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Potential consequences of overreduction of the cytoplasm in the cyanobacterium *Synechocystis* PCC6803

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Introduction

Oxygenic photosynthesis sustains electron flow from water to acceptors in the cytoplasm to serve, amongst others, NADPH production. Together with ATP from linear photophosphorylation and some contribution of so called photosystem 1 (PS1) cyclic photophosphorylation CO_2 fixation is enabled. As different from chloroplasts, that are embedded in the stabilized stroma of plant cells, cyanobacteria are free-living prokaryotes that need to cope with potential differences in the aquatic environment into which they are freely suspended. An important key to those functions that are required, such as in and efflux of ions and solutes, is ATP. From this the differences in PS1 cyclic electron flow capacity between a meager estimated maximum of 4% of the light energy conversion in chloroplasts to a likely substantially and conditionally defined higher attribute in cyanobacteria may become explained. Experiments in which salt was added to impose a higher need for ATP synthesis in ion balancing between the in- and outside of cells have rendered prove for increased capacity of PS1 cyclic [Jeanjean et al., 1999]. Equally, a relation between low CO_2 supply and PS1 cyclic has been advocated [Mi et al., 1992; Jeanjean et al., 1998]. A fairly remarkable difference between chloroplasts and cyanobacteria that would also need to become functionally explained is the difference in ratio of PS 1 to PS2 reaction centers. This ratio is very much in balance in chloroplasts, whereas in cyanobacteria PS 1 can largely outnumber PS 2. Observations and recent progress that will accelerate answering of questions like why cyanobacteria need such an excess of PS1, concern the monomer/trimer status [Rögner et al., 1990], the distribution of chlorophyll in adverse conditions such as iron limitation [Boekema et al, 2001], and great new structural details [Zouni et al, 2001]. Surprisingly, PS1 cyclic flow capacity increase following exposure to salt stress has been shown to be not very directly related to a change in PS1 centers, rather electron flow to plastoquinone (PQ) via cytoplasm-PQ reductase activity providing enzymes is at stake [Jeanjean et al., 1999]. We have studied the role of ferredoxin-$NADP^+$ reductase in enhanced PS1 cyclic function, and have shown its over expression [Van Thor et al., 2000]. Our current hypothesis is that the redox state of the cytoplasm regulates the expression of proteins that are needed in balancing of the NADPH to ATP ratio, and also for light energy dissipation in PS1 centers. In the cyanobacterium *Spirulina platensis* substantial work has been presented that relates monomer to trimer status of PS1 to long-red fluorescence emission at 760 nm [Karapetyan et al., 1997]. The latter may

present a way for heatless dissipation of light energy harvested in excess by PS1. Here we show that the induction of ferredoxin-$NADP^+$ reductase provides for additional cytoplasm-plastoquinone reductase capacity which becomes self regulatory as will be explained from properties within the primary domain structure of the enzyme. We also briefly report on new results that show for the first time conditional occurrence of 760 nm chlorophyll fluorescence emission from the genetically well accessible strain *Synechocystis*. These findings will be discussed in relation to the redox poise in the cytoplasm and the energetic status of the cells.

Materials and methods

Truncation of ferredoxin-$NADP^+$ from *Synechocystis* PCC6803 was as described in [Van Thor et al., 2000], psaE mutant making will be described elsewhere [Jeanjean et al., in preparation], photoaucoustic spectroscopy was as in [Jeanjean et al. 1998].77K fluorescence emission was studied according to [Karapetyan, 1997], with dithionite/light treatment to fully reduce the cytoplasm. Continuous culture was in a home-made set-up, growth rates of various mutants and copper depleted cells were acquired that way (details will be published elsewhere).

