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Species-dependent alteration of electron transfer in the early stages of charge stabilization in Photosystem I

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ABSTRACT

Electron transfer (ET) in Photosystem I (PS I) is bidirectional, occurring in two pseudosymmetric branches of cofactors. The relative use of two branches in the green alga *Chlamydomonas reinhardtii* and the cyanobacterium *Synechocystis* sp. PCC 6803 has been studied by changing the Met axial ligands of the chlorophyll *a* acceptor molecules, A_{0A} and A_{0B}, to His. The nature of the effect on the ET is found to be species dependent. In *C. reinhardtii*, transient absorption and transient EPR data show that in the $M688H_{PsaA}$ variant, forward ET from A_{0A} to the quinone, A_{1A}, is blocked in 100% of the PS I complexes. In contrast, in *Synechocystis* sp. PCC 6803, forward ET from A_{0A} to A_{1A} is blocked in only 50% of the PS I complexes, but in those PS I complexes in which electrons reach A_{1A}, further transfer to the iron–sulfur cluster F_X is blocked. Similar species differences are found for the corresponding B-branch variants. One possible explanation of this behavior is that it is the result of two conformers in which an H-bond between the His side chain and the O1 carbonyl group of A_1 is either present or absent. The spectroscopic data suggest that the two conformers are present in nearly equal amounts in the *Synechocystis* sp. PCC 6803 variants, while only the conformer without the H-bond is present in the same variants of *C. reinhardtii*.

1. Introduction

In oxygenic photosynthesis, Photosystem I (PS I) uses light to transport electrons across a steep thermodynamic gradient from plastocyanin or cytochrome c_6 in the lumen to ferredoxin or flavodoxin in the stroma. Electron transfer from P_{700} (a chlorophyll *a*/chlorophyll *a′* special pair) to F_X (a [4Fe–4S] cluster) occurs along two branches of cofactors located at the interface of the PsaA and PsaB subunits [1,2] (Fig. 1). Each branch contains a pair of chlorophyll *a* molecules ($ec2_A/ec3_A$ or $ec2_B/ec3_B$) and a phylloquinone (PhQ_A or PhQ_B). Spectroscopically, the $ec3_A$ and $ec3_B$ chlorophylls are referred to as A_{0A} and A_{0B} and the PhQ_A and PhQ_B phylloquinones are referred to as A_{1A} and A_{1B}. Because both branches converge at F_X, their relative use is not apparent from inspection of the structure, but has been determined experimentally by making point mutations to amino acids in PsaA and PsaB that interact with specific cofactors in the A- and B-branches [3]. These studies have provided strong evidence that both branches are active, with reported lifetimes of ~200 ns and ~20 ns for electron transfer from A_{1A} to F_X and from A_{1B} to F_X in the A- and B-branches respectively. The relative activity of B-branch is species dependent and is estimated to be <20% in the cyanobacterium *Synechocystis* sp. PCC 6803 [4] but ~40% in the green alga *Chlamydomonas reinhardtii* [5,6].

Similar or identical mutations of the Met residues $M688_{PsaA}$ and $M668_{PsaB}$, which act as the axial ligands to chlorophylls A_{0A} and A_{0B}, respectively, have been reported to produce different phenotypes in *Synechocystis* sp. PCC 6803 and *C. reinhardtii*. In the first study of the *C. reinhardtii* $M688H_{PsaA}$ and $M668H_{PsaB}$ variants, Fairclough et al. [7] observed that the EPR and ENDOR signals of photoaccumulated phyllosemiquinone were different in the two variants and that signals from the wild type were a mixture of those from the two variants. The authors of concluded that the mutations prevented electron transfer

Abbreviations: PS I, Photosystem I; DPIP, 2,6-dichlorophenolindophenol; Chl, chlorophyll; EPR, electron paramagnetic resonance; OOP-ESEEM, out-of-phase electron spin echo envelope modulation

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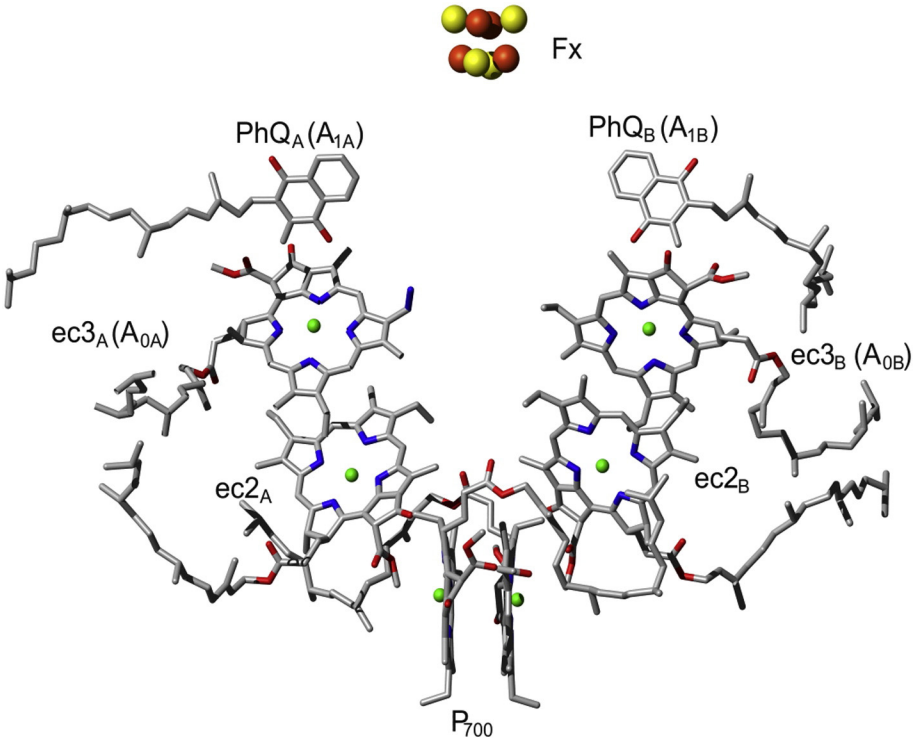

