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Electron transfer from the A_{1A} and A_{1B} sites to a tethered Pt nanoparticle requires the FeS clusters for suppression of the recombination channel

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ABSTRACT

In this work, a previously described model of electron withdrawal from the A_{1A}/A_{1B} sites of Photosystem I (PS I) was tested using a dihydrogen-producing PS I–NQ$(CH_2)_{15}$S–Pt nanoconstruct. According to this model, the rate of electron transfer from A_{1A}/A_{1B} to a tethered Pt nanoparticle is kinetically unfavorable relative to the rate of forward electron transfer to the FeS clusters. Dihydrogen is produced only when an external donor rapidly reduces P_{700}^{+}, thereby suppressing the recombination channel and allowing the electron in the FeS clusters to proceed via uphill electron transfer through the A_{1A}/A_{1B} quinones to the Pt nanoparticle. We tested this model by sequentially removing the FeS clusters, F_B, F_A, and F_X, and determining the concentration of cytochrome c_6 (Cyt c_6) at which the backreaction was outcompeted and dihydrogen production was observed. P_{700}-F_A cores were generated in a *menB* insertionally inactivated strain by removing F_B with $HgCl_2$; P_{700}-F_X cores were generated in a *menB psaC* insertionally inactivated strain that lacks F_A and F_B, and P_{700}-A_1 cores were generated in a *menB rubA* insertionally inactivated strain that lacks F_X, F_A and F_B. Quinone incorporation was measured using transient electron paramagnetic resonance spectroscopy and time resolved optical spectroscopy. Cyt c_6 was titrated into each of these PS I preparations and the kinetics of P_{700}^{+} reduction were measured. A similar experiment was carried out on PS I–NQ$(CH_2)_{15}$S–Pt nanoconstructs assembled from these PS I preparations. This study showed that the concentration of Cyt c_6 needed to produce dihydrogen was comparable to that needed to suppress the backreaction. We conclude that the FeS clusters serve to 'park' the electron and thereby extend the duration of the charge-separated state; however, in doing so, the redox advantage of removing the electron at A_{1A}/A_{1B} is lost.

1. Introduction

The Photosystem I (PS I) reaction center is a multi-subunit, pigment-protein complex found in plants and cyanobacteria that, upon illumination, generates and maintains a long-lived charge-separated state [1,2]. Its core consists of a heterodimer of the PsaA and PsaB proteins in which the electron transfer cofactors are arranged in two pseudo-C2-symmetric functionally active branches denoted A and B [3–5], spanning the thylakoid membrane [6]. The ~100 chlorophylls that form the antenna system in cyanobacterial PS I serve to absorb light and transfer energy to the primary donor (P_{700}). The kinetics and pathway of the initial charge separation are not firmly established and several different models have been proposed recently [7–9]. However, in all cases, charge separation between P_{700}, a chlorophyll (Chl) *a*′/*a* special pair (Chl *a*′ is the 13^2 epimer of Chl *a*) and either of the chlorophyll acceptors A_{0A} or A_{0B} is complete within a few ps or less. To increase the lifetime of the charge separation, the electron is transferred along a chain of cofactors that includes phylloquinone (A_{1A}/A_{1B}) and three [4Fe–4S] clusters (F_X, F_A, and F_B). The latter two clusters are bound to the PsaC subunit that is located on the stromal side of the membrane. The final charge-separated state, $P_{700}^{+}F_B^{-}$, is stable for ~65 ms, which is sufficient time for the electron to be transferred to a soluble [2Fe–2S] ferredoxin [6,10,11]. Fig. 1 provides a comprehensive view of the electron transfer kinetics in PS I.

PS I has a number of properties that make it a promising candidate for use as a photosensitizer in the production of solar biofuels.

