Biochimica et Biophysica Acta 1857 (2016) 1550–1560

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

The strontium inorganic mutant of the water oxidizing center ($CaMn_4O_5$) of PSII improves WOC efficiency but slows electron flux through the terminal acceptors☆

Colin Gates [a,b,c], Gennady Ananyev [a,b], G. Charles Dismukes [a,b,*]

[a] Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ 08854, United States
[b] Department of Chemistry and Chemical Biology, Rutgers University, Piscataway, NJ 08854, United States
[c] Department of Computational Biology and Molecular Biophysics, Rutgers University, Piscataway, NJ 08854, United States

ARTICLE INFO

Article history:
Received 4 March 2016
Received in revised form 26 May 2016
Accepted 10 June 2016
Available online 15 June 2016

Keywords:
Photosystem II
Strontium
Thermosynechococcus
Water oxidizing complex
Manganese
Oxygen

ABSTRACT

Herein we extend prior studies of biosynthetic strontium replacement of calcium in PSII-WOC core particles to characterize whole cells. Previous studies of *Thermosynechococcus elongatus* found a *lower* rate of light-saturated O_2 from isolated PSII-WOC(Sr) cores and 5–8× slower rate of oxygen release. We find similar properties in whole cells, and show it is due to a 20% larger Arrhenius activation barrier for O_2 evolution. Cellular adaptation to the sluggish PSII-WOC(Sr) cycle occurs in which flux through the Q_AQ_B acceptor gate becomes limiting for turnover rate in vivo. Benzoquinone derivatives that bind to Q_B site remove this kinetic chokepoint yielding 31% *greater* O_2 quantum yield (QY) of PSII-WOC(Sr) vs. PSII-WOC(Ca). QY and efficiency of the WOC(Sr) catalytic cycle are greatly improved at low light flux, due to fewer misses and backward transitions and 3-fold longer lifetime of the unstable S3 state, attributed to greater thermodynamic stabilization of the WOC(Sr) relative to the photoactive tyrosine Y_Z. More linear and less cyclic electron flow through PSII occurs per PSII-WOC(Sr). The organismal response to the more active PSII centers in Sr-grown cells at 45 °C is to lower the number of active PSII-WOC per Chl, producing comparable oxygen and energy per cell. We conclude that redox and protonic energy fluxes created by PSII are primary determinants for optimal growth rate of *T. elongatus*. We further conclude that the (Sr-favored) intermediate-spin S = 5/2 form of the S2 state is the active form in the catalytic cycle relative to the low-spin S = 1/2 form.

1. Introduction

Oxygenic photosynthesis is responsible for the vast majority of the Earth's biomass [1]. Photosystem II (PSII) powers oxygenic photosynthesis by using light to drive the formation of a proton gradient and the chemical reduction of plastoquinone (PQ) by the net reaction: $2H_2O + PQ \rightarrow O_2 + 2PQH_2 + 4\ (H_{inside} - H^+_{outside})$. PSII is highly conserved across a wide range of organisms that contribute to this global process, both in terms of the protein subunit composition and many specific residues comprising the active site of water oxidation, the metal-oxo cluster known as the water-oxidizing complex (WOC) [2]. The WOC is comprised of an inorganic cluster, Mn_4CaO_5, coordinated by conserved amino acid residues, that delivers electrons to the PSII reaction center via a photooxidizable tyrosine residue, Y_Z. The WOC is universally found throughout organisms which rely on PSII. The only known inorganic alteration to this cluster which results in a catalytically active WOC is the replacement of calcium (Ca) by strontium (Sr) [3], although Sr-substitution has not yet been found in vivo.

Over thirty years ago it was first observed that substitution of strontium for calcium by reconstitution in the PSII-WOC partially reactivated oxygen evolution activity in isolates of both cyanobacteria [4] and higher plants [5]. Shortly thereafter, the site was localized to the WOC by demonstration of reconstitution of $P680^+$ reduction [6] and by electron paramagnetic resonance (EPR) difference spectra which demonstrated coupling to the Mn cluster [7,8]. Historically, the isolated PSII core with strontium and artificial electron acceptors has been studied to investigate the function of calcium relative to the WOC [5,7,9], its

Abbreviations: Chl *a*, chlorophyll *a*; DMBQ, 2,5-dimethylbenzoquinone; EPR, electron paramagnetic resonance spectroscopy; FRR(F), fast repetition rate (fluorometry); LED, light emitting diode; OD, optical density; O_2, oxygen; PSII, Photosystem II; Pheo, pheophytin; RC, reaction center of PSII; ROS, reactive oxygen species; STF, single turnover flash; Tyr_Z^+, redox-active tyrosine Z cation radical; WOC, water-oxidizing complex; XRD, X-ray diffraction; Y_{O2}, flash oxygen yield; Yss, steady-state oxygen yield.

