INHIBITION OF CYTOCHROME-b_6 OXIDATION BY KCN

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1. Introduction

It has been shown by Izawa et al. [1] that treatment of chloroplasts with high concentrations of KCN results in inhibition of cytochrome-*f* photo-oxidation by photosystem I, but not its reduction by photosystem II. Reduction *P*-700 by photosystem II, on the other hand, is blocked by KCN. It was concluded that KCN prevents electron transfer between cytochrome-*f* and *P*-700, a reaction which is believed to involve plastocyanin. The view of sequential electron transfer from cytochrome-*f* to *P*-700, mediated by plastocyanin, is not universally accepted [2–4], and recently, the role of cytochrome-*f* in the electron transfer reactions between both photosystems has been questioned [5]. There seems to be agreement, however, on the site of plastocyanin function being close to the donor site of photosystem I. These conclusions are derived mainly from inhibitor and reconstitution experiments, whereas kinetic experiments for plastocyanin are still lacking.

The role of cytochrome-b_6 as a specific component of cyclic electron transport is also generally accepted and is founded mainly on the results that photosystem I can either reduce or oxidize cytochrome-b_6 under proper conditions in a DCMU-insensitive reaction [6]. Furthermore, it was shown that b_6-photooxidation includes part of the open chain electron transport [7], namely plastoquinone, on the basis of inhibitor experiments with DBMIB, and also a site of energy conservation, on the basis of crossover experiments with phosphorylation cofactors and uncouplers [6].

Using KCN as a specific inhibitor of plastocyanin-dependent reactions, the results of this report show that cytochrome-b_6 oxidation is inhibited by KCN treatment of chloroplasts; cyt-b_6 photoreduction, on the other hand, is not impaired. It is concluded that part of the linear electron transport pathway between both photosystems, including plastocyanin, mediates cytochrome-b_6 photooxidation. This is consistent with a previous analysis of the pathway of cytochrome-b_6 oxidation [6].

2. Methods

Chloroplasts were prepared from spinach grown in a controlled climate facility, as described previously [6]. Oxygen uptake was recorded with a YSI 5331 oxygen monitor at 20°C in 3 ml of standard reaction medium containing 0.1 M sucrose, 0.05 M tricine-NaOH, pH 7.8, 2 mM $MgCl_2$, 2 μM DCMU, 10 μM methylviologen. DAD/ascorbate were added at a concentration of 0.1 mM and 5 mM, respectively. Actinic illumination was defined by a red filter (RG 610, Schott); intensity 650 J/m^2 × s. Treatment of chloroplasts with KCN followed the procedure of Izawa et al. [1].

Light-induced absorbance changes were measured in a dual wavelength spectrophotometer (DW 2, Aminco) at 563 nm, with 570 nm as reference wavelength; the bandwidth of the measuring beam was 2 nm; actinic light intensity of wavelength 704 nm (Filtraflex, Balzers) incident at the cuvette: 150 J/m^2 × s. The detailed procedure for measuring b_6-photoreactions under anaerobic conditions is described in [8].

3. Results and discussion

Fig.1 shows the effect of KCN treatment of spinach chloroplasts on the photooxidation of DAD

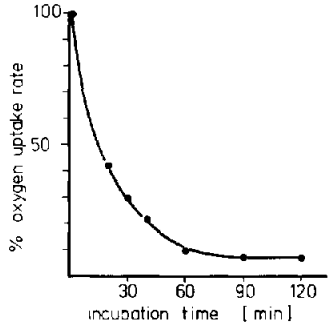

Fig.1. Inhibition of DAD oxidation by photosystem I through preincubation of spinach chloroplasts with KCN for different time intervals. Assay conditions as in Methods; chlorophyll concentration 10 μg/ml.

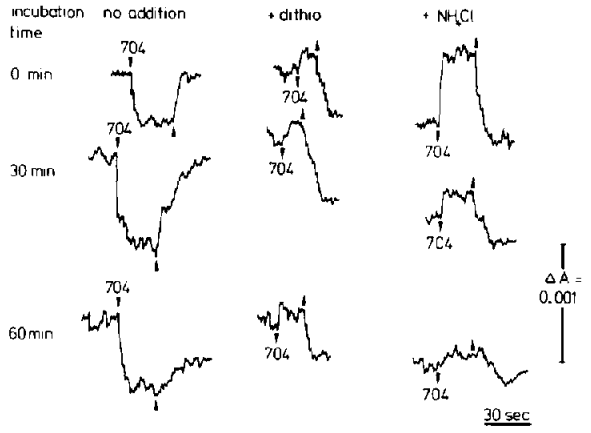

Fig.2. Effect of KCN treatment on the light-induced absorbance changes of cytochrome-b_6. The left-hand side shows the absorbance change induced by 704 nm light, in the absence and presence of KCN preincubation (0, 30 min, 60 min) under aerobic conditions. Where indicated: 2–5 μl 0.01 M dithionite were added to the cuvette under argon atmosphere to establish anaerobic conditions; NH_4Cl was added as indicated at a final concentration of 1 mM; chlorophyll concentration 50 μg/ml. Upward deflection indicates cytochrome-b_6 oxidation; downward arrows = light on, upward arrows = light off.

by photosystem I. It confirms the results of Ouitrakul and Izawa [9], where prolonged treatment with KCN resulted in inhibition of electron transport from DAD to methylviologen (cf. also [10]). The 100% value of oxygen uptake in these experiments corresponds to an activity of 570 μmoles O_2/mg chlorophyll × h. The same KCN treated chloroplast suspension was used simultaneously for measurements of light-induced absorbance changes.

Fig.2 shows that far-red light induced reduction of cytochrome-b_6, measured as an absorbance increase at 563 nm, with a 570 nm reference. Treatment with KCN results in a somewhat enhanced level in photoreduction of cytochrome-b_6, but severely inhibits its dark reoxidation.

After addition of dithionite to the chloroplast suspension under anaerobic conditions, lowering the redox potential of the mixture to about −200 mV, the photoreduction of b_6 is abolished, and a small light-induced absorbance decrease is seen instead; this absorbance decrease can be stimulated several-fold by addition of NH_4Cl at uncoupling concentrations, indicative of a rate limiting site of energy conservation in cyt-b_6 photooxidation [6]. In contrast to the effect of KCN on cyt-b_6 photoreduction, the photooxidation appears to be almost completely inhibited. The difference spectra of the absorbance changes (not shown here) are similar to the ones already published [6], confirming that cytochrome-b_6 reactions are measured.

The results are interpreted as demonstrating plastocyanin participation in the electron transport pathway of cytochrome-b_6 oxidation. They confirm the suggestion that part of the open chain electron transport is involved in cyt-b_6 oxidation, including plastoquinone, plastocyanin, and cytochrome f [6,7,9], plus the rate limiting step of energy conservation on the oxidizing site of photosystem I [6].

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