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Kinetics of phyllosemiquinone oxidation in the Photosystem I reaction centre of *Acaryochloris marina*

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ABSTRACT

Light-induced electron transfer reactions in the chlorophyll *a*/*d*-binding Photosystem I reaction centre of *Acaryochloris marina* were investigated in whole cells by pump-probe optical spectroscopy with a temporal resolution of ~5 ns at room temperature. It is shown that phyllosemiquinone, the secondary electron transfer acceptor anion, is oxidised with bi-phasic kinetics characterised by lifetimes of 88 ± 6 ns and 345 ± 10 ns. These lifetimes, particularly the former, are significantly slower than those reported for chlorophyll *a*-binding Photosystem I, which typically range in the 5–30 ns and 200–300 ns intervals. The possible mechanism of electron transfer reactions in the chlorophyll *a*/*d*-binding Photosystem I and the slower oxidation kinetics of the secondary acceptors are discussed.

1. Introduction

Chlorophyll (Chl) *d* is the most abundant chromophore bound to the photosynthetic pigment protein complexes of the cyanobacterium *Acaryochloris marina* [1–3]. Typically the Chl *d* to Chl *a* ratio exceeds 10, although the exact stoichiometry is dependent on the irradiance conditions during growth [4,5]. Chl *d* absorption, in vivo is about 30–35 nm red-shifted with respect to that of Chl *a* henceforth extending the possibility of performing oxygen evolution by the absorption of near-infrared photons (e.g. [1–6]). In addition, α-carotene is found in *A. marina* in place of β-carotene.

The Photosystem I (PS I) reaction centre of *A. marina* binds more than 90 Chl *d* molecules and 1 to 2 molecules of Chl *a* [3,7–9]. Although the majority of the Chl *d* molecules bound to PS I in *A. marina* have light harvesting function [3,6], it has been shown both by optical [7,10–12] and electron paramagnetic resonance [8,13,14] spectroscopies that the stable cation species generated following photochemical charge separation resides on a Chl *d*, or, possibly, a Chl *d*–Chl *d*′ hetero-dimer [5,8]. Based on the maximum of the differential absorption bleaching in the Q_y transition, this chemical species is referred to as $P_{740}^{(+)}$ [7,8,10,11]. The redox midpoint potential of the P_{740}/P_{740}^{+} couple was originally reported to be up-shifted by 85–155 mV with respect to stable donor cation of Chl *a*-binding PS I reaction centres [7]. However, recent re-investigations yielded values of $E^0_{P_{740}^{+}/P740}$ ranging from 425 to 450 mV [9,11,12], which are in the same range as the values reported for the P_{700}^{+}/P_{700} redox couple (reviews in Ref. [15]). As the energy input is about 100 meV smaller in Chl *d* as compared to Chl *a* containing PS I, the conserved free energy level of the cation suggests that the reducing power produced by charge separation may be lower in *A. marina* as discussed in refs. [9,11,12].

At present, the mechanism of primary charge separation in PS I of *A. marina* is not fully elucidated. Ultra-fast optical transient spectroscopy suggests that Chl *a* might be involved in electron transfer reactions in *A. marina* [8,10], either as an electron acceptor, equivalent to A_0 in Chl *a*-binding PS I, or as the primary electron donor, equivalent to the accessory Chls in PS I (see [16]). The subsequent electron acceptor, which is commonly known as A_1 has been shown to be, as in the case of Chl *a*-binding PS I reaction centres (RC), a phylloquinone (PhQ) molecule [8,9,14]. The kinetics of oxidation of A_1^- in *A.*

Abbreviations: Chl, chlorophyll; DCMU, 3-3,4 dichlorophenyl-1,1-dimethylurea; FCCP, carbonyl-cyanide-*p*-trifluoromethoxy-phenylhydrazone; PhQ, phylloquinone; PS (I/II), Photosystem (I/II); RC, reaction centres; $P^{(+)}$ or $P_{700/740}^{(+)}$, PS I electron donor (cation)

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marina, as estimated by the pulsed-EPR technique at 265 K [14], appear to be slightly slower (~450 ns) with respect to the slow phase of A_1^- oxidation in Chl *a*-binding PS I complexes (~300 ns) (e.g. [15,17]). The fast component of A_1^- oxidation, which is ~20 ns at room temperature [15,17], is generally not observable by electron paramagnetic spectroscopy. On the other hand, the kinetics of charge recombination measured at 77 K yield a value of 150 μs, which falls within the range determined for Chl *a*-binding PS I reaction centres [11]. Moreover, the functional distance between the spin densities on P_{740}^+ and A_1^- of 25.23 ± 0.05 Å [14] is essentially the same as that determined for Chl *a*-binding PS I (reviewed in Ref. [18]). Hence, the structural organisation of the electron transfer chain in PS I appears to be conserved, in PS I reaction centres whether they bind mainly Chl *d* or Chl *a*. This idea is further confirmed by the large degree of homology of the genes *psa*A and *psa*B coding for the reaction centre subunits of *A. marina* with respect to other oxygenic phototrophic organisms [19], even though they are the least homologous amongst different cyanobacterial strains. Similar homology is also observed for the *psa*C gene, which codes for the subunit binding the terminal electron acceptors, iron–sulphur clusters F_A and F_B. EPR [8,14] and optical spectroscopy [7,11] indicate that the characteristics of F_A and F_B in *A. marina* are virtually unaltered with respect to these cofactors when bound to the Chl *a*-binding RCs. Indeed, it was noticed that all PS I subunits of *A. marina* display high homology with respect to other cyanobacterial strains, except for the *PsaI* and *PsaX* genes [19].

