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Time-resolved visible and infrared difference spectroscopy for the study of photosystem I with different quinones incorporated into the A1 binding site

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ABSTRACT

Room (298 K) and low (77 K) temperature time-resolved visible and infrared difference spectroscopy has been used to study photosystem I particles with phylloquinone (2-methyl-3-phytyl-1,4-naphthoquinone), menadione (2-methyl-1,4-naphthoquinone) and plastoquinone 9 (2,3-dimethyl-5-prenyl-l,4-benzoquinone), incorporated into the A_1 binding site. Concentrated samples in short path-length (~5 μm) sample cells are typically used in FTIR experiments. Measurements were undertaken using standard "dilute" samples at 298 K, and concentrated (~5×) samples at both 298 and 77 K. No concentration induced alterations in the flash-induced absorption changes were observed. Concentrated samples in short path-length cells form a transparent film at 77 K, and could therefore be studied spectroscopically at 77 K without addition of a cryoprotectant. At 298 K, for photosystem I with plastoquinone 9/menadione/phylloquinone incorporated, $P700^+F_{A/B}^-$ radical pair recombination is characterized by a time constant of 3/14/80 ms, and forward electron transfer from A_{1A}^- to F_x by a time constant of 211/3.1/0.309 μs, respectively. At 77 K, for concentrated photosystem I with menadione/phylloquinone incorporated, $P700^+A_1^-$ radical pair recombination is characterized by a time constant of 240/340 μs, with this process occurring in 58/39% of the PSI particles, respectively. The origin of these differences is discussed. Marcus electron transfer theory in combination with kinetic modeling is used to simulate the observed electron transfer time constants at 298 K. This simulation allows an estimate of the redox potential for the different quinones in the A_1 binding site.

1. Introduction

In photosynthetic oxygen evolving organisms solar energy is captured and converted independently, but cooperatively, in two separate photosystems called photosystem I and photosystem II. Photosystem II uses light to catalyze the generation of products that eventually lead to the oxidation of water and the subsequent liberation of molecular oxygen as a by-product. Photosystem I (PSI) on the other hand uses light to catalyze the formation of reducing products that eventually leads to the reduction of carbon dioxide, and its eventual incorporation into glucose.

In each of the two photosystems light energy conversion is realized via the transfer of electrons via a series of protein bound acceptors across a biological membrane. The nature of the acceptors in terms of their electronic and structural organization within the protein environment has been a subject of interest for decades.

In this manuscript we focus on photosystem I (PSI) reaction centers (RCs). More specifically, we focus on the quinone molecule that occupies the A_1 binding site in cyanobacterial PSI particles from *Synechocystis* sp. 6803 (*S6803*). The arrangement of the electron transfer (ET) cofactors in the PSI RC is outlined in Fig. 1A. PSI contains two almost identical chains of ET cofactors bound to the protein subunits PsaA or PsaB. The cofactors bound to the PsaA or PsaB proteins are labeled with a subscript A or B, respectively. P700, the primary electron donor in PSI, is a heterodimeric Chl-*a*/Chl-*a*′ species, where Chl-*a*′ is a 13^2 epimer of Chl-*a* [1]. The primary electron acceptor, A_0, is a monomeric Chl-*a* molecule [2], and A_1 is a phylloquinone (PhQ) molecule [3]. PhQ is a 2-methyl-3-phytyl-1,4-naphthaquinone, the structure of which is outlined in Fig. 1B. F_X, F_A and F_B are (4Fe–4S) iron sulfur clusters [4,5].

In PSI, following light excitation of P700, an electron is transferred to A_1 (via A_0) in <50 ps [6,7]. To further stabilize the charge separated state, the electron is then transferred from A_1^- to F_X. A_1^- to F_X ET is characterized by two time constants of 10–25 ns and 260–340 ns at room temperature (RT) [8–12]. From F_X^- an electron is then transferred to F_A and F_B, also on a nanosecond timescale [13].

Abbreviations: 2MNQ, 2-methyl, 1,4-naphthquinone (menadione/vitamin k_3); Chl-a, chlorophyll-a; DS, difference spectra, spectrum, spectroscopy or spectroscopic; DDS, double difference spectrum; C = O, carbonyl; ET, electron transfer; FTIR, Fourier transform infrared; H bond, hydrogen bond; ms, millisecond; ns, nanosecond; PhQ, phylloquinone (vitamin k_1); PQ_9, plastoquinone 9; PSI, photosystem I; RC, reaction center; RT, room temperature; *S6803*, *Synechocystis* sp. 6803; TR, time resolved

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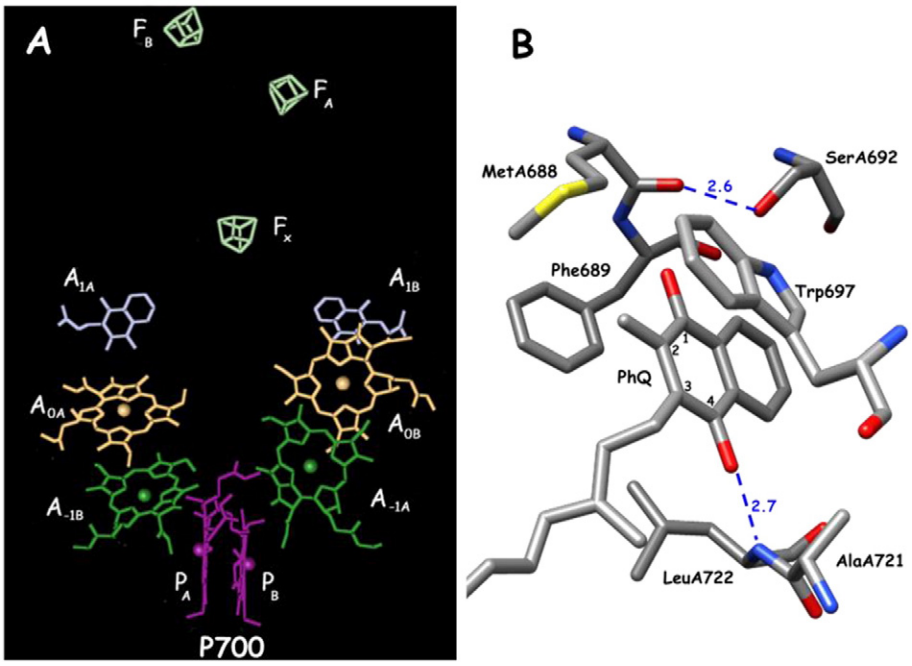

