JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

COMMUNICATION

pubs.acs.org/JACS

Redirecting Electron Transfer in Photosystem II from Water to Redox-Active Metal Complexes

Gözde Ulas and Gary W. Brudvig*

Department of Chemistry, Yale University, New Haven, Connecticut 06520-8107, United States

Supporting Information

ABSTRACT: A negatively charged region on the surface of photosystem II (PSII) near Q_A has been identified as a docking site for cationic exogenous electron acceptors. Oxygen evolution activity, which is inhibited in the presence of the herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), is recovered by adding Co^{III} complexes. Thus, a new electron-transfer pathway is created with Co^{III} as the new terminal electron acceptor from Q_A^-. This binding site is saturated at ~2.5 mM [Co^{III}], which is consistent with the existence of low-affinity interactions with a solvent-exposed surface. This is the first example of a higher plant PSII in which the electron-transfer pathway has been redirected from the normal membrane-associated quinone electron acceptors to water-soluble electron acceptors. The proposed Co^{III} binding site may enable efficient collection of electrons generated from photochemical water oxidation by PSII immobilized on an electrode surface.

Solar energy-driven chemistry provides an attractive and sustainable alternative to the increasing demand for energy in the world. Light-induced H_2O oxidation (eq 1), a reaction that provides reducing equivalents for the synthesis of green fuels, such as H_2, is invoked as a key process in the storage of solar energy. However, achieving a $4e^-$-oxidation of H_2O by the efficient removal of electrons and protons makes this reaction one of the biggest challenges in catalysis.

Photosystem II (PSII) is the only enzyme in nature that can photochemically oxidize H_2O. PSII has an inorganic Mn_4Ca core held together by μ-oxo units, called the oxygen-evolving complex (OEC). The OEC catalyzes light-driven oxidation of H_2O (eq 1) as the first step of photosynthesis.[1]

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^- \quad (1)$$

The catalytic cycle for H_2O oxidation by PSII involves 4 one-electron oxidations of the OEC that are triggered by photonic absorptions and coupled to proton-transfer reactions. The primary electron-transfer pathway is well established (Figure 1a).[1] A light-induced charge separation from the primary electron donor P_{680} to an adjacent pheophytin ($Pheo_A$) is followed by electron transfer to a permanently PSII-bound quinone, Q_A, and then to a membrane-diffusing quinone, Q_B, which is the terminal electron acceptor that shuttles the electrons away from PSII to eventually reduce $NADP^+$. Specific proton-transfer pathways have also been predicted through computational methods and from X-ray crystallographic studies[2−5] and more recent experimental evidence which supports the existence of these pathways.[6]

Interestingly in PSII, the rate-determining step (RDS) is the reduction of Q_B by $2e^-$ and $2H^+$ and its consequent diffusion out of its binding pocket into the membrane bilayer.[7] This process is 10-fold slower than the slowest catalytic step (O−O bond formation step) in the OEC.[8] Thus, it could be possible to accelerate the rate of H_2O oxidation by PSII by efficient extraction of electrons from Q_A^-, an important consideration for solar fuel production. Control over the rate of electron transfer (ET) might also offer a tool for manipulating the advancement of the oxidation states of the OEC, which could open new avenues to study the mechanism of light-driven H_2O oxidation.

One approach is to tether electron acceptors to the Q_B binding pocket, which has been accomplished with limited success.[9−11] Another approach is to redirect electron transfer from Q_A to an unnatural electron acceptor through a new binding site. This would eliminate the need for Q_B and could not only enable PSII turnover to be faster, but also prevent destructive O_2 reduction due to the longevity of the Q_A/Q_B electron-transfer intermediates. Indeed, Larom et al.[12] have reported successful electron transfer from Q_A to a genetically engineered docking site for cytochrome *c* 13 Å away on the stromal surface of cyanobacterial PSII. In thylakoid membranes, this mutation, in conjunction with cyt *c* and a herbicide capable of blocking the Q_B site, significantly reduced oxidative damage.