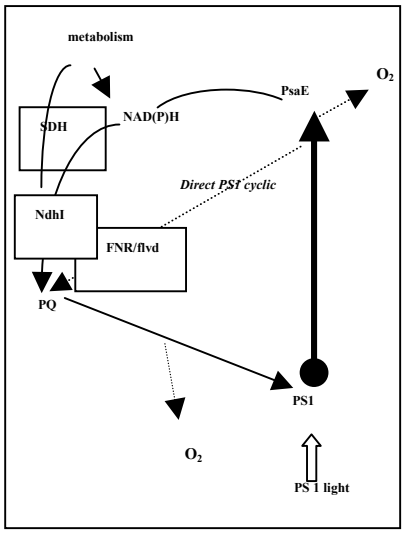
Results and discussion

Wild-type and mutants impaired in PQ-reductase activity were studied in continuous culture (Table 1, Fig. 1). Salt stress was imposed to enhance the impact of impaired cyclic function on the yield of cell growth on light. A 42 % lower yield was observed in the presence of salt for the WT. In the NDH 1 impaired M55 mutant the growth rate, with ample CO_2 supply, was not much different from the wild type. With salt present the growth rate was nearly 60% lower than for M55 without salt. As depicted in Fig. 2, the primary structure of FNR from Synechocystis PCC6803 and more from cyanobacteria in general is extended in comparison to FNR from chloroplasts. The amino terminal domain plays a role in attachment of FNR to the phycobilisomes [Schluchter and Bryant, 1997; Van Thor et al, 1999a] and as follows from the work here also in the attachment of FNR to the thylakoid membranes in view of a proposed PQ-reductase function [Van Thor et al., 2000].

Tab.1, Fig.1. Growth rates and PS-1 cyclic electron flow capacity in various mutants of the cyanobacterium *Synechocystis* PCC6803. Growth was in BG 11 medium, the dilution rates have been determined in steady state conditions. The growth data compared well to the estimated cyclic rates that were measured by photoacoustic spectroscopy.

Strain	Growth		Energy Storage	
	-NaCl	+ NaCl	-NaCl	+NaCl
Wild-type	0.046	0.027	18	24
M55 (del. *NdhB*)	0.038	0.018	7	11
SM8 (del. *petH*)	0.044	0.025	17	17
DM4 (del. *petH* + *ndhB*)	0.032	0.003	3	3
del.*psa*E	0.046	0.028	27	24
del.*psa*E			12*	22*

*measured in an atmosphere of nitrogen



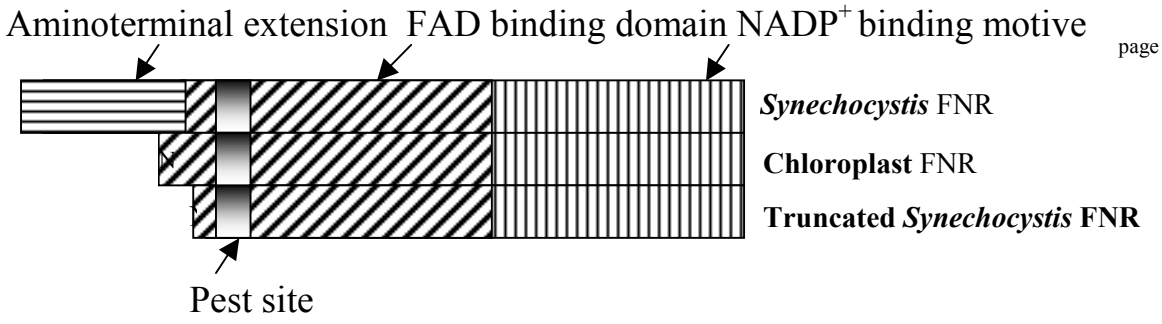


Fig.2. Primary structure of ferredoxin-$NADP^+$ reductase (FNR) typically shown from a cyanobacterium and for a plant chloroplast.The N-terminal extension shown here for *Synechocystis* PCC6803 is common for all cyanobacteria studied. The third domain is homologous to the cpcD phycobilisome linker polypeptide. FNR attaches to phycobilisomes that way. Binding to thylakoids of FNR in function of cytoplasm-plastoquinone reductase has been revealed from the properties of the truncated mutant.