☆ *We dedicate this manuscript to the memory of Fabrice Rappaport, on whose work this text stands.*

* Corresponding author at: Waksman Institute of Microbiology, Rutgers, The State University of New Jersey, 190 Frelinghuysen Rd., Piscataway, United States.
E-mail address: dismukes@chem.rutgers.edu (G.C. Dismukes).

http://dx.doi.org/10.1016/j.bbabio.2016.06.004
0005-2728/

relationship to S-state transition times [10], restructuring events [11,12], and free energy gaps [13], and especially the effect of substitution on the overall rate of oxygen production [5,7,14,15].

The WOC inorganic cluster is unstable when removed from its protein scaffold. However, it can be reassembled from the individual elements in vitro, starting with the cofactor-depleted apo-WOC PSII complex, plus free Mn^{2+}, Ca^{2+}, and HCO_3^- in the presence of light and electron acceptors. This in vitro process is called photoassembly and is believed to mimic biogenesis and repair in vivo [3,16–19]. Ca^{2+} was shown to be essential for forming the functional WOC cluster by restricting the number of photooxidizable Mn^{2+} to precisely 4.0 per site [20]. In the absence of Ca^{2+}, Mn^{2+} photooxidation incorporates as many as 20 Mn^{2+} and eventually forms an amorphous oxyhydroxide polymer containing Mn^{3+} and Mn^{4+}, $[MnO_x(OH)_y]_{n>20}$, that is catalytically inactive [21,22]. This inactivity is due to suppression of formation of the catalytically active form of Mn. Therefore, Ca^{2+} plays an indispensable role in templating the formation of the correct structure of the Mn_4O_5 WOC core.

Curiously, strontium replacement of calcium accelerates the rate of photoassembly of the WOC [23]. Sr^{2+} was shown to bind selectively to the Ca^{2+} effector site of spinach apo-WOC-PSII particles, causing a 5-fold acceleration of the rate of cluster photoassembly compared to Ca^{2+}, as measured by the recovery of O_2 evolution [23]. Mechanistically, it was found that this occurs because Sr^{2+} is five times faster than Ca^{2+} in accelerating the net flux through the first two sequential assembly steps: k_1 – the photo-oxidation of the first $Mn^{2+} \rightarrow Mn^{3+}$, and k_2 – the subsequent dark step in which Ca^{2+} affinity increases by 10× and forms the first stable intermediate, $Ca^{2+}(OH)_2Mn^{3+}$. Thus, this second (dark) process is rate-limiting overall for recovery of O_2 evolution function. A protein conformational change was postulated to occur on the dark step, but not shown directly [10]. Deactivation by charge recombination from this intermediate was shown to be 2× slower in the presence of Sr^{2+} than Ca^{2+}, and postulated to arise from its greater thermodynamic stability. The resulting photoassembled Sr-WOC-PSII has a 65% lower O_2 evolution yield per flash than Ca^{2+} samples, which is also similar to the slower light-saturated O_2 evolution rate in the Sr-exchanged holo-enzyme, noted above [7]. No metal ion other than Sr^{2+} has been found to functionally replace Ca^{2+} in water oxidation. Neither Mg^{2+} nor Ba^{2+} bind to the Ca^{2+} effector site, while VO^{2+} and Cd^{2+} do bind, but do not activate O_2 evolution activity.

The mechanism by which Sr creates these major functional changes and whether the influence it has extends also to controlling flux through the electron acceptor side of PSII remain unclear. However, detailed structural information has appeared recently which can contribute to this understanding. The crystal structures of PSII with calcium or strontium in the WOC (PDB IDs: 3WU2 and 4IL6, respectively) have been determined with relatively high resolution for *Thermosynechococcus vulcanus* (the nearest relative of our selected strain) [24,25]. A somewhat lower resolution structure of the native calcium PSII core from *Thermosynechococcus elongatus* is also available (PDB ID: 4V62) [26,27]. These data provide a structural platform for prediction of function and analysis of spectroscopic data. In particular, detectable structural shifts of the location of Sr^{2+} closer to Y_Z may cause redox energy changes for the WOC (S-states) relative to Y_Z. Specifically, since the WOC is closer to Y_Z, the substitution can be expected to increase the electrical potential gradient and the electronic coupling, thereby facilitating forward electron transfer and slowing charge recombination between the acceptor and donor sides. This prediction is contrary to the observation of slower light saturated O_2 evolution. Redox potential shifts within the WOC across the S2/S3 transition and between Q_A and Q_A^- stemming from Sr^{2+} substitution have previously been demonstrated [13].

