Chimaeric CP47 mutants of the cyanobacterium *Synechocystis* sp. PCC 6803 carrying spinach sequences: Construction and function

Wim F.J. Vermaas¹, Gaozhong Shen¹,³ & Itzhak Ohad²

¹Department of Botany, and Center for the Study of Early Events in Photosynthesis, Arizona State University, Tempe AZ 85287-1601, USA; ²Department of Biological Chemistry, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel; ³Present address: Department of Molecular and Cell Biology, Pennsylvania State University, University Park PA 16802, USA

Received 24 October 1995; accepted in revised form 6 December 1995

**Key words:** gene replacement, Photosystem II, photosynthesis, thylakoid membranes, transformation

**Abstract**

Chimaeric mutants of the cyanobacterium *Synechocystis* sp. PCC 6803 have been generated carrying part or all of the spinach *psbB* gene, encoding CP47 (one of the chlorophyll-binding core antenna proteins in Photosystem II). The mutant in which the entire *psbB* gene had been replaced by the homologous gene from spinach was an obligate photoheterotroph and lacked Photosystem II complexes in its thylakoid membranes. However, this strain could be transformed with plasmids carrying selected regions of *Synechocystis psbB* to give rise to photoautotrophs with a chimaeric spinach/cyanobacterial CP47 protein. This process involved heterologous recombination in the cyanobacterium between *psbB* sequences from spinach and *Synechocystis* 6803; which was found to be reasonably effective in *Synechocystis*. Also other approaches were used that can produce a broad spectrum of chimaeric mutants in a single experiment. Functional characterization of the chimaeric photoautotrophic mutants indicated that if a decrease in the photoautotrophic growth rates was observed, this was correlated with a decrease in the number of Photosystem II reaction centers (on a chlorophyll basis) in the thylakoid membrane and with a decrease in oxygen evolution rates. Remaining Photosystem II reaction centers in these chimaeric mutants appeared to function rather normally, but thermoluminescence and chlorophyll *a* fluorescence measurements provided evidence for a destabilization of Qₐ. This illustrates the sensitivity of the functional properties of the PS II reaction center to mild perturbations in a neighboring protein.

**Abbreviations:** diuron = 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F_v – variable chlorophyll *a* fluorescence; HEPES = N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid); (k)bp = (kilo)base pairs; PS II = Photosystem II; QA = primary electron-accepting plastoquinone in Photosystem II; QB = secondary electron-accepting plastoquinone in Photosystem II; SDS = sodium dodecyl sulfate

**Introduction**

Photosystem II (PS II) is the pigment-protein complex in thylakoid membranes that catalyzes the light-induced reduction of plastoquinone by water. Light is absorbed by antenna pigments (such as chlorophylls *a* and *b* in plants, and chlorophyll *a* and phycobilins in cyanobacteria), and light energy is transferred to P680, the primary donor in the PS II reaction center (reviewed by Nixon et al. 1992; Barber 1992; Vermaas 1993). Two chlorophyll-binding antenna proteins, CP43 and CP47 (approximately 43 000 and 47 000 M_w, respectively), are associated directly with the D1 and D2 proteins that form the PS II reaction center (reviewed by Bricker 1990). Of these two chlorophyll-binding antenna proteins, CP47 appears to be closer to the reaction center complex as PS II reaction centers retaining CP47 but lacking CP43 can be isolated (Ghanotakis et
al. 1989; Dekker et al. 1989). The relatively peripheral arrangement of CP43 in PS II complexes is in agreement with ultrastructural observations (Boekema et al. 1994, 1995).