Even though a clear picture of the structural and chemical nature of the electron transfer cofactors bound to the PS I reaction centre of *A. marina* is emerging, there is a lack of clear understanding of the dynamics of electron transfer reactions. In particular, secondary electron transfer involving phylloquinone A_1 and successive electron acceptors has only been investigated in frozen samples (265 K and 77 K, [14]). The value reported for a close-to-physiological temperature, was determined in time-resolved EPR experiments, which, in general, suffer from relatively limited time-resolution (~50 ns). Furthermore the lifetime determined by such techniques might be biassed by magnetic relaxation processes which, together with the electron transfer reaction rates, contribute to the decay of the electron spin echo signal (for a discussion see Ref. [20]).

In order to characterise the oxidation kinetics of A_1^- in the PS I reaction centre of *A. marina* at room temperature further we have investigated electron transfer reactions in living cells of this organism using optical pump-probe spectroscopy having a temporal resolution of ~5 ns. Results are compared with those obtained in a Chl *a*-binding cyanobacterial model system, *Synechocystis sp.* PCC 6803. It is shown that the kinetics of A_1^- oxidation are bi-phasic, characterised by slower lifetimes than are seen in Chl *a* PS I, 88 ± 6 ns and 345 ± 10 ns compared to 18 ± 4 and 285 ± 10, respectively. Possible models of secondary electron transfer in the Chl *d*-binding PS I RC are discussed.

2. Material and methods

Cultures of *A. marina* were grown under continuous illumination (15 μE m^{-2} s^{-1}) in K + ESM medium [21], except that the $FeSO_4.7H_2O$ level was increased to 4 mg L^{-1}. The cells were shaken vigorously to allow sufficient aeration. Cultures of *Synechocystis sp.* PCC 6803 were grown in BG11 medium, under continuous illumination and aerated by air bubbling. The cultures were harvested during semi-logarithmic growth by centrifugation at 5500 *g* for 5 min, and immediately suspended in the growth medium supplemented with 20% W/V Ficoll to avoid sedimentation during the measurements. 3-(3,4 dichlorophenyl)-1,1-dimethylurea (DCMU) (20 μM) and hydroxylamine (1 mM) were added to the cell suspension to suppress PS II photochemistry and carbonyl-cyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP) (8 μM) was used to dissipate transmembrane ionic gradients.

2.1. Optical spectroscopy

Light-induced optical transients were monitored with a home-built pump-probe spectrometer which has been previously described in detail [22]. In brief, the actinic flash is provided by a dye laser (LDS 698) pumped by a frequency double Nd-YAG laser (Quantel, Brilliant). The excitation wavelength was 700 nm, the bandwidth of the actinic pulse is ~7 ns and the intensity is adjusted to excite ~70% of the reaction centres. The transients are probed by the out-put of an Optical Parametric Oscillator (OPO), pumped by a frequency tripled Nd-YAG laser (Continuum, Surelite). For measurements in the near-UV the output of the OPO is frequency doubled. The pump-probe delay is controlled by a home-built pulse programmer. The excitation (pump) flashes were fired with a frequency of 0.2 Hz, which allows for the cellular electron transfer chain to return to the dark-adapted condition between each flash. The kinetic traces are acquired firstly by scanning the pump-probe sequence from short to long delays followed by a reverse (long to short) series and each pump-probe point is sampled twice. Typically, four to eight of these sequences were averaged depending of the signal-to-noise at a given wavelength. The increased averaging, and hence the increased experimental time, is generally required in the near UV.

2.2. Data analysis

Optical transients acquired at multiple wavelengths were fitted to a sum of exponential functions by a global minimisation routine as previously described [23,24].

3. Results

Laser flash-induced transient absorption kinetics, were recorded in living cells of *A. marina* (Fig. 1) and *Synechocystis sp.* PCC 6803 (Fig. 2) and monitored through the near-UV and the visible spectrum. The kinetic traces were fitted by a sum of exponential functions using a global fitting routine. In *A. marina* a satisfying agreement is obtained by considering three decay components characterised by lifetimes of 88 ± 4 ns, 345 ± 10 ns and 11 ± 0.7 μs, as shown in Fig. 1 for selected wavelengths. In *Synechocystis sp.* PCC 6803 the best fit yields lifetimes of 18 ± 4 ns, 285 ± 10 ns and 7.5 ± 0.5 μs, also shown at selected wavelengths in Fig. 2. Moreover, in both organisms, a non-decaying component (within the experimental time window of 10 ns—20 μs) needs to be considered. Extending the time window to 1 ms, it was determined that this signal decays with a lifetime of 125 ± 25 μs in *A. marina* cells, and 140 ± 15 μs in *Synechocystis sp.* PCC 6803 cells (Fig. 3). The decay associated spectra (DAS) resulting from the global fit of the kinetics in the 10 ns–20 μs interval are presented in Fig. 4.

3.1. Long-living and microsecond decay components

The spectra associated with the non-decaying component (up to 20 μs) recorded in vivo are consistent with difference spectra arising from oxidation of the meta-stable electron donor, P_{740}, of *A. marina* and P_{700} in *Synechocystis*. The reduction lifetime of 125 ± 25 μs derived from an experiment performed on an extended time scale is also consistent with the reduction of the meta-stable oxidised electron donor occurring through a diffusion-limited process involving a soluble electron donor, which in the case of *A. marina* is probably plastocyanin ([12], but see also Ref. [25] for discussion of electron donors).

Diffusion controlled rates of P^+ reduction are most commonly observed in prokaryotes (reviewed in Ref. [26]). In agreement with this, the rate of P_{700}^+ reduction in *Synechocystis* is determined as 140 ± 15 μs. The DAS associated with the non decaying component (in the 10 ns–20 μs time window, Fig. 4D) obtained in *Synechocystis sp*. PCC 6803 suggests that the electron donor, under the growth