Here, we report the successful rescue of the activity of herbicide-inhibited higher plant PSII core complexes by the addition of cationic redox-active metal complexes. Once the natural ET pathway is blocked by using a herbicide that binds tightly in the Q_B site, the rescue of PSII activity has been possible due to the redirection of electron transfer from H_2O to metal complexes that putatively bind to a negatively charged docking site on the surface of PSII, 9 Å away from Q_A and conserved in cyanobacterial and higher plant PSII. This provides a new example of using redox-active, water-soluble complexes to harvest electrons extracted from H_2O via an unnatural electron-transfer pathway in PSII without the requirement of any mutations.

To identify a surface-binding site close to Q_A, we examined the surface electrostatic potential and searched for a cluster of charged amino acid residues that were within ET distance from Q_A. By using the 2.9 Å X-ray crystal structure of cyanobacterial PSII as a template,[13] we generated electrostatic potential surface maps of the enzyme using the Adaptive Poisson−Boltzmann Solver (APBS)[14] and viewed the solvent-exposed surfaces of

Received: May 28, 2011
Published: August 02, 2011

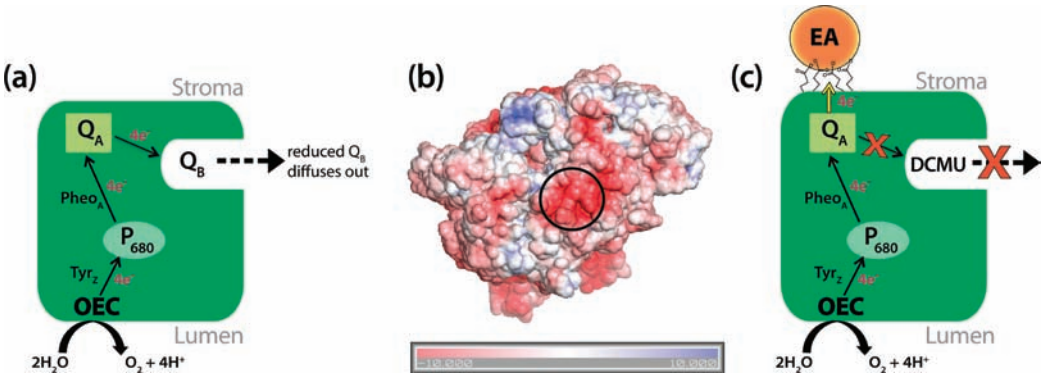

Figure 1. (a) A simplified scheme of PSII showing a view along the membrane plane of the electron-transfer pathway from the oxygen-evolving complex (OEC) to the membrane-diffusing quinone, Q_B. (b) Electrostatic potential of the solvent-exposed stromal surface of PSII shown at −10 kT/e (in red) and +10 kT/e (in blue). The patch outlined within the black circle was identified as a putative binding site for cationic small molecules. (c) Redirection of the electron-transfer pathway within PSII. DCMU, a potent herbicide, binds to the Q_B site and blocks electron transfer. Catalytic activity can be restored by electron transfer to an exogenous electron acceptor (EA) that binds to a surface site near Q_A (as shown bound by the black circle in panel b).

PSII. We identified a predominantly negatively charged patch on the stromal surface of PSII (Figure 1b), which consists of four conserved glutamic acid residues (see Supporting Information). These residues constitute a patch that is ∼5 Å in diameter and 9 Å away from Q_A. We then screened a series of Co^{III} complexes that were suitable in size, stability, and solubility with a well-poised $E°$ for efficient ET from Q_A^- (E_m = −90 mV vs NHE for herbicide-bound PSII).[15,16]