CP47 is also important for stable assembly of the PS II complex: In mutants in which the psbB gene (encoding CP47) has been interrupted or deleted, D1 and D2 are virtually absent from the thylakoid membrane (Vermaas et al. 1988). Structurally, CP47 is quite conserved throughout evolution: The amino acid sequence of CP47 from Synechocystis sp. PCC 6803 is 76% identical compared to CP47 from spinach (Vermaas et al. 1987). However, functionally the constraints on the chlorophyll-binding proteins in the PS II core complex may be different in cyanobacteria as compared to that in plants. In the first place, the peripheral antenna complex is different (phycobilisomes in cyanobacteria and the integral chlorophyll-binding light-harvesting complex LHC II in plants; see Gantt (1988) and Thornber et al. (1988)), and thus recognition between the core (CP47/CP43) and peripheral antenna may be different in the two groups of organisms. Secondly, CP47 is involved in binding extrinsic proteins associated with the water-splitting apparatus, particularly the '33 kDa' manganese-stabilizing protein (Bricker 1990; Vermaas et al. 1993). The manganese-stabilizing protein occurs both in plants and cyanobacteria, but its sequence is rather divergent between the two phyla. Also, two proteins in contact with the manganese-stabilizing protein, the 16 and 23 kDa proteins, are present in plants but absent in cyanobacteria. In cyanobacteria, these proteins may have been functionally replaced by cytochrome c-550 (Shen and Inoue 1993). Thus, an evolutionary divergence between cyanobacteria and higher plants may have occurred with respect to CP47 to optimize, for example, the energy transfer efficiency and the interaction with extrinsic components at the donor side of PS II.

To test the exchangeability of CP47 between Synechocystis and higher plants, we set out to determine to which extent CP47 from spinach can functionally replace cyanobacterial CP47 in Synechocystis through replacing part or all of the psbB gene from Synechocystis by that from spinach. One of the methodologies to create such chimaeric Synechocystis/spinach psbB genes involves recombination between the non-identical spinach and cyanobacterial psbB sequences. Homologous recombination by means of double-crossover events occurring at sequences that are identical between genome and plasmid is known to occur in Synechocystis 6803 at a high frequency (Shes-takov and Reaston 1987; Williams 1988). The length of sequence identity required for efficient recombination generally is found to be ≥ 100 nucleotides at each recombination site. However, in this study double recombination in Synechocystis was found to occur also if the sequences in the domains where crossover takes place are similar, but non-identical. This corroborates and extends observations made when creating chimaeric CP43 mutants (Carpenter et al. 1993).

Functional characterization of the chimaeric CP47 mutants indicates that at least parts of the Synechocystis CP47 protein are needed for stability of the PS II reaction center of Synechocystis 6803 and for optimal function of the secondary quinone-type electron acceptor in PS II, Q_B.

Materials and methods

Growth conditions and transformation protocols of Synechocystis sp. PCC 6803 have been described (Vermaas et al. 1987). Cyanobacterial growth rates in liquid culture were determined by measurements of the optical density (light scattering) at 730 nm as a function of time in a Shimadzu UV-160 spectrophotometer. Linearity between optical density and cell number was demonstrated up to an optical density of 0.4 at 730 nm (not shown). At optical densities exceeding 0.4, cell cultures were diluted in the cuvette before measurements were made.

After restriction digestion of genomic DNA from cyanobacteria, Southern blotting to GeneScreen Plus (NEN-Du Pont) was performed, and blots were hybridized with a nick-translated cyanobacterial psbB probe according to the manufacturer's recommendations. The hybridized blots were washed at reduced stringency (two 10-min washes in 0.45 M NaCl/45 mM Na-citrate (pH 7.0), followed by two 30-min washes in 0.3 M NaCl/30 mM Na-citrate (pH 7.0) and 0.5% (w/v) sodium dodecyl sulfate (SDS), and two 30-min washes in 15 mM NaCl/1.5 mM Na-citrate (pH 7.0); all washes were done at room temperature) to retain some hybridization between the cyanobacterial psbB probe and the genomic spinach psbB present in some mutants.

Oxygen evolution measurements were performed on a Gilson oxygraph (model KM) using intact cells suspended in 25 mM N-(2-hydroxyethyl)pipperazine-N'-(2-ethanesulfonic acid) (HEPES)/NaOH, pH 7.0. The chlorophyll concentration was 10 μg/ml, and the suspension was kept at 30 °C during the measurement.