Role of pigments and subunits in the cytochrome $b_6f$ complex of

*Synechocystis PCC6803*

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**Introduction**

The cytochrome $b_6f$ complex of cyanobacteria consists of the four main subunits cytochrome $f$ (PetA), cytochrome $b_6$ (PetB), the Rieske protein (PetC), subunit IV (PetD) and additional small subunits named PetG, PetM and PetN. In contrast to higher plants and algae, no gene encoding the subunits PetL and PetO can be found in the genome of the completely sequenced cyanobacterium *Synechocystis* PCC 6803. The function of the additional small subunits in cyanobacteria is not yet clear: While PetM seems to have a regulatory role (Schneider et al., 2001), deletion of *petG* and *petN* did not yield completely segregated mutants (Schneider, unpubl.). In contrast to all other subunits which are encoded by single genes, the genome of *Synechocystis* shows a family of three *petC* genes (*sll1316* = *petC1*, *slr1185* = *petC2*, *sll1182* = *petC3*), the reason for which is unknown. Also, the role of one chlorophyll and one carotenoid per monomeric $b_6f$ complex in both pro- and eukaryotic cyt $b_6f$ preparations (Bald et al., 1992; Pierre et al., 1997, Zhang et al., 1999) is still unresolved.

**Results and discussion**

**Hemes, chlorophyll and carotenoids**

Reduction of the highly purified cyt $b_6f$ complex (Wenk et al., 1998) with dithionite caused a 1-nm red shift in the absorbance spectrum of the chlorophyll molecule (Fig. 1A). As such a shift was not observed with ascorbate, which reduces cyt $f$ but not cyt $b_6$, a charge interaction of the chlorophyll molecule with one or both hemes of cyt $b_6$ is strongly suggested. This is supported by identical kinetics of the chlorophyll absorbance shift and the cyt $b_6$ redox change (Fig. 1B), yielding a linear relationship between these two events as shown in Fig. 1C.

The role of the bound carotenoid was investigated in more detail with a *Synechocystis* mutant strain containing an interrupted *crtO* gene. This gene codes for the β-carotene ketolase, CrtO, which is required for the synthesis of echinenone, the carotenoid selectively bound by the cyt $b_6f$ complex of *Synechocystis*. Pigment analysis of the cyt $b_6f$ complex isolated from this mutant showed the replacement of echinenone by three other carotenoids: β-carotene, zeaxanthine and a mono-hydroxy β-carotene (possibly cryptoxanthine). All three were 9-cis isomers, showing a characteristic 4-5 nm blue-shift, increased absorption at 340 nm and decreased absorption at 280 nm similar to β-carotene.
Fig. 1. A: 4 K absorbance spectrum of chlorophyll associated with the cyt b6f complex. Solid line: Sample oxidized by 100 µM ferricyanide, followed by reduction of cyt f with 2 mM ascorbate. Dashed line: Cyt b6 reduced by dithionite. Dotted line: Difference spectrum of solid and dashed line. B: Reoxidation kinetics (by air) of cyt b6 combined with the kinetics of chlorophyll absorbance shift after reduction by 0.5 mM dithionite. C: Kinetics of the cyt b6 redox change plotted against the chlorophyll absorbance shift.

A characteristic difference in the carotenoid content was also suggested by the absorbance spectrum of the isolated mutant cyt b6f complex (Fig. 2): Reduction of cyt b6 caused a red shift by about 1.5 nm of the bands at 496 and 462 nm, which did not occur upon reduction of cyt f. Such small band shifts could not be observed in the WT cyt b6f complex containing echinenone due to the structureless absorbance spectrum of echinenone.

To characterize the specific binding sites of the two pigments, the isolated cyt b6f complex was dissociated into its individual subunits by a mild detergent treatment, followed by chromatographic separation of the native proteins as outlined in (Boronowsky et al., 2001).

Characterization of the native subunits showed that both pigments are exclusively bound to the cyt b6 subunit. This location fulfils all requirements which have been imposed by previous results:

1) The extremely short fluorescence lifetime of the chlorophyll (Peterman et al., 1998) suggested a binding of Chl in a specific pocket of the cyt b6f complex, where a heme or an
amino acid is able to quench the excited state of the chlorophyll in order to protect the protein from oxidative damage.

2) The red-shift of the chlorophyll peak simultaneously with the reduction of the \( b \)-type hemes suggests a short distance between these two components.

3) Also, the red shift of the carotene peaks with the reduction of the \( b \)-type hemes suggests a short distance between them.

4) The proximity of both pigments is required for the suggested function of the carotenoid to prevent the generation of singlet oxygen by photoexcited chlorophyll \( a \) (Zhang et al., 1999).

Modelling of the \( b_{6} \)-structure based on the known \( bc_{1} \)-complex structure reveals that the most probable Chl binding site is located just in between the two heme groups. This enables speculations on a possible role of Chl as light sensor, which could have impact on the Q-cycle of the \( b_{6}f \) complex.

### Rieske proteins

In order to investigate the role of multiple Rieske genes in *Synechocystis*, all three encoded proteins were heterologously overexpressed in *E. coli* BL21(DE3). In addition, for *in vivo* studies, *Synechocystis* mutant strains lacking one or two Rieske genes were created and characterized.

After overexpression of the three full length Rieske proteins, two of them (PetC2 and PetC3) were found in a native form in the cytoplasmic membrane of *E. coli* with incorporated iron-sulfur cluster. As the third protein (PetC1) could not be obtained in an active form, the overproduced protein was purified from inclusion bodies and the Fe-S cluster was reconstituted enzymatically *in vitro* (Schneider et al., 2000). EPR-measurements showed the typical g-values for all three Rieske proteins (Fig. 3) and enabled the determination of the redox potentials by titration: In contrast to the main Rieske protein PetC1 (+320 mV) and to PetC2 (about +300 mV) with rather high \( E_{m} \) values, PetC3 showed an unusual low midpoint potential of only +135 mV. In consequence, plastoquinone would not be able to donate electrons to PetC3 and only menaquinone is a potential electron donor due to its low redox potential.

Further experiments were done to elucidate the physiological role of the three Rieske proteins in the cytochrome \( b_{6}f \) complex. Expression studies showed that all three proteins are expressed (Schneider et al., 2001). Single gene deletion experiments revealed a nonessential function of any of the individual Rieske proteins. However, deletion of the main gene petC1 affected the cells considerably more than deletion of petC2 which had no phenotype: The \( \Delta petC1 \) strain showed effects on the activity of the cyt \( b_{6}f \) complex, PS2 and the cyt \( bd \) oxidase in the thylakoid membrane. The observation that the double gene deletion mutants \( \Delta petC1/C3 \) and \( \Delta petC2/C3 \), but not \( \Delta petC1/C2 \) completely segregated also confirm that PetC2 can partly replace the function of PetC1.

The existence of free MQ in thylakoid or cytoplasmic membrane and of a special MQ-oxidizing cyt \( b_{6}f \) complex remains further to be investigated. In combination with the different Rieske proteins they may represent mechanisms of physiological adaptations to environmental (stress) conditions as has already been shown for three copies of the \( psbA \) gene in *Synechocystis*, coding for the D1 protein in PS2.
Fig. 3. EPR spectra of *Synechocystis* Rieske proteins after purification and reconstitution (PetC1) or of *E. coli* membranes with incorporated PetC2 and PetC3 and of control membranes recorded at 15 K.

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**References**