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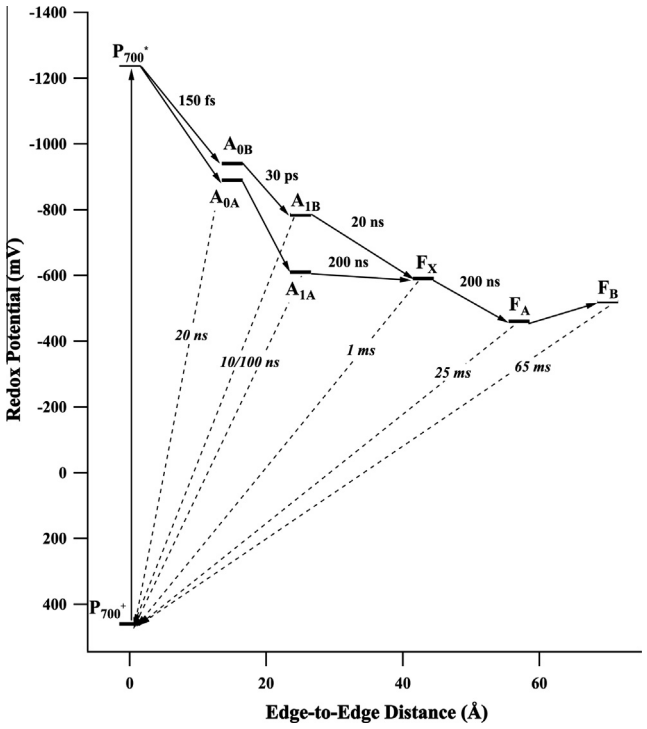

Fig. 1. Edge-to-edge distance and midpoint potential of PS I cofactors. Solid lines represent forward electron transfer lifetimes and dashed lines represent charge recombination lifetimes. The A and B branches are depicted as A_{0A}/A_{1A} and A_{0B}/A_{1B}, respectively. It is not known whether A_{0A} and A_{0B} have different reduction potentials; they are separated here for clarity purposes only.

Not only does it have a high quantum yield of charge separation, estimated to be >0.98 [12], but it is exceptionally stable to high light and high temperatures. Additionally, the midpoint potential of the final acceptor, F_B, is sufficiently negative to reduce protons to dihydrogen in the presence of a suitable catalyst [1].

Greenbaum and colleagues were the first to exploit this capability by depositing Ru, Os, and Pt catalysts on thylakoid membranes and on PS I particles and observing dihydrogen production on exposure to light [13–19]. More recently, a number of groups have successfully coupled both enzymatic and inorganic catalysts to PS I for dihydrogen production [20–32]. All of these attempts focused on obtaining the electron from the reduced F_B cluster at the terminus of the transfer chain. However, Gibbs free energy is lost as the electron progresses from A_0 to F_B (Fig. 1); hence, if the electron could be extracted earlier in the electron transfer chain, it could be obtained at a more reducing potential. For example, direct electron transfer from the A_{1B} quinone would allow the electron to be available at a midpoint potential of −845 mV [5].

Terasaki and coworkers were the first to pioneer a system of electron withdrawal from the A_1 sites for use in a biophotosensor [33]. In their work, PS I was treated with diethyl ether to remove phylloquinone from the A_{1A} and A_{1B} sites, which were then reconstituted with a synthesized naphthoquinone derivative. However, chemical extraction removes all of the carotenoids and most of the Chls [34–40], which leads to a decrease in the optical cross-section for photon capture. Additionally, the lack of carotenoids renders the reaction center susceptible to oxidative damage. Thus, we attempted to reconstitute the A_{1A} and A_{1B} sites in a larger and more robust system containing all of the carotenoid and Chl molecules by using the *menB* strain of PS I.