In all experiments of Table 1 ample CO_2 supply was provided. Limiting CO_2 supply basically showed similar relative differences between the wild-type and the mutants (data not presented). The data support the proposed function of PS1 cyclic flow in energy generation in case of stress, also including the case of growth rate limiting CO_2 supply. The major PQ reductase is NDH1 [Mi et al., 1992]. The SDH box in Fig. 1 represents a recently established PQ reductase pathway that proceeds via succinate dehydrogenase [Cooley et al., 2000, 2001]. The data in Table 1 would exclude a prominent role for SDH in the present work. Yet, in the absence of NDH1 still a large part of PS1 cyclic flow is present, especially with salt added. We have proposed that FNR contributes this additional activity [Jeanjean et al. 1999] Moreover, we suggest that it provides for regulation of the capacity of PS1 cyclic flow. FNR in its long form is proposed to play an important role in inducible direct PS1 cyclic electron flow. The SM8 truncation mutant showed only a slight decrease in the growth rate, however the double mutant DM4 grew very slowly. The impact of the ferredoxin-$NADP^+$ -reductase truncation thus follows from the outspoken phenotype of the double mutant DM4. Truncated FNR functions totally normal in linear electron transfer [Van Thor et al., 1999b]. A study of the FNR enzyme, presented as a diagram in Fig. 2 has revealed a PEST site, highly sensitive to proteolysis, and most likely cause of the large variation in molecular mass estimates, that are revealed from the multiple bands of FNR visible in PAGE, that have appeared in literature. It is attractive to propose that FNR plays a role in the regulation of the capacity of PS1 cyclic flow through *de novo* sunthesis on the one hand and potential variable protection of its PEST site to proteolytic enzymes on the other hand. Newly synthesized enzyme with its highly positively charged aminoterminal domain easily attaches to the negatively charged thylakoid membranes. The regulation springs from the fact that the actual negative charge of the stroma exposed thylakoids relies on the presence or absence of the shielding ion Mg^{2+}. Those negative charges are exposed if Mg^{2+} ion resides in the lumen, which corresponds to a low energetic state of the thylakoids. That is the case when ATP synthesis demand is high and if maintaining of the trans-thylakoid proton flux is rate limiting. In that state the additional PQ reductase contribution of FNR is instrumental to raise the capacity for proton accumulation in the lumen. Notably, given ATP demand and proton motive force, the leaking out of Mg^{2+} ion from the lumen, if the proton motive force increases capacity or if the proton gradient is less exploited, causes the release of FNR of the stroma exposed thylakoids. This becomes non-reversible after cleavage at the PEST site. Finally, Table 1 shows that only in the PsaE mutant, absence or presence of oxygen in the gas atmosphere above the sample in the PAS chamber rendered adverse effects on the so-called energy storage, the percentage of light that actually is retained (‘stored’) by the cells. A very high storage in air actually springs from deviating electron efflux to oxygen in a Mehler type of reaction. In the presence of salt, the PsaE mutant behaved like the wild-type.

