

# Interfacial Photoredox Molecular Interactions: A New Class of Hill Reagents for Photosystem II Reaction Centers

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Photosynthetic reaction centers are molecular photovoltaic structures that are of significant interest in the field of nanoscale science and technology. We report here the results of an extensive experimental survey and characterization of over 20 species of transition-metal compounds for their activity in the Hill reaction at the lipid–water interface of Photosystem II (PSII) membrane fragments. Four new PSII Hill reagents were identified:  $\text{PtCl}_4$ ,  $[\text{PtCl}_6]^{2-}$ ,  $[\text{OsCl}_6]^{2-}$ , and  $[\text{RuCl}_6]^{2-}$ . Each can be photosynthetically reduced by PSII. The negative charge of the metal compound species is apparently essential for their interaction with the reducing site of PSII. When  $\text{PtCl}_4$  is dissolved in water, it combines with water molecules to form  $\text{H}_2[\text{PtCl}_4(\text{OH})_2]$ , which can dissociate to the negatively charged species  $[\text{PtCl}_4(\text{OH})_2]^{2-}$  at neutral pH. These findings could have significant implications for photosynthesis, nanoscale science, and nanofabrication in the field of biometallocalysis.

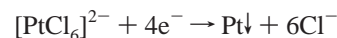
## Introduction

Photosynthesis is the key biological process that converts the electromagnetic energy of sunlight to useful chemical energy, thereby supporting essentially all life on earth. The primary events of photosynthesis occur in the Photosystem I (PSI) and Photosystem II (PSII) reaction centers. Photosynthetic reaction centers are small (5–10 nm), extremely fast (picosecond response time), and highly efficient (photon to electron quantum yield is close to 100%) photovoltaic devices.<sup>1</sup> The architecture and functions of PSII reaction centers arguably surpass those of semiconductor devices. They can generate photoinduced charge separation within about 30 ps, a speed that is 100 times faster than that of a silicon photodiode.<sup>2</sup> These properties of photosynthetic reaction centers are attractive for ultrafast photodetection, photoelectronic sensing, and communication. Significant progress in nanofabrication using photosynthetic reaction centers has recently been made. Metallic binding, molecular “welding”, and orientation of isolated photosynthetic reaction centers have been demonstrated at nanometer scales through the use of a chemical platinization reaction.<sup>3–5</sup>

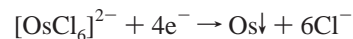
With advances in single biomolecule science, it is also possible to transform photosynthetic reaction centers into nanoscale devices to produce fuels and chemicals by biometallocalysis.<sup>1,6</sup> Using a chemical platinization reaction, such a transformation has been demonstrated for photosynthetic membranes (thylakoids) that contain both PSII and PSI reaction centers.<sup>7</sup> Through chemical deposition of colloidal metallic platinum at the reducing side of PSI reaction centers in thylakoid membranes, a new photosynthesis was created for simultaneous photoproduction of  $\text{H}_2$  and  $\text{O}_2$  by water splitting and biometallocalysis. Using metallic platinum particles in direct contact with the reducing site of PSI in platinized thylakoids, photosynthetically generated electrons from PSI have been output to an external circuit.<sup>8–10</sup>

As illustrated in Figure 1, a second technique<sup>11,12</sup> was developed to specifically precipitate metallocalysts at the

reducing site of PSI through photosynthetic reduction of hexachloroplatinate,  $[\text{PtCl}_6]^{2-}$ , or hexachloroosmate,  $[\text{OsCl}_6]^{2-}$ . This technique utilized  $[\text{PtCl}_6]^{2-}$  or  $[\text{OsCl}_6]^{2-}$  as a Hill oxidant,<sup>13</sup> which accepts electrons directly from PSI. Unlike the common Hill reagent ferricyanide ( $[\text{Fe}(\text{CN})_6]^{3-}$ ), hexachloroplatinate and hexachloroosmate are able to accept four electrons consecutively from PSI, thereby converting  $[\text{PtCl}_6]^{2-}$  and  $[\text{OsCl}_6]^{2-}$  anions to zerovalent metallic platinum (Pt) and osmium (Os) at the reducing site of PSI, according to the following reactions:<sup>11,12</sup>