In the present manuscript, we extend the earlier functional studies to examine for the first time the kinetics of electron/proton transfer in native Ca and Sr-substituted WOCs in vivo, within intact cells of *T. elongatus*. This approach, although more challenging, eliminates both alterations caused by biochemical isolation in detergents and the use of artificial electron acceptors essential for in vitro studies. Kinetic studies of WOC turnover can now be extended to intact cells by application of a Chl fluorescence method that allows detection of the WOC catalytic cycle [28–31]. Together, these methods allow precise determination of PSII turnover energetics and kinetics in its native environment. As such, we can examine the influence of the electron acceptor side on PSII flux from water oxidation and its potential regulation of WOC turnover.

2. Materials and methods

All cultures used were grown at 45 °C in replete or Sr-substituted (380 μM Ca or Sr) DTN medium [32] in volumes of 150 mL with bubbling of 1.0% CO_2-supplemented air under 40 μmol m^{-2} s^{-1} continuous light from Philips Silhouette cool white fluorescent lamp, following the method of Sugiura with modifications [33]. All cultures were derived from a *Thermosynechococcus elongatus* His-tagged CP-47 mutant strain provided by Dr. Sugiura (henceforth referred to as *T. elongatus*). Our method here varies from that of Sugiura in that *T. elongatus* is very light-sensitive and grown at below normal growth temperatures (48–55 °C normal). In Sr-containing medium cells only grow stably at lower light intensities. For both cultures, we had good success at ~40–45 μmol m^{-2} s^{-1} continuous light at a growth temperature of 45 °C. All samples were taken from culture in mid-exponential growth. For determination of growth phase of sampled cultures, optical density (OD) was measured at 730 nm using a Thermo Scientific Evolution-60 spectrophotometer. Chlorophyll *a* (Chl *a*) concentration was determined using this instrument, after methanol extraction from culture [34].

Quantification of the D1 subunit of PSII was carried out via two methods (Fig. S1). Polyacrylamide gel electrophoresis and Western blot were performed against a D1 protein standard (Agrisera) using primary antibodies targeted to a conserved PSII sequence and horseradish peroxidase secondary antibody/luminol detection. Western blots were conducted in quadruplicate and results averaged. All samples were adjusted to equivalent chlorophyll concentrations of 3 μg/mL before blotting for ease of comparison to other data. Quantification of the tyrosine-D radical was performed by EPR spectroscopic measurements against a standard curve of Fremy's salt (potassium nitrosodisulfonate) using a Bruker Elexsys E580 spectrometer; conditions: 10 mW microwave power, 70 dB receiver gain, and 100 kHz modulation frequency at 100 K.

Oxygen was detected electrochemically by custom-made Clark-type electrodes using single turnover flashes (STF) of 20 μs duration applied at a range of flash frequencies [31]. The LED spectral range was 660 ± 20 nm FWHM, and the light intensity was 32,000 μmol photons per second per square meter. Individual flash oxygen traces were integrated to obtain absolute quantum yields per flash by comparing to a standard of known concentration. Steady-state oxygen yields from multiple flashes in a train of flashes following 2 min dark incubation were also averaged and plotted separately as in Fig. 4. Lastly, for comparing the relative oscillations in oxygen yield from different pulse trains, as in Fig. 2, the individual flash yields were normalized to the same steady-state value after oscillations decayed to zero.

The absolute O_2 quantum yields (mol O_2 per flash/mol PSII-D1) were obtained by normalizing to the number of PSII centers measured by Chl *a* concentration and EPR spectroscopy. Current generated by the reduction of O_2 following a single flash was integrated to obtain the total charge, which corresponds to O_2 consumed at the electrode (4 e^- per molecule).

S-state decay lifetimes were determined by advancing a dark-acclimated culture to the desired state using flashes, followed by dark incubation for variable time in this state, followed by rapidly advancing to oxygen evolution via flashes [28,30,31,35]. The populations were corrected using the WOC inefficiency parameters listed in Table 1. For certain measurements, cultures were supplemented with 250 μM