PSII activity assays were developed using the property that when the natural ET pathway is blocked by binding of a herbicide to the Q_B site, the activity could be rescued only if a secondary route for ET is available (Figure 1c). In a conventional activity assay, two types of electron acceptors are used: DCBQ (2,5-dichloro-*p*-benzoquinone), which binds to the Q_B site to accept electrons and exchanges with the bulk once it is reduced, and $[Fe(CN)_6]^{3-}$, used to reoxidize DCBQ. This ET process can be disrupted when a herbicide is used to block the Q_B site (Table 1); in our experiments, we used DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea), a potent inhibitor of PSII turnover (K_d = 40 nM).[17] DCMU binding to the Q_B site results in the blockage of the natural ET pathway. Once this site is blocked, DCBQ cannot bind to the Q_B site or accept electrons from PSII, and hence, PSII turnover is stopped. The only way activity can be rescued in these herbicide-bound PSII samples is through an unnatural ET pathway with an electron acceptor that is capable of turnover at another binding site.

The efficiency of a series of electron acceptors was assayed using PSII core complexes isolated from spinach,[18] and the rate of O_2 production was initiated with visible light (λ > 400 nm) (see Supporting Information) and measured with a Clark-type electrode with the following general scheme: (1) The activity of an aliquot of PSII was first measured in the presence of the conventionally used electron acceptors, 250 μM DCBQ and 1 mM $[Fe(CN)_6]^{3-}$, and reflects the turnover frequency (TOF) of untreated PSII. (2) PSII activity was then measured in the presence of 1 mM DCMU and 1 mM $[Fe(CN)_6]^{3-}$. DCMU completely inhibits the PSII turnover when only $[Fe(CN)_6]^{3-}$ is present. (3) These activities were then compared to that of a DCMU-bound PSII sample that was measured in the presence of 1 mM of various Co^{III} complexes. The complexes screened for their potency to rescue the activity of DCMU-inhibited PSII core complexes were: $[Co(NH_3)_6]Cl_3$, $[Co(NH_3)_5Cl]Cl_2$, $[Co(phen)_3]Cl_3$ and $[Co(terpy)_2]Cl_3$ (where phen is 1,10-phenanthroline, and terpy is 2,2′;6′,2″-terpyridine). The results are displayed in Table 1.

Table 1. Turnover Frequency of Spinach PSII Core Complexes in the Presence of Electron Acceptors

electron acceptor[a]	herbicide (DCMU) concentration	$E°$ (vs NHE)[b]	TOF (mmol O_2/(mol PSII·s))[c]
DCBQ + $[Fe(CN)_6]^{3-}$[d]	None	-	7620 ± 66 (100%)
$[Co(terpy)_2]^{3+}$	1 mM	+310 mV	570 ± 60 (8%)
$[Co(phen)_3]^{3+}$	1 mM	+420 mV	530 ± 50 (7%)
$[Co(NH_3)_6]^{3+}$	1 mM	+108 mV	0
$[Co(NH_3)_5Cl]^{2+}$	1 mM	+38 mV	0
$[Ru(NH_3)_6]^{3+}$	1 mM	+100 mV	130 ± 60 (2%)
$[Co(EDTA)]^-$	0.1 mM	+380 mV	0
$[Fe(CN)_6]^{3-}$	1 mM	+358 mV	0
DCBQ	1 mM	+720 mV[e]	250 ± 80[f] (3%)

[a] All the activity assays were done in 50 mM MES buffer (NaOH, pH = 6.0) without any additional ions. The designated electron acceptor (all in 1 mM concentration, except for DCBQ at 250 μM), and DCMU were added to the assay solution right before the addition of the PSII aliquot, and the mixture was let incubate for 1 min in the dark before the start of the experiment. [b] Standard reduction potentials taken from refs 19−22. [c] The mol O_2 was detected experimentally by a Clark-type electrode. Illumination was performed with white light in all cases (λ > 400 nm) as described in ref 27. TOF was calculated using 37 chlorophylls/PSII. [d] Standard activity assay conditions were used for this assay, where [DCBQ] = 250 μM, and [$[Fe(CN)_6]^{3-}$] = 1 mM. [e] The reduction potential for DCBQ is pH-dependent. In our experiments pH = 6.0 and the E_m for DCBQ is +366 mV. [f] This activity is ascribed to the binding of hydrophobic DCBQ at the Q_B site, which is not observed for charged electron acceptors.