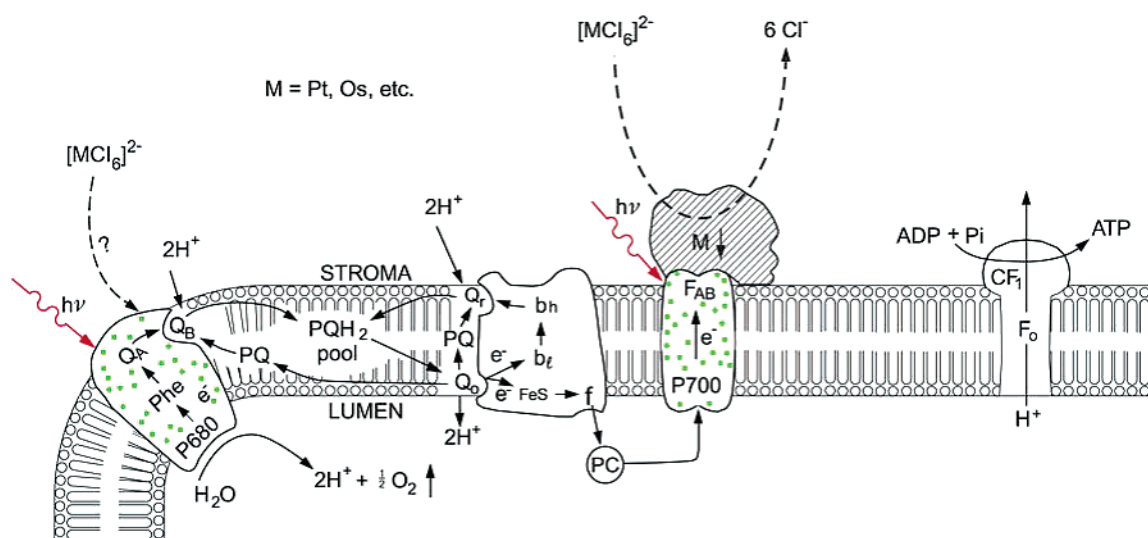


and



In the present work, we investigated whether the photochemical activity of PSII reaction centers per se could directly provide electrons for reduction of these types of water-soluble transition-metal compounds. As illustrated in Figure 1, light energy captured by P680 photochemistry (about 1.8 eV) drives the process that oxidizes water to protons and molecular oxygen ( $\text{O}_2$ ). Electrons acquired from water oxidations are transported vectorially from P680 to the  $\text{Q}_\text{B}$  site through the intermediate carriers pheophytin (Phe) and  $\text{Q}_\text{A}$  in a PSII reaction center pigment–protein complex. Thermodynamically, it should be possible to use these photosynthetically generated electrons to reduce the metal compounds for the formation of nanoscale catalytic metal particles at the reducing site of PSII, which has both theoretical and practical significance. We report here an extensive survey and characterization of more than 20 species of transition-metal compounds that was conducted in search of their potential ability to act as Hill reagents for PSII reaction centers. New PSII Hill reagents and species for active metallocalysis were identified. In addition to the known Hill reagent  $\text{K}_3\text{Fe}(\text{CN})_6$ , transition-metal compounds  $\text{PtCl}_4$ ,  $[\text{PtCl}_6]^{2-}$ ,  $[\text{OsCl}_6]^{2-}$ , and  $[\text{RuCl}_6]^{2-}$  were also found to be active in the PSII Hill reaction. These findings have potentially important

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**Figure 1.** Photosynthetic thylakoid membrane and the concept of photoredox chemical interactions of water-soluble heavy transition-metal species with PSII and PSI reaction centers.

implications for renewable fuels production using photosynthetic reaction centers.

