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Mutagenesis of histidine-469 in the photosystem II chlorophyll *a*-binding protein CP47 in *Synechocystis* sp. PCC 6803

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Introduction

The photosystem II (PSII) chlorophyll-binding proteins, CP43 and CP47, serve as a core antenna and possess structural homology to the N-terminal domains of the photosystem I PsaA and PsaB reaction center proteins (reviewed in Barber *et al*., 2000). In the case of CP47 it has been estimated that about 15 chlorophyll molecules bind to the protein (Zheleva *et al*., 1998; Rhee *et al*., 1998). In addition, from a hydropathy analysis of CP47, there are expected to be 12 His residues located in six membrane–spanning domains as indicated in Fig. 1 (Bricker, 1990; but see Vermaas, 1993). Based on other chlorophyll-binding proteins the imidazole nitrogens of these His residues may serve as axial ligands to chlorophyll (e.g., McDermott *et al.* (1995) and Jordan *et al*. (2001)). Additionally, a role for histidyl ligation of chlorophyll in CP47 has been supported by mutagenesis studies (Eaton-Rye and Vermaas, 1992; Shen *et al*., 1993; Shen and Vermaas, 1994; Wu *et al*., 1999). However, other residues, including Gln, act as chlorophyll ligands (e.g., Kühlbrandt *et al*. (1994) and Jordan *et al*. (2001)).

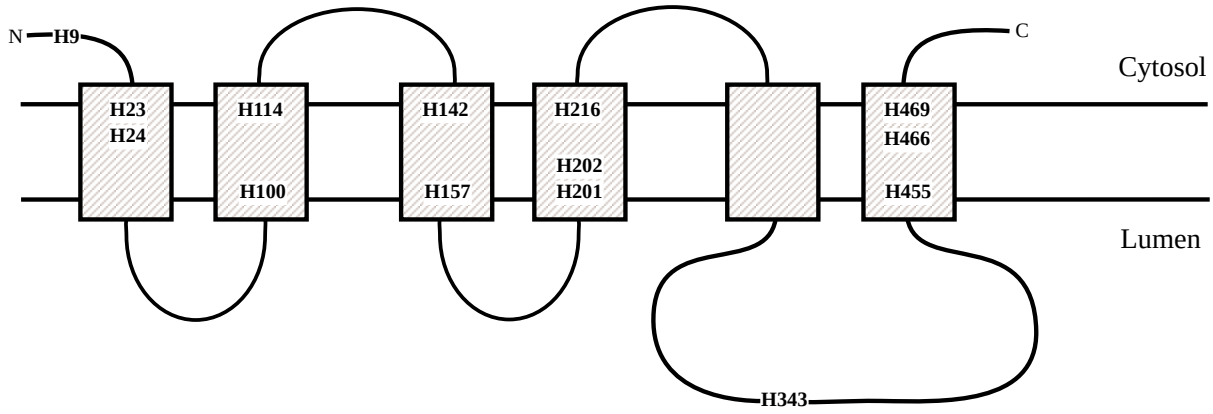

Fig. 1. Diagram of CP47 showing the conserved histidine residues. The membrane-spanning helices are shown as shaded boxes and are numbered as one to six from left to right.

To investigate if the His residues in helix six of CP47 serve as chlorophyll ligands His-455, His-466 and His-469 were replaced by Gln and the H455Q, H466Q and H469Q strains were characterized (Eaton-Rye and Vermaas, 1992). Whereas the phenotypes of H455Q and H466Q were similar to wild type, the mutant H469Q showed a five-fold reduction in the number of PSII centers assembled and a reduction in the corresponding rate of photoautotrophic growth. Moreover, photoautotrophic growth was abolished in the mutants

H455T, H455Y, H469Y, and the double mutant, F430L,H466R (Wu *et al*., 1999). Glutamine is a conservative substitution for His and the similarity between the H455Q and H466Q mutants and the wild-type phenotype suggest that the His-455 and His-466 residues may serve as chlorophyll ligands. However, the inability of Gln to substitute for His-469 may be indicative of a different role for this residue. Interestingly, modeling based on an 8 Å electron density map, obtained by electron crystallography, suggests that His-455, His-466 and His-469 are located at 6.97 Å, 6.47 Å and 7.45 Å, respectively, from the nearest chlorophylls and are therefore unlikely to act directly as chlorophyll-binding ligands (Barber *et al*., 2000).

