COPPER INHIBITION OF PHOTOSYSTEM II ACTIVITY IN THE CYANOPHAGE AS-1-RESISTANT MUTANT

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Copper inhibited the photoreduction of DCPIP (PS II activity) in a spheroplast preparation of cyanophage AS-1-resistant mutant (An/AS-1). The inhibitory action of Cu^{2+} was reversed by supplementation with manganese but not with DPC. The results suggest that the site of inhibition was either between the Mn^{2+} and DPC donation site(s) or close to DPC donation site. Protection of the Hill activity by EDTA further suggests that Cu^{2+} interacted with the reaction center.

In general, all heavy metals at higher concentrations are toxic to algae. Some heavy metals including copper (Cu^{2+}) are required in trace amounts by cyanobacterial species for various metabolic processes. The discovery of a number of cyanophages (1-3) provides an ideal system to understand the physiological and genetic nature of cyanobacteria and cyanophage resistant mutants (4). Recently it has been demonstrated in the unicellular cyanobacterium *Anacystis nidulans* and its cyanophage AS-1 resistant mutant that Cu^{2+} specifically affects the growth, photosynthetic pigment synthesis (5), uptake and reduction of nitrate (6), acid and alkaline phosphatase activities (7). Pleiotropic behaviour of the cyanophage AS-1 resistant mutant has also been reported (8). Although there are many reports suggesting that Cu^{2+} has a specific inhibitory effect on photosynthetic reactions such as the Hill reaction, when it is added in toxic amounts (9, 10), the possible action site of Cu^{2+} remains uncertain. The present communication deals with the action of Cu^{2+} on the Hill activity carried out with the spheroplasts of the cyanophage AS-1-resistant mutant.

MATERIALS AND METHODS

Organism and culture conditions. The cyanophage AS-1-resistant mutant,

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designates as An/AS-1, was isolated by double-agar layer techniques following the infection of cyanophage AS-1 with the unicellular cyanobacterium *Anacystis nidulans* ATCC 27144, which was obtained through the courtesy of Dr. R. S. Safferman, Environmental Protection Agency, University of Cincinnati, Ohio, USA. The An/AS-1 mutant was isolated with a frequency of 4×10^{-6} . No reversion mutation frequency has been observed during the subsequent subculturing. The mutant was grown and maintained in Allen's medium (11) supplemented with A-5 trace elements. The cultures were maintained at $24 \pm 1^\circ\text{C}$ and illuminated for 14 days with cool daylight fluorescent tubes (energy flux density 3.87 W/m^2 on the surface of culture vessel).

Preparation of spheroplasts. Log phase cultures ($10^7 \text{ cells} \cdot \text{ml}^{-1}$) were harvested by centrifugation, suspended in phosphate buffer (40 mM, pH 7) containing 0.05–0.1% lysozyme and sucrose (0.4 M) and incubated at 37°C . After 3 h of incubation, the suspension was centrifuged at a low speed ($1,000 \times g$, 5–10 min) to remove cell debris and unbroken cells. The pellet was discarded and the supernatant was again centrifuged ($3,500 \times g$, 15 min). The pellet was resuspended in the preparation medium containing phosphate buffer (pH 7.2, 40 mM), sucrose (0.2 M) and NaCl (0.2 M).

Measurement of PS II activity. Light from a projection lamp (500 W) was filtered through a 2.5 cm thick water column and focussed on the 2.5 ml cuvette. The light intensity of the surface of vessel was 20 W/m^2 and the temperature was kept between 25 – 27°C . In the reaction mixture containing spheroplasts (eqvt. to 2.1–2.26 mg Chl $\cdot \text{ml}^{-1}$), 2,6 dichlorophenol indophenol (DCPIP) was added in such a way that its final concentration became 40 mM. Before illumination the reaction mixtures containing spheroplasts were preincubated for 1 h. The dye reduction was followed spectrophotometrically at 680 nm at regular intervals of 30 or 60 s. Reaction mixture incubated in dark served as the control. Manganese (Mn^{2+}), 1,5 diphenyl carbazide (DPC) and ethylene diamine tetra acetic acid (EDTA) were added in the reaction mixture as needed.