## Materials and Methods

**Sources of Chemicals.** Three chemicals were purchased from the Aldrich Chemical Co., Inc. (Milwaukee, WI): potassium hexachlororuthenate(III),  $K_3RuCl_6$ ; potassium ferricyanide,  $K_3Fe(CN)_6$ ; and sodium hexachloroiridate(IV),  $Na_2IrCl_6$ . The following 19 chemicals were purchased from Johnson Matthey Co. (Ward Hill, MA): ammonium hexachloroiridate(III),  $(NH_4)_3IrCl_6$ ; ammonium hexachlororhodate(III),  $(NH_4)_3RhCl_6$ ; ammonium hexachlororuthenate(IV),  $(NH_4)_2RuCl_6$ ; ammonium molybdenum oxide,  $(NH_4)_6Mo_7O_{24}$ ; platinum(IV) oxide,  $PtO_2$ ; platinum(II) chloride,  $PtCl_2$ ; platinum(IV) chloride,  $PtCl_4$ ; sodium hexachloropalladate(IV),  $Na_2PdCl_6$ ; sodium hexachloroosmate(IV),  $Na_2OsCl_6$ ; sodium hexachloroplatinate(IV),  $Na_2PtCl_6$ ; potassium tetrachloropalladate(II),  $K_2PdCl_4$ ; sodium tetrachloroaurate(III),  $NaAuCl_4$ ; rhenium(III) chloride,  $ReCl_3$ ; osmium(III) chloride,  $OsCl_3$ ; nickel(II) chloride,  $NiCl_2$ ; cobalt chloride,  $CoCl_2$ ; tetraammineplatinum(II) chloride,  $Pt(NH_3)_4Cl_2$ ; tetraamminepalladium(II) chloride,  $Pd(NH_3)_4Cl_2$ ; and ammonium tetrachloropalladate(II),  $(NH_4)_2PdCl_4$ . Each of these compounds was dissolved in water to make a 50 mM stock solution for the assays with thylakoids.

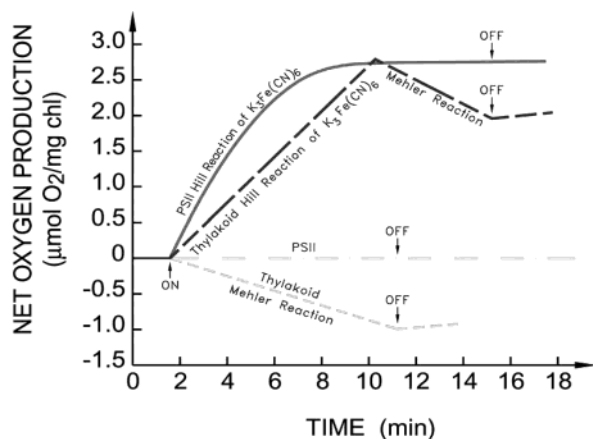
**Preparation of Thylakoid Membranes.** Thylakoids were isolated from spinach leaves using the procedure described by Reeves and Hall.<sup>14</sup> The thylakoids were suspended in Walker's assay medium<sup>15</sup> and adjusted to a final chlorophyll (chl) concentration of 40 or 600  $\mu g/mL$  for Hill reaction assays with a Clark oxygen electrode or photoprecipitation of metallocatalysts for production of  $H_2$  and  $O_2$  with a reactor-flow detection system, respectively. The hydrogen detector was a tin oxide gas-sensitive semiconductor purchased from Figaro Corp. (Evanston, IL). The chl content was determined in 90% acetone extracts.<sup>16</sup>

**Preparation of PSII Membrane Fragments.** PSII reaction centers were isolated from the above thylakoid samples by the surfactant-assisted solubilization and centrifugation techniques described by Gleiter et al.,<sup>17</sup> Haag et al.,<sup>18</sup> and Volker et al.<sup>19</sup> Briefly, thylakoids were solubilized by resuspending the thylakoid material in a Triton X-100 containing buffer (Volker et al.) to a chl concentration of 2 mg/mL with a camel hair brush

and then gently stirring the suspended solution in ice and darkness for 30 min. The solubilized thylakoids were then centrifuged at 20K rpm ( $35\,000 \times g$ ) with an SS-34 rotor for 20 min. The green pellet resulting from the centrifugation was retained (the supernatant was discarded) and resuspended in a Triton-free buffer with a camel hair brush. The resuspended PSII-containing material was then centrifuged at 8K rpm ( $5000 \times g$ ) with a SS-34 rotor for 10 min. The supernatant resulting from this slow centrifugation step was collected (discarding the non-PSII pellet). The PSII-containing supernatant was then centrifuged again at high speed ( $35\,000 \times g$ ) for 20 min. The resulting green pellet, PSII membrane fragments, was retained and resuspended to a final chl concentration of 40 or 600  $\mu g/mL$  in Gleiter's suspending medium for the following Hill reaction assays with a Clark oxygen electrode or photoprecipitation of metallocatalysts for production of  $H_2$  and  $O_2$  with a reactor-flow detection system, respectively.

