in chloroplasts is involved in the recovery of photosynthetic activity which has been impaired by strong irradiance or other environmental stresses [3–6].

Here we report that CAP mediates rapid electron transfer from photosystem I (PSI) to molecular oxygen and consequently serves as an inhibitor of photosynthesis. The potential of CAP to affect other biological redox reactions is also suggested.

Abbreviations: CAP, chloramphenicol; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; MES, 2-(*N*-morpholino) ethanesulfonic acid; PSI, photosystem I; PSII, photosystem II; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCIP, 2,6-dichlorophenol indophenol.

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2. MATERIALS AND METHODS

Oryza sativa L. cv. 'Nipponbare' was grown in a greenhouse for 2 weeks as described previously [7]. Fully expanded third leaves were used. Leaf blades were cut into segments of 2 cm long and floated on 10 mM MES-NaOH (pH 5.7) containing indicated concentrations of CAP at 30°C in the dark.

Photosynthetic oxygen evolution of leaf segments was measured with a Hansa Tech gas phase oxygen electrode at 30°C according to the method described in [8] except that the gas chamber was filled with air containing about 10% CO_2. Leaf segments were illuminated with saturating white light of 1500 $\mu E \cdot m^{-2} \cdot s^{-1}$.

The induction of chlorophyll *a* fluorescence was determined at room temperature with a laboratory-built apparatus. A He-Ne laser (Nippon-Kagaku Engineering) was the source of an excitation beam at 632.5 nm. A fiber optic was used to transmit excitation beam to, and fluorescence from, the upper surface of a leaf segment. Fluorescence at 685 nm was measured with a Hamamatu R636 photomultiplier through a monochromator (Nikon P250).

Light-induced O_2 uptake in the thylakoids was measured with a Clark-type O_2 electrode at 30°C [9]. Thylakoid membranes were prepared as described in [10]. The reaction medium contained 50 mM HEPES-NaOH buffer (pH 7.5), 1 mM NaN_3, 10 mM methylamine, 0.4 M sucrose, 10 mM $MgCl_2$, 10 mM NaCl and thylakoid membranes equivalent to 10 μg chlorophyll per ml. PSI electron transport was determined in the same medium supplemented with 12.5 μM DCIP, 2 mM ascorbate and 10 μM DCMU. The thylakoids were illuminated with white light of a saturating intensity (2000 $\mu E \cdot m^{-2} \cdot s^{-1}$).

3. RESULTS

Fig. 1 shows changes in the rate of photosynthetic oxygen evolution in leaf segments of rice which were floated on MES-NaOH buffer containing 3 mM CAP. Experiments were carried out at 30°C in the dark. The rate of oxygen evolution gradually decreased with a half inactivation time of about 3 h. The inactivation can be ascribed to CAP because there was no significant change in the oxygen evolving activity of leaves which had been treated similarly but in the absence of CAP for 24 h. The inhibition was incomplete and a low rate of oxygen evolution persisted even after 24 h incubation. The activity was slowly but fully restored when CAP-

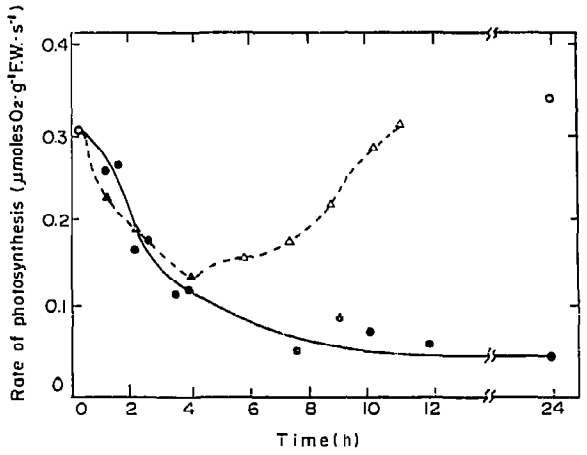

Fig. 1. Effects of CAP on photosynthetic oxygen evolution in rice leaves. Leaf segments were floated on 10 mM MES-KOH buffer (pH 5.7) (○), or the buffer containing 3 mM CAP (●) for indicated periods of time. In experiments shown by the dashed line, leaf segments were treated with CAP for 4 h (▲), then well rinsed with water and transferred to the buffer containing no CAP (△). Experiments were carried out at 30°C in the dark.