Chemicals. 2,6 Dichlorophenol indophenol (DCPIP), 1,5-diphenyl carbazide (DPC) and ethylene diamine tetraacetic acid (EDTA) were obtained from Sigma Chemicals. Cu^{2+} (used as $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$) and Mn^{2+} (used as $\text{MnCl}_2 \cdot 2\text{H}_2\text{O}$) were obtained from British Drug Houses, India.

RESULTS AND DISCUSSION

The measurement of electron transport from H_2O to DCPIP showed that Cu^{2+} inhibited PS II activity in a concentration-dependent manner, i.e., linearly against logarithmic increase in Cu^{2+} concentrations. The concentration required for 50% inhibition was about $5 \times 10^{-6} \text{ M}$ and complete inhibition was achieved when Cu^{2+} reached $5 \times 10^{-5} \text{ M}$ (Fig. 1).

The addition of DPC ($2.5 \mu\text{M}$) or Mn^{2+} ($2.5 \mu\text{M}$) increased the dye reduction in comparison to the control (Figs. 2 and 3). The dye reduction in the presence of DPC was $8 \mu\text{mol} \cdot \text{mg Chl}^{-1}$ after 4 min of treatment, which comes down to $7.5 \mu\text{mol} \cdot \text{mg}$

Fig. 1.

Fig. 2.

Fig. 1. Inhibition of DCPIP reduction by different concentrations of Cu^{2+} in the spheroplasts of An/AS-1. The data are based on rate of dye-reduction (μmol DCPIP reduced per mg of Chl $\cdot \text{min}^{-1}$).

Fig. 2. Time course of DCPIP reduction in spheroplasts of An/AS-1 in presence ($\times$, $\blacktriangle$) or absence ($\circ$, $\bullet$) of DPC, with or without ($\circ$, $\times$) Cu^{2+} .

Chl^{-1} when Cu^{2+} was added. Thus, in the presence of Cu^{2+} , DPC had no stimulatory effect. It is quite possible that the mode of PS II inhibition by Cu^{2+} in cyanobacterial cells could be very similar to that in spinach chloroplasts (10). The conclusion stems from the observation that Mn^{2+} partially reversed the inhibitory action of Cu^{2+} . The recovery of Hill activity by Mn^{2+} was rapid. Within 2 min, a 54% stimulation was observed in comparison to Cu^{2+} inhibition (Fig. 3). Mn^{2+} is known to donate electrons between the water splitting site and the PS II reaction centre (12), and competes successfully with water as an electron donor to PS II (13). The presence or absence of EDTA (5–50 μM) has little effect on DCPIP reduction (Fig. 4). In contrast, when EDTA was added to the reaction mixture containing Cu^{2+} , the dye reduction was higher than in the presence of Cu^{2+} alone. Considering the level of Cu^{2+} inhibition (40%) the protection afforded by EDTA was approximately 25% during the course of the experiment. DPC did not protect the system from Cu^{2+} inhibition. This indicates that Cu^{2+} inhibited the electron transport before the Mn^{2+} donation site. The above observations have summarized in a tentative model (Fig. 5) which illustrates the possible target site and mode of action of the different chemicals used and the probable site of Cu^{2+} inhibition.

Fig. 3.

Fig. 4.

Fig. 3. Time course of DCPIP reduction in spheroplasts of An/AS-1 in presence ($\times$, Δ) or absence ($\circ$, $\bullet$) of Mn^{2+} with ($\bullet$, Δ) or without ($\circ$, $\times$) Cu^{2+} . Mn^{2+} (Δ) was added at the time indicated by the arrow.

Fig. 4. Time course of DCPIP reduction in spheroplasts of An/AS-1 in the absence ($\circ$, $\times$) or presence ($\bullet$, Δ) of Cu^{2+} with ($\times$, Δ) or without ($\circ$, $\bullet$) EDTA.

Fig. 5. Model for possible target site of Cu^{2+} action.

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