**Hill Reaction Assay with Clark Oxygen Electrode.** Hill reactions of the compounds were assayed by measuring  $O_2$  evolution in thylakoids or PSII membrane fragments using a Clark oxygen electrode system (YSI model 53). Calibration of the oxygen electrode system was accomplished using water deoxygenated with  $Na_2S_2O_4$  for the zero baseline and air-saturated water as a standard ( $0.0310\text{ L } O_2/L\text{ H}_2O$  at  $20\text{ }^\circ C$ ).<sup>20</sup> After calibration, a 1.8-mL sample was placed in the water-jacketed reaction chamber at  $20\text{ }^\circ C$ . To eliminate supersaturation of oxygen, the assay medium was deoxygenated by bubbling helium through it for about 10 min. The deoxygenated medium was then mixed with an appropriate amount of thylakoids and the stock solution of the compound being tested for Hill reaction activity to give a final concentration of 0.5 mM for the potential Hill reagent and 40  $\mu g$  chl/mL for the thylakoids. The initial oxygen level of the assay sample was about 40% that of air-saturated water, which was low enough to prevent oxygen saturation during the Hill reaction assay. After allowing the oxygen level to stabilize in darkness, the reaction chamber was illuminated at about  $100\text{ } \mu E/(m^2 \cdot s)$  by a dichroic-reflector halogen lamp projected through a glass fiber-optical cable. The rate and extent of oxygen evolution (if any) were recorded by the Clark electrode oxygen detection system.

**Assays for Biometallocalysis.** Photoprecipitation of metallocatalysts for the production of  $H_2$  and  $O_2$  was performed in



**Figure 2.** Characterization of thylakoids and PSII membrane samples through  $O_2$  measurements of Hill and Mehler reactions. The initial concentration of the Hill reagents was 0.50 mM. The chl concentration of the samples was 40  $\mu\text{g/mL}$ . The upward and downward arrows indicate the times at which actinic illumination [PAR, 100  $\mu\text{E}/(\text{m}^2\cdot\text{s})$ ] was turned on and off, respectively.

a helium atmosphere using a reactor-flow detection system as described previously.<sup>6,12</sup> Ten milliliters of PSII membrane suspension (600  $\mu\text{g chl/mL}$ ) was placed in the reactor. The prospective Hill reagent was added to the PSII suspension for a final concentration of 2 mM. The reaction vessel was water-jacketed and held at 20  $^\circ\text{C}$  with a temperature-controlled water bath (Lauda RM6, Brinkmann Instruments, Germany). The reaction medium (PSII suspension plus a Hill reagent) was gently stirred by a magnetic stirring bar and purged with a helium flow (50 mL/min) in the headspace above the liquid. The rate of helium flow was controlled by an MKS gas-flow control system (type 260, MKS Instruments, Inc., Andover, MA). Possible photoprecipitation of metallocatalysts was initiated by actinic illumination [50  $\mu\text{E}/(\text{m}^2\cdot\text{s})$ ] provided by a tungsten lamp. Production of  $H_2$  and  $O_2$  was monitored as the change of their concentration in the effluent gas flow as detected by the tin oxide hydrogen sensor and galvanic cell.

## Results and Discussion

**Characterization of Thylakoids and PSII Membrane Fragments by Clark Oxygen Electrode Assays.** The thylakoid membranes and PSII membrane fragments were characterized with Clark oxygen electrode assays. As shown by the oxygen-production data presented in Figure 2, both the thylakoids and PSII samples were capable of photoevolution of molecular oxygen ( $O_2$ ) in the presence of a common Hill reagent, 0.5 mM  $K_3Fe(CN)_6$ . However, the two oxygen-production curves are qualitatively different. The PSII Hill reaction of  $K_3Fe(CN)_6$  typically demonstrates a nonlinear rise of  $O_2$  production after the actinic light is turned on (indicated by the upward arrow of Figure 2). The curve gradually levels off when the Hill reagent [ $K_3Fe(CN)_6$ ] is consumed. No Mehler reaction (photodependent consumption of  $O_2$ ) was observed in the PSII samples. Furthermore, in the PSII control experiment where the Hill reagent [ $K_3Fe(CN)_6$ ] was omitted, neither light-dependent consumption nor production of  $O_2$  was observed as indicated by the flat line. This result demonstrated that unlike PSI, PSII does not support a Mehler reaction, suggesting that molecular oxygen does not react with the electrons at the reducing side of PSII. Only certain Hill reagents such as  $K_3Fe(CN)_6$  can readily accept electrons from PSII and, thus, support photoevolution of  $O_2$ .