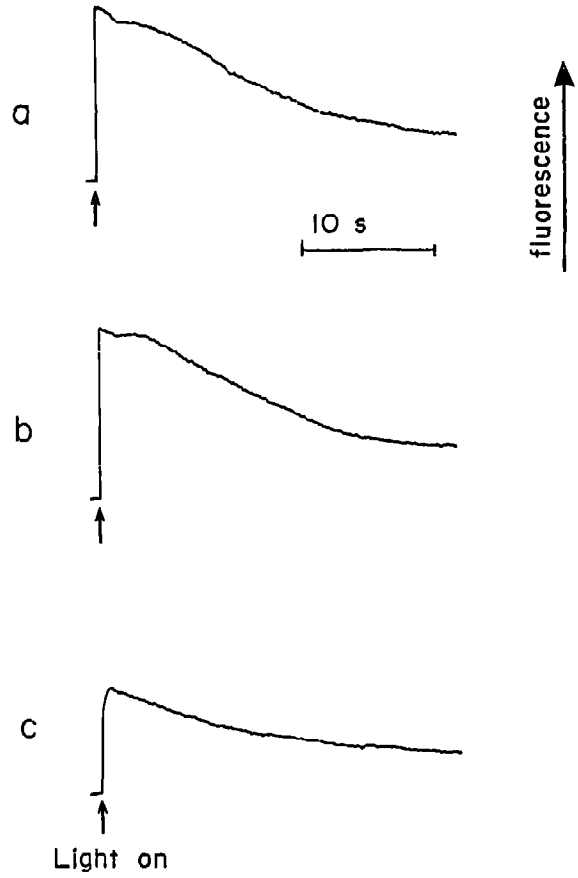

Fig. 2. Effects of CAP on the fluorescence induction of rice leaves. (a) An untreated leaf which was dark-adapted for 30 min prior to measurement. (b, c) Leaves were floated on MES buffer containing no or 3 mM CAP, respectively, at 30°C in the dark for 5 h.

treated leaf segments had been rinsed with water and transferred to buffer containing no CAP. Thus, the inhibition is reversible in the dark.

CAP is a specific inhibitor of protein synthesis in chloroplasts. However, it is unlikely that the inactivation and reactivation of leaf photosynthesis observed above are consequences of inhibition of protein synthesis and its reversal because the occurrence of a functional protein of photosynthesis which turns over with a half life time of 3 h in the total darkness is not known. On the other hand, evidence suggesting that CAP directly affects photosynthetic electron transport was obtained from experiments in which the induction kinetics of chlorophyll *a* fluorescence in leaves were determined (Fig. 2). Dark-adapted leaves showed typical fluorescence transients (the Kautsky effect) consisting of a rapid initial rise, subsequent small transitional changes and a final slow decrease to a low steady state level (trace a). The initial rapid rise in the fluorescence yield reflects accumulation of reduced Q_A, which serves as a quencher of fluorescence in the oxidized form [11]. Q_A is rapidly reduced at the onset of illumination because electron transport beyond PSI is blocked in dark-adapted leaves [12,13]. CAP treatment resulted in a marked decrease in the magnitude of the initial fluorescence rise, indicating an inhibition of Q_A reduction (trace c). Because the redox state of Q_A is determined by a balance between its reduction by PSII and its oxidation by electron transport to PSI, the suppression of Q_A reduction suggests either an inhibition of PSII or enhancement of electron flow through PSI by CAP.

No evidence was obtained to indicate that CAP inhibited PSII (data not shown but see below). Instead, the following experiments indicated that CAP enhanced electron transport through PSI. When the thylakoid membranes were illuminated in the presence of CAP, substantial rates of oxygen-uptake occurred (Fig. 3). The rate of oxygen uptake increased as the concentration of CAP was raised above 100 μM and was saturated at about 1 mM. CAP-dependent oxygen uptake involves PSII because the reaction was completely abolished in the presence of 10 μM DCMU. The effect of CAP resembles that of methyl viologen, which serves as an electron acceptor of PSI and induces a rapid oxygen uptake. A net oxygen uptake occurs in the presence of methyl viologen because evolution of 1 oxygen molecule from 2 molecules of water is accompanied by reduction of 4 methyl viologen molecules which in turn reduce 2 oxygen molecules to hydrogen peroxide. Note that the maximum rate attained in the presence of CAP was comparable to that observed in the presence of 1 mM methyl viologen. The result suggests that CAP serves as a good electron acceptor of PSI and, once reduced, transfers its electron to oxygen. It is also evident that CAP has no inhibitory effect on PSII electron transport.

That CAP serves as an electron acceptor of PSI was

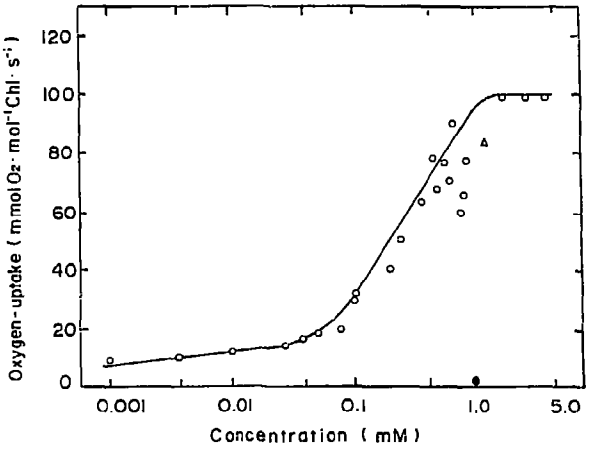

Fig. 3. Light-induced oxygen uptake in thylakoids in the presence of various concentrations of CAP. The activity was determined in the presence of indicated concentrations of CAP (○), 1 mM methyl viologen (but no CAP) (△) or 1 mM CAP and 10 μM DCMU (●).