The results in Table 1 show that the catalytic turnover of DCMU-inhibited PSII core complexes is rescued in the presence of cationic redox-active Co^{III}-based compounds that are known to exhibit reversible reduction−oxidation behavior. The rescued activities in the cases of $[Co(phen)_3]Cl_3$ and $[Co(terpy)_2]Cl_3$ are not significantly different from each other, suggesting that the turnover rate is not governed by the rate of electron transfer. With Co^{III} complexes containing monodentate ligands, we do

dx.doi.org/10.1021/ja2049226 |J. Am. Chem. Soc. 2011, 133, 13260–13263

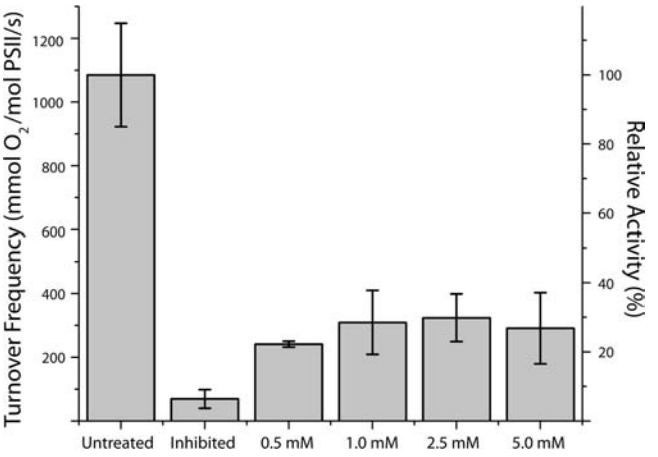

Figure 2. PSII core activity as a function of $[Co(terpy)_2]^{3+}$ concentration. The untreated sample solution includes 250 μM DCBQ and 1 mM FeCN as the electron acceptors, the inhibited sample contains 1 mM DCMU + 250 μM DCBQ + 1 mM FeCN. The rest of the samples contain the designated concentration of $[Co(terpy)_2]^{3+}$, 250 μM DCBQ and 1 mM DCMU each. All other assay conditions were the same as described in Table 1. The observed values show the effect of the concentration of $[Co(terpy)_2]^{3+}$ on PSII turnover frequency. Saturation is observed at ~2.5 mM Co^{III} concentration, indicating a low-affinity binding site which enables electron transfer from Q_A^- to Co^{III}. Differences in the recovered PSII activities shown in Table 1 and this figure are ascribed to the higher rate of Co^{II} formation in the more active sample used for the measurements in Table 1.

not observe any significant recovery of activity. This is attributed to their lower reduction potentials and the irreversible reduction of the Co^{III} complexes, due to ligand loss in the Co^{II} state, and subsequent inactivation of the OEC by aqueous Co^{II},[23−25] or to their slow self-exchange rates of electron transfer.[26] An analogous Ru^{III} complex with faster self-exchange rates of electron transfer[26] is able to recover some PSII activity.

In addition, $[Fe(CN)_6]^{3-}$ and $[Co(EDTA)]^-$, both negatively charged and substitutionally inert complexes with suitably poised reduction potentials, are unable to rescue the activity of PSII inhibited by DCMU (Table 1). This is consistent with the requirement of a cationic electron acceptor to bind a negatively charged docking site on PSII.