However, photodependent  $O_2$  consumption was clearly observed with the thylakoid sample under the stated experi-

mental conditions. As shown in Figure 2, upon actinic illumination in the absence of the Hill reagent  $K_3Fe(CN)_6$ , the  $O_2$  curve of the control thylakoid sample (bottom curve) decreased because of  $O_2$  consumption by the Mehler reaction, which is believed to be associated with PSI reaction centers in the thylakoid membranes. It is known that a photosynthetically energized electron emergent from PSI is capable of reducing molecular oxygen ( $O_2$ ) to the superoxide ( $O_2^{\cdot-}$ ) anion—a known mechanism of the Mehler reaction. Consequently, for every four electrons transported from PSII to PSI, four molecules of  $O_2$  could be reduced to  $O_2^{\cdot-}$  by PSI whereas only one molecule of  $O_2$  is generated by water oxidation at PSII. Therefore, the combined result is a net consumption of three molecules of  $O_2$  for every four electrons transported from PSII to PSI. This explains why, upon actinic illumination, the Mehler reaction consumes  $O_2$  as observed in the thylakoid sample in the absence of the Hill reagent  $K_3Fe(CN)_6$  (bottom curve of Figure 2). When the actinic light was turned off, the Mehler reaction stopped and the  $O_2$  concentration rose slightly. The slight rise in  $O_2$  concentration after the light was turned off indicates that some of the Mehler reaction product ( $O_2^{\cdot-}$ ) may slowly revert back to molecular oxygen  $O_2$ . The photoreduction of  $K_3Fe(CN)_6$  is a kinetically quicker sink for electrons from PSI than that of the Mehler reaction. In the presence of 0.5 mM  $K_3Fe(CN)_6$ , the thylakoid Hill reaction was the dominant process until the added  $K_3Fe(CN)_6$  was fully consumed. As illustrated in Figure 2, the oxygen production curve of the thylakoid [ $Fe(CN)_6$ ]<sup>3-</sup> Hill reaction rose almost linearly upon actinic illumination and then decreased sharply at the point of maximum oxygen production. This abrupt turning point marks complete conversion of [ $Fe(CN)_6$ ]<sup>3-</sup> into [ $Fe(CN)_6$ ]<sup>4-</sup>, which cannot be further reduced by PSI, and the beginning of oxygen consumption by the Mehler reaction, in which emergent electrons from PSI begin to reduce molecular oxygen.

Note the rate (initial slope) of the PSII  $K_3Fe(CN)_6$  Hill reaction (34  $\mu\text{mol } O_2/\text{mg chl/h}$ ) was greater than that of the thylakoid  $K_3Fe(CN)_6$  Hill reaction (21  $\mu\text{mol } O_2/\text{mg chl/h}$ ). This probably reflects the fact that the PSII membrane material had more PSII reaction centers/mg chl than the thylakoid sample because of the removal of PSI (chl's) in the preparation of the PSII membrane fragments. The absence of a Mehler reaction in the PSII sample indicated that the PSII preparation is essentially free of PSI and suitable for this study.

**New PSII Hill Reagents Identified by Clark Oxygen Electrode Assays.** Among the more than 20 compounds of heavy transition metals tested, four species,  $PtCl_4$ ,  $Na_2PtCl_6$ ,  $Na_2OsCl_6$ , and  $(NH_4)_2RuCl_6$ , were found to be active in the PSII Hill reaction (Table 1). The rate of the Hill reaction can be measured as the initial rate of oxygen evolution in PSII membrane fragments using the Clark oxygen electrode (Figures 3–5). The Hill reaction rates of  $PtCl_4$ ,  $Na_2PtCl_6$ ,  $Na_2OsCl_6$ , and  $(NH_4)_2RuCl_6$  were in a range of 5.5–105% that of the classic Hill reagent  $K_3Fe(CN)_6$  (Table 1). The results indicated that these transition-metal compounds, like ferricyanide, are Hill reagents that can accept electrons from photosynthetic water splitting in PSII membrane fragments.