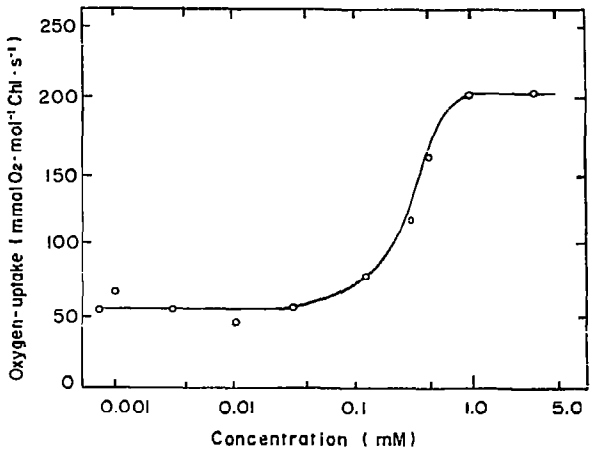

Fig. 4. Photosystem I electron transport supported by various concentrations of CAP. Experiments were carried out as in Fig. 3 except that 10 μM DCMU, 12.5 μM DCIP and 2 mM sodium ascorbate were added.

more clearly demonstrated in Fig. 4. In this experiment, PSII electron transport was blocked by DCMU and the DCIP–ascorbate couple was added as an electron donor to PSI. CAP induced high rates of oxygen-uptake at concentrations above 100 μM. It is concluded therefore that CAP mediates electron flow from PSI to molecular oxygen and as a consequence inhibits leaf photosynthesis.

4. DISCUSSION

The present study indicates that CAP serves as an electron acceptor of PSI. The capacity of CAP for electron acceptance is ascribed to a nitro group attached to the benzene ring of the molecule. Wessels [14] showed that various aromatic nitro compounds serve as electron acceptors of PSI. These substances were reduced by illuminated chloroplasts to corresponding amino derivatives only under anaerobic conditions. This indicated that a reduction product of nitro compounds, nitroso or hydroxyamine derivative, was auto-oxidizable. Thus, the couple of CAP and its reduction intermediate constitutes the redox system responsible for electron transfer from PSI to molecular oxygen.

CAP inhibits photosynthesis by competing with the CO_2 reducing cycle for electrons from PSI. The effect of CAP on photosynthetic electron transport becomes significant above 100 μM and reaches the maximum at 1 mM. This is the range of concentrations which has been used in experiments to examine the inhibitory effect of CAP on the protein synthesis in vivo. Under the present experimental conditions, where CAP had been administered to leaves simply by keeping their abaxial surface in contact with CAP solution, the inhibition of leaf photosynthesis developed slowly with a half time of 3 h. Because CAP, once inside the chloroplasts, is expected to serve immediately as an electron acceptor of PSI, the slow development of inhibition could be ascribed to a slow diffusion of CAP from the leaf surface to the stroma compartment of chloroplasts. Thus, CAP should exert its inhibitory action more rapidly when leaves are infiltrated with, or algal cells are suspended in, a CAP solution. When assayed by measuring oxygen production, photosynthesis is particularly sensitive to CAP because the inhibitor not only suppresses CO_2 reduction but also induces oxygen uptake. Nevertheless, CAP inhibited only incompletely photosynthetic oxygen evolution in rice leaves. This suggests that the CO_2 reducing cycle can significantly compete with CAP for electrons in vivo. An alternative is that a proportion of chloroplasts present in leaves are inaccessible to CAP. At any event, the present work strongly cautions that photosynthesis cannot accurately be determined in the presence of CAP.

Furthermore, CAP is expected to exert another harmful effect on biological activities of plants. Reduction of oxygen by the reduction intermediate of CAP is a one-electron process and hence results in production of O_2^-. Prolonged illumination of plant leaves or cells in the presence of CAP will lead to massive production of 'active oxygen', which has detrimental effects on various biological activities. The previous study showed that aromatic nitro compounds are non-enzymatically reduced with reduced ferredoxin or flavin [14]. Thus, CAP has a potential to interact with various biological redox reactions, including the respiratory chain, which produce strong reductants. We recommend therefore the use of inhibitors of protein synthesis other than CAP for investigation of the de novo synthesis of proteins in chloroplasts or mitochondoria.

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