Fig. 1. Arrangement of the electron transfer cofactors in Photosystem I. The labels, $ec3_A$, $ec3_B$, PhQ_A and PhQ_B are the crystallographic nomenclature for the acceptors in the A- and B-branches. The labels A_{0A}, A_{0B}, A_{1A} and A_{1B} are the corresponding spectroscopic labels. The figure was constructed from the 2.5-Å resolution X-ray structure of PS I from *T. elongatus* (PDB ID: 1JB0) [2] using molmol [39].

past A_0 in the branch carrying the mutation. A time-resolved optical study of the same variants [8,9] revealed the presence of a chlorophyll *a* anion with a lifetime that was significantly longer than that of A_0^- irrespective of which branch the mutation was made in. The authors of these studies also concluded that the mutations prevented electron transfer beyond A_0, thus extending the lifetime of the A_{0A}^- and A_{0B}^- chlorophyll anions from ~30 ps to several nanoseconds. In contrast, we recently showed that similar variants in *Synechocystis* sp. PCC 6803 caused only partial blockage of electron transfer in the branch carrying the mutation and also altered the rate of electron transfer from the downstream quinone to F_X [10]. Transient EPR, transient absorbance, and molecular dynamics simulations provided evidence that alteration of the A_1 to F_X kinetics is due to the formation of an H-bond between the δ-nitrogen of the introduced His and the O1 carbonyl oxygen of the adjacent phylloquinone. This H-bonding arrangement can be seen in Fig. 2, which shows a comparison of the protein-cofactor binding of the A_{0A} chlorophyll and the A_{1A} phylloquinone in the X-ray structure of wild type *T. elongatus* (top) and a structural model of the $M688H_{PsaA}$ variant (bottom).

Differences in the behavior of these PS I variants at low temperature have also been reported between species [11,12]. An electron spin echo envelope modulation (ESEEM) study of whole cells of the wild type and $M668H_{PsaB}$ variant in *Synechocystis* sp. PCC 6803 at 100 K suggested that electron transfer is strongly biased toward the A-branch (A:B branching ratio of <0.2) and signals from the $M688H_{PsaA}$ variant could not be found in whole cells [12]. In contrast, a similar study of the same variants in whole cells of *C. reinhardtii* suggested a branching ratio of 0.42, and a spectrum assigned to $P_{700}^+ A_{1B}^-$ was found if the cells were illuminated before freezing [12]. These results hint at a species-dependent difference in the phenotype of the mutation, but it is difficult to compare the two datasets, which have been collected under different conditions and in different laboratories.

We therefore decided that a systematic study of the behavior of the $M688H_{PsaA}$ and $M668H_{PsaB}$ variants in *C. reinhardtii* and *Synechocystis* sp. PCC 6803 under the same conditions and measured using the same instruments was warranted. Here, we show that there are indeed significant differences between the Met to His variants between the two species. We propose that in *C. reinhardtii*, only the coordination bond to A_{0A}/A_{0B} from the introduced His residue is present whereas in *Synechocystis* sp. PCC 6803, the H-bond to A_{1A}/A_{1B} and the coordination bond to A_{0A}/A_{0B} are present in approximately equal amounts.

2. Materials and methods

2.1. Construction and growth of the C. reinhardtii $M684H_{PsaA}$ and $M664H_{PsaA}$ variant strains

The $M684H_{PsaA}$ and $M664H_{PsaB}$ mutations were created as described previously [13]. Briefly, the wild type strain CC-125 was used as the recipient for clones containing relevant portions of the PsaA or PsaB genes following site-directed mutagenesis using the QuickChange site-directed mutagenesis kit (Stratagene Inc.) and the aminoglycoside adenyl transferase (*aadA*) gene. Transformants generated by biolistic bombardment were identified by growth on spectinomycin and streptomycin and were brought to homoplasmicity as the concentrations of antibiotics were increased stepwise over several passages. Homoplasmicity was verified by digesting the PCR products of the putative mutation site to specific restriction endonucleases to test for the presence or absence of novel cut sites introduced during site-directed mutagenesis. Cells were harvested and broken in a chilled French press at 3000 psi. Thylakoid membranes were solubilized on ice in darkness with 0.9 % (wt/vol) n-dodecyl-β-D-maltoside at 0.8–1.0 mg Chl mL^{-1} for 20 min and the solubilized fraction was then isolated from the insoluble debris by ultracentrifugation at 65,000 ×*g* for 25 min. Solubilized membrane proteins were laid on sucrose density gradients formed by freeze-thaw of tubes containing 5 mM Tricine–NaOH (pH 8.0), 0.3 M sucrose, 0.3 M betaine, and 0.05% n-dodecyl-β-