Driving force for solar water oxidation in biology

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Photosynthesis provides the chemical free energy and the molecular oxygen that most organisms on Earth need for their survival. It also generated the organic matter that converted over geological time scales into the fossil fuels upon which most present human societies completely depend. Understanding the biological process of converting solar energy into chemical fuels, and how it can inspire artificial devices, may thus be of utmost importance.

Water is the source for photosynthetic oxygen production and for the electrons required for CO_2 assimilation. Its light-driven oxidation to molecular oxygen is powered by charge separations in the reaction center of photosystem II (PSII) that are induced by the absorption of visible light in the associated antenna complexes and subsequent excitation energy transfer. The PSII reaction center comprises the photochemical electron donor P680, which is made of four chlorophyll-*a* molecules (Chl_{D1}, P_{D1}, P_{D2}, Chl_{D2}), and the primary electron acceptor molecule pheophytin (Pheo). Absorption of visible light by the PSII-associated antenna and subsequent excitation energy transfer to the reaction center leads to the primary charge separation, i.e. to the formation of the radical pair $P_{680}^{\bullet+}Pheo^{\bullet-}$. The $P_{680}^{\bullet+}/P_{680}$ couple has an estimated oxidizing potential of about + 1200 mV, the highest known in biology. For minimizing harmful and wasteful charge recombination reactions, PSII has a set of electron redox-active cofactors that allow spatial and energetic separation of the radical pair. The oxidized photochemical electron donor $P_{680}^{\bullet+}$ is reduced by a redox active tyrosine known as Y_Z, which is H-bonded to D1-His190. Y_Z oxidation leads to the formation of the $Y_Z^{\bullet}His190^{+}$ pair by moving the proton within the H-bond. This pair, that we denote Y_Z^{ox}, then oxidizes a chair-shaped cluster associating four manganese ions, one calcium ion and five bridging oxo-groups (Mn_4CaO_5 cluster). Together with its protein environment and surrounding water molecules this inorganic cluster forms the oxygen-evolving complex (OEC).

Using this general arrangement, photosynthetic water oxidation to O_2 is carried out by PSII over a reaction cycle involving four photochemical steps that drive the OEC through five redox states S_i (i = 0, .., 4). For understanding the catalytic strategy of biological water oxidation it is important to elucidate the energetic landscape of PSII and in particular that of the final $S_4 \rightarrow S_0$ transition. In this short-lived chemical step the four oxidizing equivalents accumulated in the

preceding photochemical events are used up to form O_2, two protons are released and at least one substrate water molecule binds to the Mn_4CaO_5 cluster.

The driving force for O_2 formation in the $S_4 \rightarrow S_0$ transition has been a controversial issue for several years. In 2004, Clausen and Junge reported an experiment aimed at estimating this driving force by testing the effect of high oxygen pressure [1]. Increasing the concentration of the product will lower the equilibrium constant of the reaction, possibly bringing it to a stall if the effect is large enough. And such seemed to be the case, as the reaction was found half inhibited by only 2.3 bar O_2 partial pressure (i.e. ~ 10-fold the physiological oxygen concentration). This pointed to a rather small driving force (ΔG_0) of about 80 meV (i.e. an equilibrium constant $K \approx 20$) for this final step (expressed under normal atmospheric conditions), and suggested that PSII is operating close to reversibility. The finding of a low equilibrium constant was in line with an earlier study by Vos et al., using electroluminescence, where K was estimated to be about 65 [2].

However, the results of Clausen and Junge, which relied on the assignment of UV difference spectra to Mn oxidation state changes, were subsequently challenged by three independent studies. Haumann et al. employed time-resolved X-ray absorption measurements to follow the Mn oxidation changes directly during the turnover of PSII at O_2 pressures of up to 16 bar. These experiments revealed no blockage of the $S_4 \rightarrow S_0$ transition under any of the tested conditions [3]. Similarly, Kolling et al., using chlorophyll fluorescence to follow the S state turnover, found no evidence for product inhibition up to 11 bar O_2 [4]. Finally, some of us demonstrated by membrane-inlet mass spectrometry (MIMS) that the production of $^{18}O_2$ from $H_2{}^{18}O$ occurs at the same rate in the presence of 20 bar O_2 as with a similar N_2 pressure [5]. Thus, all three experiments point to a more substantial driving force than estimated by Vos et al. [2] and Clausen and Junge [1], but its exact magnitude remained unclear.

In this study [6] we probed the probability to form S_4 from S_0 and O_2 by incubating Y_D-less PSII in the S_0 state for 2-3 days in the presence of $^{18}O_2$ and $H_2{}^{16}O$. The absence of any measurable $^{16,18}O_2$ formation by water-exchange in the S_4 state suggests that the S_4 state is hardly ever populated. On the basis of a detailed analysis we determined that the equilibrium constant K of the $S_4 \rightarrow S_0$ transition is larger than 1.0×10^7 so that this step is highly exergonic. We argue that this finding is consistent with current knowledge of the energetics of the S_0 to S_4 reactions, and that the high exergonicity is required for the kinetic efficiency of PSII.

[1] J. Clausen, W. Junge, Nature 430 (2004) 480-483.

[2] M.H. Vos, et al., Biochim. Biophys. Acta 1056 (1991) 27-39.

[3] M. Haumann, et al., Proc. Natl. Acad. Sci. USA 105 (2008) 17384-17389.

[4] D.R.J. Kolling, et al., Biochemistry 48 (2009) 1381-1389.

[5] D. Shevela, et al., Proc. Natl. Acad. Sci. USA 108 (2011) 3602-3607.

[6] H. Nilsson, et al., Biochim. Biophys. Acta 1857 (2016) 23-33.