The *menB* gene encodes 1,4-dihydroxy-2-naphthoyl-CoA synthetase, a necessary enzyme in the phylloquinone biosynthesis pathway [41,42]. When the *menB* gene is inactivated, phylloquinone is not synthesized, and the A_{1A} and A_{1B} sites are occupied by plastoquinone-9 (PQ-9) [43–47], a mobile electron carrier that shuttles electrons from Photosystem II to the cytochrome b_6f complex and transports protons from the stroma to the lumen. This substituted benzoquinone binds loosely to the A_{1A} and A_{1B} sites of PS I and can be readily displaced with a variety of substituted napthoquinones [46,48,49]. We took advantage of this ability to displace PQ-9, to introduce a menaquinone with a head group identical to that of the native phylloquinone, but with a carbon chain long enough to reach the surface of the protein. When a Pt nanoparticle is bound to the thiolated end of the substituted menaquinone and the resulting PS I–$NQ(CH_2)_{15}S$–Pt nanoconstruct is illuminated, it evolves dihydrogen in the presence of a sacrificial electron donor [32].

In a previous publication [32] we proposed a hypothesis for how this system might function. In the model proposed, the rate of electron transfer to the attached nanoparticle is kinetically unfavorable relative to the rates of forward electron transfer from A_{1A}^- and A_{1B}^- to the FeS clusters ($\tau_{A1A \rightarrow FX}$ = 200 ns; $\tau_{A1B \rightarrow FX}$ = 20 ns) and to the rate of charge recombination from the FeS^- clusters to P_{700}^+ (∼65 ms). Under these conditions, the electron in the FeS clusters ultimately recombines with P_{700}^+, and no dihydrogen is produced. However, if charge recombination is suppressed by reducing P_{700}^+ rapidly with an external donor, the electron is transferred instead to the Pt nanoparticle. In essence, the FeS clusters serve to 'park' the electron, thereby extending the lifetime of the charge-separated state and making it possible for an external donor to reduce P_{700}^+. The key to dihydrogen production, then, is the suppression of the charge recombination channel.

If this model is correct, then faster charge recombination times from earlier acceptors should require higher concentrations of Cyt c_6 to produce dihydrogen. In this work, we test the proposed model by sequentially removing the FeS clusters F_B (P_{700}-F_A cores), F_A/F_B (P_{700}-F_X cores), and F_X, F_A/F_B (P_{700}-A_1 cores) from PS I and measuring the backreaction kinetics and dihydrogen production as a function of Cyt c_6 concentration for each sample.

2. Materials and methods

2.1. Cell growth and PS I purification

Strains used for the purification of PS I include the wild type (WT) strain, the *menB* insertionally inactivated strain of *Synechocystis* sp. PCC 6803 (hereafter *menB* strain), and a *menB rubA* insertionally inactivated strain of *Synechococcus* sp. PCC 7002 (hereafter *menB rubA* strain). The *Synechocystis* sp. PCC 6803 *menB* gene was disrupted with the *aadA* gene conferring spectinomycin resistance. The *Synechococcus* sp. PCC 7002 *menB rubA* strain was created by the insertional inactivation of *menB* and *rubA* by antibiotic resistance genes for gentamicin resistance (*aacC1*) and for kanamycin resistance (*aphII*), respectively. These strains were grown and PS I was purified as described previously [43,50,51]. Cells were harvested by centrifugation, broken by three passes through an M-110EH-30 microfluidizer processor (Microfluidics). Cell debris was removed via low speed centrifugation (2000*g*) and the resulting supernatant was centrifuged at 158,000*g* for 1 h to pellet the thylakoid membranes. Membranes were solubilized with 1% (w/v) β-dodecylmaltoside (β-DM) for 1 h at 4 °C in the dark. The PS I trimers were purified on a 5–20% sucrose density gradient in 50 mM Tris–HCl buffer (pH 8.3), 0.05% (w/v) β-DM, subsequently pelleted, and suspended in 50 mM Tris–HCl buffer (pH 8.3) containing 20% (w/v) glycerol and 0.05% (w/v) β-DM. The *menB rubA* strain did not produce PS I trimers; hence, PS I monomers were collected from the first gradient, dialyzed, and the contaminating PS II was removed using a DEAE-Sepharose