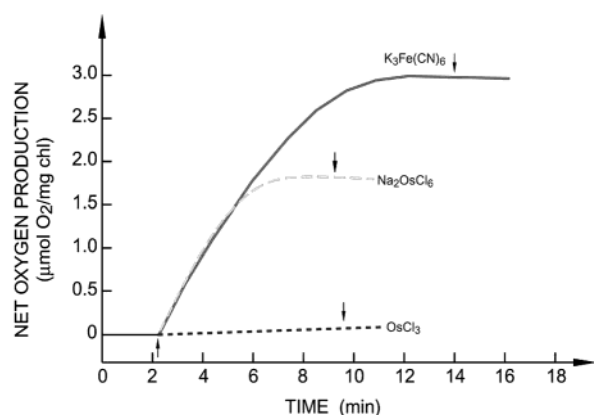
These newly identified Hill reagents, however, have specific characteristics.  $Na_2OsCl_6$  supports steady net oxygen production (Figure 3). The initial rate of  $Na_2OsCl_6$  PSII Hill reaction (105%), to within experimental error, was about same as that of the control [ $K_3Fe(CN)_6$  PSII Hill reaction]. A related species,  $OsCl_3$ , which supports the thylakoid Hill reaction,<sup>21</sup> can only slightly support  $O_2$  photoevolution in the PSII sample (Figure 3, bottom curve).



**TABLE 1: Test Results for 23 Metal Compounds, Surveyed by Measuring Their Ability to Support Oxygen and Hydrogen Evolution in PSII Membranes<sup>a</sup>**

initial species	% rate of Hill reaction	H <sub>2</sub>	redox system	E <sub>o</sub> (V)
PtCl <sub>4</sub>	5.5	0	PtCl <sub>4</sub> + 4e <sup>-</sup> = Pt↓ + 4Cl <sup>-</sup>	
OsCl <sub>3</sub>	0	0	OsCl <sub>3</sub> + 3e <sup>-</sup> = Os↓ + 3Cl <sup>-</sup>	
Na <sub>2</sub> PtCl <sub>6</sub>	26.5	0	[PtCl <sub>6</sub> ] <sup>2-</sup> + 4e <sup>-</sup> = Pt↓ + 6Cl <sup>-</sup>	+0.68
Na <sub>2</sub> OsCl <sub>6</sub>	105	0	[OsCl <sub>6</sub> ] <sup>2-</sup> + 4e <sup>-</sup> = Os↓ + 6Cl <sup>-</sup>	
(NH <sub>4</sub> ) <sub>2</sub> RuCl <sub>6</sub>	25	0	[RuCl <sub>6</sub> ] <sup>2-</sup> + 4e <sup>-</sup> = Ru↓ + 6Cl <sup>-</sup>	
K <sub>3</sub> RuCl <sub>6</sub>	0	0	[RuCl <sub>6</sub> ] <sup>3-</sup> + 3e <sup>-</sup> = Ru↓ + 6Cl <sup>-</sup>	
Na <sub>2</sub> PdCl <sub>6</sub>	0	0	[PdCl <sub>6</sub> ] <sup>2-</sup> + 4e <sup>-</sup> = Pd↓ + 6Cl <sup>-</sup>	
Na <sub>2</sub> IrCl <sub>6</sub>	0	0	[IrCl <sub>6</sub> ] <sup>2-</sup> + 4e <sup>-</sup> = Ir↓ + 6Cl <sup>-</sup>	+0.86
(NH <sub>4</sub> ) <sub>3</sub> IrCl <sub>6</sub>	0	0	[IrCl <sub>6</sub> ] <sup>3-</sup> + 3e <sup>-</sup> = Ir↓ + 6Cl <sup>-</sup>	+0.77
(NH <sub>4</sub> ) <sub>3</sub> RhCl <sub>6</sub>	0	0	[RhCl <sub>6</sub> ] <sup>3-</sup> + 3e <sup>-</sup> = Rh↓ + 6Cl <sup>-</sup>	+0.44
K <sub>2</sub> PdCl <sub>4</sub>	0	0	[PdCl <sub>4</sub> ] <sup>2-</sup> + 2e <sup>-</sup> = Pd↓ + 4Cl <sup>-</sup>	+0.62
(NH <sub>4</sub> ) <sub>2</sub> PdCl <sub>4</sub>	0	0	[PdCl <sub>4</sub> ] <sup>2-</sup> + 2e <sup>-</sup> = Pd↓ + 4Cl <sup>-</sup>	+0.62
Pd(NH <sub>3</sub> ) <sub>4</sub> Cl <sub>2</sub>	0	0		
ReCl <sub>3</sub>	0	0		
NiCl <sub>2</sub>	0	0		
CoCl <sub>2</sub>	0	0		
PtO <sub>2</sub>	0	0		
PtCl <sub>2</sub>	0	0		
Pt(NH <sub>3</sub> ) <sub>4</sub> Cl <sub>2</sub>	0	0		
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub>	0	0		
NaAuCl <sub>4</sub>	0	0		
K <sub>2</sub> PtCl <sub>4</sub>	0	0	[PtCl <sub>4</sub> ] <sup>2-</sup> + 2e <sup>-</sup> = Pt↓ + 4Cl <sup>-</sup>	+0.73
K <sub>3</sub> Fe(CN) <sub>6</sub>	100 ± 7	0	[Fe(CN) <sub>6</sub> ] <sup>3-</sup> + e <sup>-</sup> = [Fe(CN) <sub>6</sub> ] <sup>4-</sup>	+0.36

