Alternative cyanide-sensitive oxidase
interacting with photosynthesis in *Synechocystis* PCC6803.
Ancestor of the terminal oxidase of chlororespiration?

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Abstract

Influence of respiration on photosynthesis in *Synechocystis* PCC6803 was studied by measuring the redox transients of cytochrome f (cyt f) upon excitation of the cells with repetitive single turnover flashes. Upon the addition of KCN the flash-induced oxidation of cyt f was increased and the reduction of cyt f⁺ was accelerated. Dependence of these effects on the concentration of KCN clearly demonstrated the existence of two cyanide-sensitive oxidases interacting with photosynthesis: cyt aa₃, which was sensitive to low concentrations of cyanide, and an alternative oxidase, which could be suppressed by using ≥1 mM KCN. The interaction between the photosynthetic and the respiratory electron transport chains was regulated mainly by the activity of the alternative cyanide-sensitive oxidase. The oxidative pathway involving the alternative cyanide-sensitive oxidase was insensitive to salicyl hydroxamic acid and azide. The close resemblance of the inhibition pattern reported here and that described for chlororespiration in algae and higher plants strongly suggest that an oxidase of the same type as the alternative cyanide-sensitive oxidase of cyanobacteria functions as a terminal oxidase in chloroplasts.

Additional key words: absorbance; azide; cyanobacteria; cytochromes; flash irradiation; KCN; NaN₃; phylogeny; respiration; salicyl hydroxamic acid.

Introduction

In cyanobacteria, the cytochrome (cyt) aa₃ type terminal oxidase of the respiratory electron transport and photosystem I (PSI) competes for electrons from the

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**Abbreviations:** cyt = cytochrome; *C₅₀* = concentration for half-maximal effect; PS = photosystem; SHAM = salicyl hydroxamic acid.

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plastoquinone pool (cf. Scherer 1990, Schmetterer 1994). This oxidase is similar to the mitochondrial terminal oxidase and should therefore be sensitive to low concentrations of KCN (Peshek et al. 1989). Using 1 μM KCN, the influence of this oxidase on PS1 activity could indeed be demonstrated for different species of cyanobacteria (Geerts et al. 1994, Schubert et al. 1995). However, using a cyt aa3 deficient mutant of *Synechocystis* PCC6803, Schmetterer et al. (1994) found evidence for the existence of an alternative cyanide-sensitive oxidase, which was present after the deletion of the cox-genes; this oxidase was sensitive to high concentration (7 mM) of KCN.

High concentrations (≥1 mM) of KCN affect the activities of PS1 and cyt f in chloroplasts of some algae (Bennoun 1982, Büchel and Garab 1995a) and higher plants (Garab et al. 1989). This effect has been attributed to the inhibition of the terminal oxidase of the respiratory electron transport of chloroplasts, i.e., of chlororespiration (Bennoun 1982; for recent review see Büchel and Garab 1997). Electrons can be donated to the plastoquinone pool from the NAD(P)H-plastoquinone oxidoreductase (Godde and Trebst 1980, Friedrich et al. 1995, Seidel-Guye et al. 1997). However, attempts to identify and isolate the terminal oxidase of chlororespiration have failed, thus the mechanism of oxidation of the plastoquinone pool in the dark is still unclear.

The interaction between the chlororespiratory and the photosynthetic electron transport systems in higher plants and some algae resembles the interaction between respiration and photosynthesis in cyanobacteria (Lajkó et al. 1997). However, systematic investigations in cyanobacteria under the conditions comparable to those done earlier in green algae, higher plant protoplasts, and chloroplasts have not been carried out. In this work, we studied the effects of respiratory inhibitors on the photosynthetic activity in a cyanobacterium, *Synechocystis* PCC6803, and investigated the possibility of involvement of an alternative cyanide-sensitive oxidase.

**Materials and methods**

**Growth conditions:** *Synechocystis* PCC6803 was grown photoautotrophically under "white light" of 70 μmol m⁻² s⁻¹ (PAR) at 35 °C in BG 11 medium supplemented with 20 mM Hepes-NaOH (pH 7.5). Air-lift cultures were supplied with 1 % CO₂ in sterile air. Cells were harvested in the logarithmic growth phase by centrifugation at 1000 x g for 5 min. Pelleted cells were diluted with the culture medium to A₁₀₀₅ ≈ 0.8 (measured in a Shimadzu UV-160 spectrophotometer), and stored at 35 °C under "white light" of 70 μmol m⁻² s⁻¹ until the measurements.

**Oxygen measurements:** Uncoupled respiration of intact cells was measured in a Clark-type oxygen electrode (Hansatech) at 25 °C after 5 min of dark adaptation using 2 mM NH₄Cl. Inhibitors were added immediately before measurements.

**Flash induced absorbance changes:** induced by single turnover flashes at a repetition rate of 1 Hz were measured using an equipment described in Büchel and Garab.