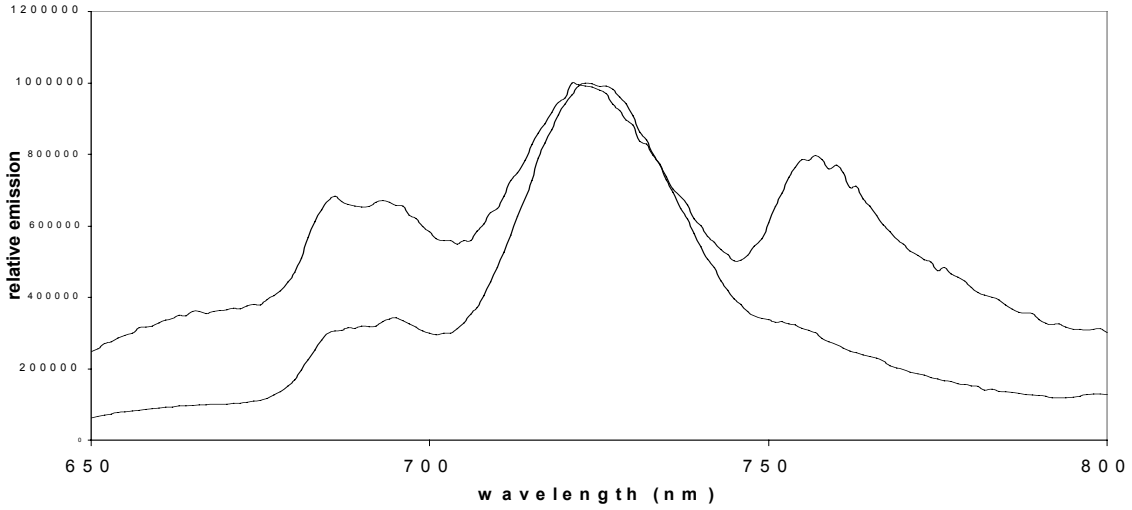


Fig. 3. Fluorescence emission spectra of control, (lower trace) and copper depleted cells of the cyanobacterium *Synechocystis* PCC 6803. The samples were taken directly from continuous culture, excitation was at 430 nm. Spectra were recorded at 77K with an Aminco Bowman AB2 spectrofluorimeter. Treatment with dithionite and light was as in [Karapetyan et al., 1997]

We have recently started to question the role of the cytoplasmic redox state in PS1 cyclic function. To that end we have cultured *Synechocystis* without copper, which stops electron efflux via cytochrome-c-oxidase, cf. fig. 1. Omission of copper has consequences for the PS1 cyclic flow, to be reported elsewhere [Jeanjean et al., in preparation]. The largest surprise in copper free cells came from 77K fluorescence emission studies. It has been long-term known that in *Spirulina platensis* reduction of the cytoplasm with dithionite and light renders fluorescence emission at 760 nm [Karapetyan et al., 1997]. In *Synechocystis* such a signal is absent. However, in the copper depleted cells a large 760 nm emission became apparent, which disappeared in time after re-addition of copper. Continued analysis of this observation, also using other methods to manipulate the cytoplasmic redox poise have shown that reducing conditions give rise to the 760 nm emission, provided that cells are exposed to those reducing conditions for a time span of several hours. A need for protein synthesis prior to the onset of 760 nm emission has been proven from experiments in the presence of protein synthesis inhibitor lincomycin (Ardelean et al., manuscript in preparation).

References

Boekema, E.J., Hifney A., Yakushevskaya, A.E., Piotrowski, M., Keegstra, W., Berry, S., Michel, K.-P., Pistorius, E.K. and Kruip, J. (2001) *Nature* in press.

Cooley, J.W., Howitt, C.A., Vermaas, W.F.J. (2000) *J. Bacteriol.* **182**, 714-722

Cooley, J.W. and Vermaas, W.F.J. (2001) *J. Bacteriol.* in press (july issue)

Jeanjean, R., Van Thor, J.J., Havaux, M., Joset, F. and Matthijs, H.C.P. (1999) in: The Phototrophic Prokaryotes (Peschek et al. eds.) Kluwer/Plenum Publ. New York pp. 251-258.

Mi, H., T. Endo, Schreiber,U., Ogawa,T., and Asada, K. (1992) *Plant Cell Physiol* **33,** 1233-1237.

Jeanjean, R., Bédu, S., Havaux, M., Matthijs, H.C.P. and Joset, F. (1998) *FEMS Microbiol. Lett.* **167,** 131-137.

Karapetyan, N.V., Dorra, D., Schweitzer, G., Bezsmertnaya, I.N., and Holzwarth, A.R. (1997) *Biochemistry* **36**, 13830-13837.

Rögner M, Mühlenhoff U, Boekema, EJ and Witt HT (1990) *Biochim. Biophys. Acta* **1015,** 415-424

Schluchter, W.A. and Bryant, D.A. (1992) *Biochemistry* **31**, 3092-3102

van Thor, J.J., Jeanjean, R., Havaux, M., Sjollema, K.A., Joset, F., Hellingwerf, K.J., Matthijs, H.C.P. (2000) *Biochim.Biophys. Acta* **1457**, 129-144.

van Thor, J.J., Gruters, O.W.M., Matthijs, H.C.P. and Hellingwerf, K.J. (1999a) *EMBO J.* **18,** 4128-4136.

van Thor, J.J, Geerlings, T.H., Matthijs, H.C.P. and Hellingwerf, K.J. (1999b) *Biochemistry* **38**, 12735-12746.

Zouni, A., Witt, H.-T., Kern, J., Fromme, P., Krauss, N., Saenger, W., Orth, P. (2001) *Nature* **409**, 739-743