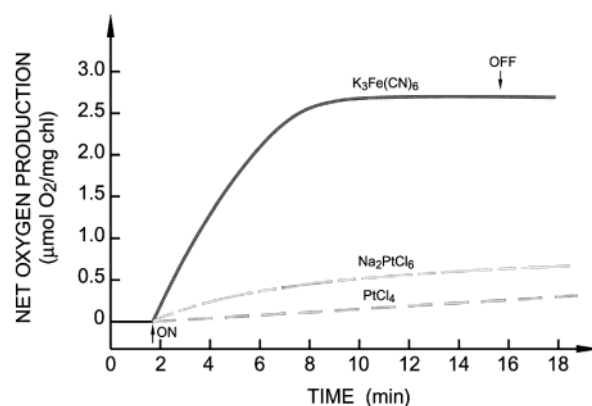
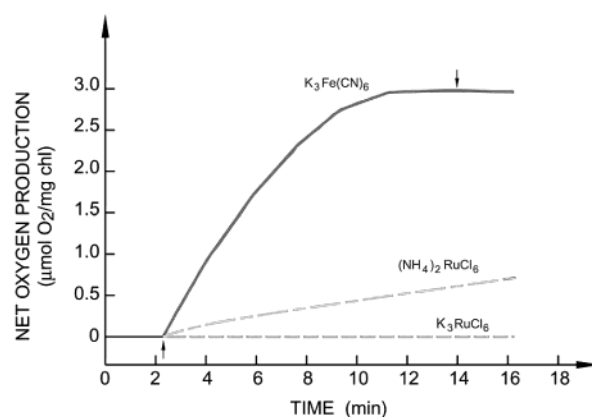
<sup>a</sup> The initial O<sub>2</sub> production rates by Hill reactions of 0.5 mM test compounds were measured with a Clark oxygen electrode and normalized to the nonlight-saturated standard (34.3 μmol O<sub>2</sub>/mg chl/h) using 0.5 mM [Fe(CN)<sub>6</sub>]<sup>3-</sup>. The values of the midpoint redox potentials are from Charlot et al.<sup>25</sup> and Milazzo and Caroli.<sup>26</sup> The detection sensitivity (limit) of the H<sub>2</sub>-production assay was ±2 nmol/mg chl/h.