Chemical rescue of PSII activity at different concentrations of $[Co(terpy)_2]^{3+}$ is shown in Figure 2, in comparison to the activities of untreated PSII (PSII + 250 μM DCBQ + 1 mM $[Fe(CN)_6]^{3-}$), and inhibited PSII core complexes (PSII + 250 μM DCBQ + 1 mM $[Fe(CN)_6]^{3-}$ + 1 mM DCMU). Saturation behavior is observed at ~2.5 mM $[Co^{III}]$, consistent with the presence of a docking site for the cationic Co^{III} complex. The $[Co^{III}]/[PSII]$ is ~10^4, which suggests that this docking site has very low affinity for the Co^{III} complex and is consistent with a site on the solvent-exposed surface, allowing for rapid exchange of the Co^{III} complexes. There is a possibility that Co^{III} complexes nonselectively bind to PSII surfaces; however, our screening method is selective for Co^{III} that is within suitable distance from the ET pathway (most likely through Q_A) and thus, capable of rescuing PSII activity.

Although the proposed site has an affinity for the Co^{III} complexes, the activity of rescued PSII is lower than that of uninhibited PSII. As PSII catalysis requires the transfer of $4e^-$ for the release of a single molecule of O_2, each mole of O_2 formed consumes 4 mol Co^{III}. One possibility for the slower turnover at saturating Co^{III} concentrations is rate-limiting dissociation from the PSII surface.

Altogether, this report demonstrates the possibility of short-circuiting the natural ET pathway in PSII to introduce a novel pathway for cationic small molecules to harvest the electrons extracted from H_2O. We suggest that a predominantly negatively charged, solvent-exposed surface site that is already naturally present in higher plant PSII serves as the binding site of substitutionally inert, cationic, redox-active Co^{III} complexes. The observation that only cationic complexes are capable of rescuing the catalytic activity of PSII whose natural ET pathway has been blocked, together with saturation behavior, shows that there is an alternative docking site on PSII that is negatively charged and is in close proximity to a cofactor on the ET pathway, most likely Q_A, which is closest to the surface. These results are consistent with predictions from electrostatic potential maps generated from the 2.9-Å PSII X-ray crystal structure. The proposed Co^{III} binding site opens a new avenue for harvesting electrons from H_2O through immobilization of PSII on electrode surfaces and is complementary to work in which photosystem I has been wired for photochemical H_2 production.[28,29]

■ ASSOCIATED CONTENT

Supporting Information. Synthetic methods, activity assay conditions, sequence alignment, crystallographic data for $[Co(terpy)_2]^{3+}\cdot[Fe(CN)_6]^{3-}$ (cif). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
gary.brudvig@yale.edu

■ ACKNOWLEDGMENT

We thank Julie Thomsen for help with preliminary experiments, Dr. Cheng-I (Sophia) Lee for the PSII core complexes, Dr. Julian Tirado-Rives and Dr. Markus K. Dahlgren for their help in small molecule docking screens, Dr. Christopher Incarvito for the X-ray crystal structure of $[Co(terpy)_2]^{3+}\cdot[Fe(CN)_6]^{3-}$, and Dr. Siddhartha Das for many useful discussions. This work was supported by U.S. Department of Energy grant DE-FG02-05ER15646.

■ REFERENCES

(1) McEvoy, J. P.; Brudvig, G. W. *Chem. Rev.* **2006**, *106*, 4455–4483.
(2) Guskov, A.; Kern, J.; Gabdulkhakov, A.; Broser, M.; Zouni, A.; Saenger, W. *Structure* **2009**, *17*, 1223–1234.
(3) Ho, F. M. *Photosynth. Res.* **2008**, *98*, 503–522.
(4) Ishikita, H.; Saenger, W.; Loll, B.; Biesiadka, J.; Knapp, E.-W. *Biochemistry* **2006**, *45*, 2063–2071.
(5) Murray, J. W.; Barber, J. *J. Struct. Biol.* **2007**, *159*, 228–237.
(6) Ulas, G.; Brudvig, G. W. *Biochemistry* **2010**, *49*, 8220–8227.
(7) de Wijn, R.; van Gorkom, H. J. *Biochemistry* **2001**, *40*, 11912–11922.
(8) Haumann, M.; Liebisch, P.; Müller, C.; Barra, M.; Grabolle, M.; Dau, H. *Science* **2005**, *310*, 1019–1021.
(9) Bocarsly, J. R.; Brudvig, G. W. *J. Am. Chem. Soc.* **1992**, *114*, 9762–9767.
(10) Karki, L.; Lakshmi, K. V.; Szalai, V. A.; Brudvig, G. W. *J. Am. Chem. Soc.* **2000**, *122*, 2069–2077.