A spontaneous revertant, H469Qrev, was obtained that restored photoautotrophic growth in the H469Q mutant. Sequencing confirmed that the change responsible was not in the *psbB* gene and therefore the H469Qrev strain was a pseudorevertant that contained an intergenic suppressor mutation (Eaton-Rye and Vermaas, 1992). The current study was undertaken to characterize the specificity of this suppressor mutation and different substitutions were introduced at His-469 in both the control (see Methods) and pseudorevertant genetic backgrounds. In addition, the effect of the suppressor mutation on the phenotype of a strain with a deletion from Ser-471 to Thr-473 was investigated. The Δ(S471-T473) mutant had previously been shown to exhibit significantly reduced levels of oxygen evolution and only possessed 50% of the PSII centers found in the wild type. Interestingly, this mutant appeared to have impaired electron transfer between the PSII plastoquinone electron carriers Q_A and Q_B (Clarke, 2000). Finally, several candidate genes for the intergenic suppressor were investigated.

Materials and methods

Mutations were introduced into the *psbB* gene, encoding CP47, as described in Eaton-Rye and Vermaas (1991) and measurements of oxygen evolution, in whole cells, were performed as in Morgan *et al*. (1998). The relative levels of assembled PSII reaction centers were determined by measuring the variable chlorophyll fluorescence yield (Nixon and Diner, 1992). The control was identical to the wild type except for a kanamycin-resistance cassette located downstream of the *psbB* gene (Eaton-Rye and Vermaas, 1991).

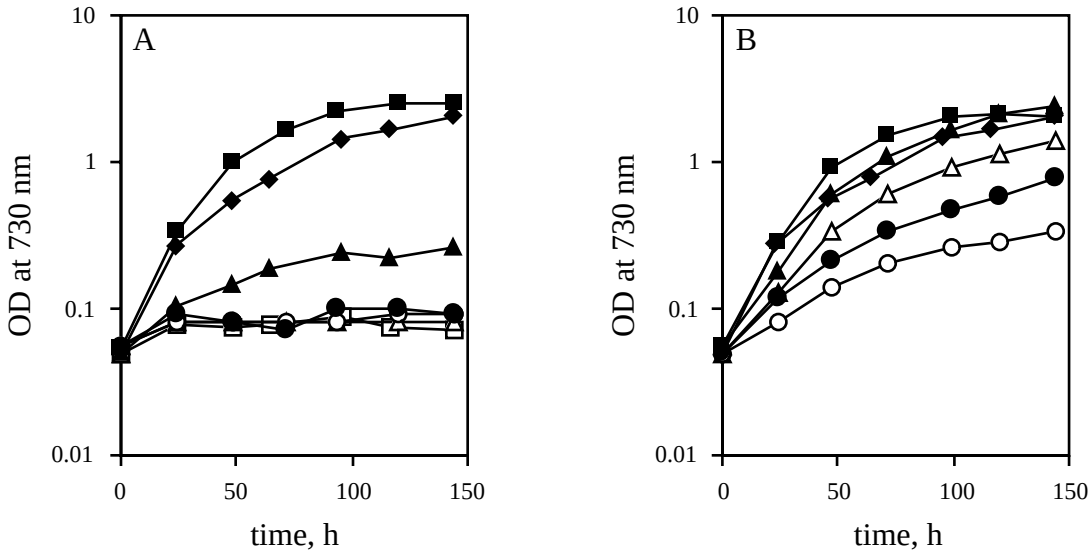

Fig. 2. Photoautotrophic growth curves of *Synechocystis* sp. PCC 6803 strains as measured by the optical density at 730 nm in the control (A) or pseudorevertant (B) genetic backgrounds. A. Control (solid squares), Δ*psbB* (open squares), H469K (solid circles), H469P (open circles), H469Q (solid triangles), H469Y (open triangles) and Δ(S471-T473) (solid diamonds). B. Control (solid squares), H469Krev (solid circles), H469Prev (open circles), H469Qrev (solid triangles), H469Yrev (open triangles) and Δ(S471-T473)rev (solid diamonds).

Results

The initial step in this work was the creation of a *psbB* deletion mutant in the genetic background of the H469Qrev strain. This was achieved by following the procedures described in Eaton-Rye and Vermaas (1991). Oligonucleotide-directed mutagenesis was then used to introduce Lys, Pro and Tyr substitutions at His-469 in both the control and pseudorevertant backgrounds. In addition, the deletion between Ser-471 and Thr-473 was introduced into the pseudorevertant background.

Table 1. Comparison of oxygen evolution and PSII assembly in different mutants in the control and pseudorevertant genetic backgrounds.