Fig. 1. (A) Arrangement of the two branches of ET cofactors in PSI. Number subscript refers to cofactor. Letter subscript refers to protein subunit (PsaA or B) to which cofactor is bound. The A/B-branch refers to the set of ET cofactors on the left/right side, respectively. Fig. 1A is generated using the 2.5 Å x-ray crystal structure of trimeric PSI particles from the cyanobacterium *Synechococcus elongatus* (PDB file accession number PDB ID: 1JB0) [5]. (B) View of PhQ in the A_{1A} binding site. Possible H-bonding interactions are shown (*dotted*), and PhQ numbering scheme is indicated. Nitrogen/oxygen/sulfur atoms are blue/red/yellow, respectively.

As mentioned, in cyanobacterial PSI at RT, forward ET from A_1^- to F_X is characterized by two phases with time constants of 10–25 ns and 260–340 ns [8–12]. These "fast" and "slow" kinetic phases have been associated with ET down the B and A branches, respectively [8–12]. As the temperature is lowered, in a portion of the RCs, forward ET diminishes and is replaced by a $P700^+A_1^-$ direct recombination reaction, which is characterized by a time constant of ~245 μs at 77 K [8]. This time constant is found using PSI particles from *Synechococcus elongatus*. In PSI from *S6803* the corresponding time constant is ~285–340 μs (see below and [9]). In cyanobacterial PSI particles at 77 K, the $P700^+A_1^-$ state recombines in ~45% of the particles, the $P700^+F_X^-$ state recombines in greater than 5 ms in ~20% of the PSI particles, and in ~35% of the PSI particles ET is irreversible [8]. The degree to which each branch is active in ET is becoming clearer [14,15], with the 260–340 ns/285–340 μs components at RT/77 K, respectively, being associated with ET along the A branch [14–16]. The 10–25 ns phase and the irreversible fraction at RT and 77 K, respectively, have been associated with ET along the B branch [16,17]. The different rates of ET from A_1^- to F_X at RT, as well as the different functionality at 77 K is primarily ascribed to different midpoint potentials of PhQ in the A_1 binding site on the A and B branches.

The reported range of the midpoint potential for PhQ occupying the A_1 binding site is −670 to −850 mV (see [16] for a review), making it one of the most reducing quinones in biology. The estimated difference in the midpoint potentials between A_{1A} and A_{1B} ranges from 25 to 173 mV, with ET from A_{1A}^- to F_X being "thermodynamically uphill" while A_{1B}^- to F_X is "thermodynamically downhill" [17,18] (see below).

The unprecedented redox potential of PhQ in the A_1 binding site is in part a result of interactions of PhQ with the surrounding protein environment. Fig. 1B shows a view of PhQ in the A_{1A} binding site and several of the surrounding amino acids. The B-side is similar. Fig. 1B indicates that the C_1=O group of PhQ is not H-bonded whereas the C_4=O is H-bonded to the backbone NH group of LeuA722 (*S. elongatus* numbering).

Recently it has been demonstrated that different quinones can be incorporated into the A_1 binding site in *menB* null mutant PSI particles simply by incubating the particles in a large molar excess of the quinone of interest [19–22]. This quinone incubation method relies on mutant cells (from the cyanobacterium *Synechocystis* sp. 6803) in which genes that code for enzymes involved in PhQ biosynthesis have been disrupted [23,24]. For example, in mutants where the *menB* gene has been deactivated PhQ biosynthesis is inhibited and plastoquinone-9 (PQ_9) is recruited into the A_1 site instead [23–25]. PSI particles from these mutant cells will be referred to as $menB^-$ PSI particles. In $menB^-$ PSI particles foreign quinones can displace PQ_9 in the A_1 binding site, simply by incubating particles in the presence of the quinone of interest [19–22].

In this manuscript we describe work undertaken using regular $menB^-$ PSI particles, with PQ_9 in the A_1 binding site, and $menB^-$ PSI particles with PhQ or menadione [2-methyl-1,4-naphthquinone (2MNQ)] incorporated into the A_1 binding site. This manuscript focuses on measurement of the kinetics of forward and reverse ET in PSI samples with the three different quinones incorporated, at both RT and 77 K. The kinetics of ET are sensitive to the redox potential of the quinone in the A_1 binding site, and here we use Marcus theory in combination with a quasi-equilibrium model (of radical pair states) to estimate the redox potentials of the quinones in the A_1 binding site.

2 . Materials and methods

Trimeric PSI particles from *menB* null mutant cells from *Synechocystis* sp. PCC 6803 (*S6803*) were isolated and stored as described previously [23]. To incorporate quinones into the A_1 binding site, PSI particles are incubated in the presence of a ~1000× molar excess of the quinone of interest (quinone/RC ratio). Quinones were dissolved in ethanol and added in such a way that the ethanol concentration never exceeds 2% of the total volume. PSI particles were