(11) Reifler, M. J.; Szalai, V. A.; Peterson, C. N.; Brudvig, G. W. *J. Mol. Recognit.* **2001**, *14*, 157–165.
(12) Larom, S.; Salama, F.; Schuster, G.; Adir, N. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 9650–9655.
(13) Guskov, A.; Gabdulkhakov, A.; Broser, M.; Zouni, A.; Saenger, W. *Nat. Struct. Mol. Biol.* **2009**, *16*, 334–342.
(14) Baker, N. A.; Sept, D.; Joseph, S.; Holst, M. J.; McCammon, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 10037–10041.
(15) Shibamoto, T.; Kato, Y.; Sugiura, M.; Watanabe, T. *Biochemistry* **2009**, *48*, 10682–10684.
(16) Ishikita, H.; Hasegawa, K.; Noguchi, T. *Biochemistry* **2011**, *50*, 5436–5442.
(17) Tischer, W.; Strotmann, H. *Biochim. Biophys. Acta* **1977**, *460*, 113–125.
(18) Ghanotakis, D. F.; Yocum, C. F. *FEBS Lett.* **1986**, *197*, 244–248.
(19) Gafney, H. D.; Adamson, A. W. *J. Am. Chem. Soc.* **1972**, *94*, 8238–8239.
(20) Maki, N.; Tanaka, N. In *Encyclopedia of Electrochemistry of the Elements*; Bard, A. J., Lund, H., Eds.; Marcel Dekker, Inc.: New York, 1973; Vol. 3, pp 43−55.
(21) Vanýsek, P. In *CRC Handbook of Chemistry and Physics*; 91st ed.; Lide, D. R., Ed.; Chapman & Hall/CRC Press: Boca Raton, FL, 2010−2011; pp 8-20−8-29.
(22) Clark, W. M. *Oxidation-Reduction Potentials of Organic Systems*; The Williams & Wilkins Company: Baltimore, MD, 1960.
(23) Lilie, J.; Shinohara, N.; Simic, M. G. *J. Am. Chem. Soc.* **1976**, *98*, 6516–6520.
(24) Tripathy, B. C.; Bhatia, B.; Mohanty, P. *Biochim. Biophys. Acta* **1983**, *722*, 88–93.
(25) Hoganson, C. W.; Casey, P. A.; Hansson, Ö. *Biochim. Biophys. Acta* **1991**, *1057*, 399–406.
(26) Basolo, F.; Pearson, R. G. *Mechanisms of Inorganic Reactions*; 2nd ed.; John Wiley and Sons, Inc.: New York, 1967.
(27) Beck, W. F.; de Paula, J. C.; Brudvig, G. W. *Biochemistry* **1985**, *24*, 3035–3043.
(28) Lubner, C. E.; Knorzer, P.; Silva, P. J. N.; Vincent, K. A.; Happe, T.; Bryant, D. A.; Golbeck, J. H. *Biochemistry* **2010**, *49*, 10264–10265.
(29) Utschig, L. M.; Dimitrijevic, N. M.; Poluektov, O. G.; Chemerisov, S. D.; Mulfort, K. L.; Tiede, D. M. *J. Phys. Chem. Lett.* **2011**, *2*, 236–241.