Strain	Rate of Oxygen Evolution[1]	PSII Centers Determined by the Variable Fluorescence Yield[2]
control	1.00	1.00
Δ*psbB*	0.00	0.00
H469K	0.02	0.02
H469P	0.01	0.02
H469Q	0.30	0.15
H469Y	0.03	0.02
Δ(S471-S473)	0.10	0.40
H469Krev	0.42	0.35
H469Prev	0.39	0.25
H469Qrev	0.76	0.85[3]
H469Yrev	0.62	0.37
Δ(S471-T473)rev	0.10	0.65

[1]The rate of oxygen evolution for the control was 490 μmol 0_2 (mg of chlorophyll)$^{-1}$ h^{-1}.
[2]Normalized to a variable fluorescence yield of 0.55.
[3]Determined by [^{14}C]diuron-binding (Eaton-Rye and Vermaas, 1992).

In Fig. 2A the photoautotrophic doubling time for the control strain was 12 h; whereas, the Δ*psbB* strain (lacking *psbB*) was an obligate photoheterotroph and did not assemble PSII centers (Table 1; Eaton-Rye and Vermaas (1991)). However, the Δ(S471-T473) strain had a photoautotrophic doubling time of 15 h. In contrast, substitutions at His-469 resulted in a significant disruption of photoautotrophic growth. The mutant H469Q was found to have a doubling time of ~50 h, in agreement with Eaton-Rye and Vermaas (1992), and the mutants H469K, H469P and H469Y, were obligate photoheterotrophic strains. The data in Table 1 demonstrated that the number of PSII centers in the H469K, H469P and H469Y strains was close to zero and hence almost no oxygen evolution was detected. In the case of H469Q the number of PSII centers was reduced by 85% when compared to the control strain but the oxygen evolution rate was only reduced by 70% (cf. Eaton-Rye and Vermaas (1992)).

Figure 2B compares the corresponding rates of photoautotrophic growth for the different mutants in the genetic background of the pseudorevertant strain H469Qrev with the control strain. As reported earlier the H469Qrev mutant has a doubling time of approximately 14 h (Eaton-Rye and Vermaas, 1992). In Fig. 2B the Δ(S471-T473)rev strain has a similar doubling time to Δ(S4712-T473) and therefore the suppressor mutation did not significantly

alter the phenotype of this mutant. This is also demonstrated by the data in Table 1, although a slight increase in the number of PSII centers was detected for this mutant in the pseudorevertant background. However, the strains H469Yrev, H469Krev, and H469Prev were able to grow photoautorophically with doubling times of 18 h, 24 h and 34 h, respectively, and therefore the suppressor mutation was able to partially restore photoautotrophic growth to these strains. The data in Table 1 also show that the number of functional PSII centers, and the rates of oxygen evolution, detected in the H469Krev, H469Prev, H469Qrev and H469Yrev strains had increased in the pseudorevertant background. However, the rates of oxygen evolution in the Δ(S471-T473) and Δ(S471-T473)rev mutants were unchanged.

To initiate a search for the gene containing the suppressor mutation several candidates were selected. The *psbA2* and *psbA3* genes, that both encode D1, and the *psbD1* and *psbD2* genes, encoding D2, were examined. In addition, the *psbC* gene, encoding CP43, and the *btpA* gene were tested. A mutation in the *btpA* gene had previously been shown to recover a mutant strain of *Synechocsytis* sp. PCC 6803 with a severely reduced PSI content (Bartesvich and Pakrasi, 1997). Each of the candidate genes were obtained by PCR from genomic DNA obtained from the H469Qrev mutant and spotted onto a lawn of H469Q cells using the dot transformation technique of Dzelzkalns and Bogorad (1988). However, none of these genes rescued the H469Q mutant (data not shown).

Discussion

The substitution of CP47:His-469 by Lys, Pro or Tyr produced obligate photoheterotrophic mutants that could be partially recovered by the suppressor mutation in the H469Qrev strain. Nevertheless, a precise role for His-469 in the PSII complex remains to be established and it is not yet known if the suppressor is specific for mutations at His-469; however, the phenotype of the Δ(S471-T473) mutant was not rescued.

The identity of the suppressor mutation may provide information on the role of CP47 in the assembly and stability of the PSII complex. A promising approach is the mapping of the second site mutation using the complementation method described by Vermaas (1998). In particular, the inability of the H469K, H469P and H469Y strains to grow photoautotrophicallly will facilitate the identification of large restriction fragments that carry the secondary mutation by screening for the recovery of photoautotrophic growth. This, in turn, would enable the identification of the gene carrying the mutation.

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