**Figure 3.** Comparison of O<sub>2</sub> curves for the newly identified PSII Hill reagents Na<sub>2</sub>OsCl<sub>6</sub>, OsCl<sub>3</sub>, and K<sub>3</sub>Fe(CN)<sub>6</sub>. The data were obtained by O<sub>2</sub> electrode assays in the PSII sample in the presence of these water-soluble metal compounds. The initial concentration of the Hill reagents was 0.50 mM. The chl concentration of the PSII suspension was 40 μg/mL. The upward and downward arrows indicate the times at which actinic illumination [PAR, 100 μE/(m<sup>2</sup>·s)] was turned on and off, respectively.

The data of Figure 4 demonstrate that Na<sub>2</sub>PtCl<sub>6</sub> and PtCl<sub>4</sub> can also support steady net oxygen production in the PSII sample. The rates of PSII O<sub>2</sub> photoevolution with Na<sub>2</sub>PtCl<sub>6</sub> and PtCl<sub>4</sub> are only about 5.5 and 26.5% of the K<sub>3</sub>Fe(CN)<sub>6</sub> PSII Hill reaction (100%).

The O<sub>2</sub>-photoevolution assay demonstrated that (NH<sub>4</sub>)<sub>2</sub>RuCl<sub>6</sub> can also support steady net oxygen production for PSII reaction centers (Figure 5). The rate of PSII O<sub>2</sub> photoevolution with (NH<sub>4</sub>)<sub>2</sub>RuCl<sub>6</sub> is about 25% of the K<sub>3</sub>Fe(CN)<sub>6</sub> PSII Hill reaction.

A noteworthy aspect of our data is that platinum chloride (PtCl<sub>4</sub>) was able to slowly accept photosynthetically generated electrons from PSII (Figure 4). This finding is of theoretical

**Figure 4.** Comparison of O<sub>2</sub> curves for newly identified PSII Hill reagents Na<sub>2</sub>PtCl<sub>6</sub>, PtCl<sub>4</sub>, and K<sub>3</sub>Fe(CN)<sub>6</sub>. The data were obtained by O<sub>2</sub> electrode assays in the PSII sample in the presence of these water-soluble metal compounds. The initial concentration of the Hill reagents was 0.50 mM. The chl concentration of the PSII suspension was 40 μg/mL. The upward and downward arrows indicate the times at which actinic illumination [PAR, 100 μE/(m<sup>2</sup>·s)] was turned on and off, respectively.**Figure 5.** Results of PSII Hill reaction assays with reagents (NH<sub>4</sub>)<sub>2</sub>RuCl<sub>6</sub>, K<sub>3</sub>RuCl<sub>6</sub>, and K<sub>3</sub>Fe(CN)<sub>6</sub>. The data were obtained by O<sub>2</sub> electrode assays in the PSII sample in the presence of these water-soluble metal compounds. The initial concentration of the Hill reagents was 0.50 mM. The chl concentration of the PSII suspension was 40 μg/mL. The upward and downward arrows indicate the times at which actinic illumination [PAR, 100 μE/(m<sup>2</sup>·s)] was turned on and off, respectively.

significance because PtCl<sub>4</sub> is structurally and electrostatically quite different from the other PSII Hill reagents [PtCl<sub>6</sub>]<sup>2-</sup>, [OsCl<sub>6</sub>]<sup>2-</sup>, [RuCl<sub>6</sub>]<sup>2-</sup>, and [Fe(CN)<sub>6</sub>]<sup>3-</sup>. These four active PSII Hill reagents are octahedrally coordinated and negatively charged. The negative charge might be required to interact with the reducing site of PSII. Comparative studies with [Pt(NH<sub>3</sub>)<sub>4</sub>]<sup>2+</sup> and [PtCl<sub>6</sub>]<sup>2-</sup> have previously suggested that the negative charge of [PtCl<sub>6</sub>]<sup>2-</sup> is required for its interaction with the reducing site of PSI and that the positively charged species, [Pt(NH<sub>3</sub>)<sub>4</sub>]<sup>2+</sup>, is not photoactive in thylakoids.<sup>7</sup> As shown in Table 1, the positively charged [Pt(NH<sub>3</sub>)<sub>4</sub>]<sup>2+</sup> species was not photoactive in PSII either. Therefore, the same requirement is probably also true for PSII. Apparently, PtCl<sub>4</sub> satisfies this requirement by reacting with water. When it is dissolved in water, it combines with water molecules to form H<sub>2</sub>[PtCl<sub>4</sub>(OH)<sub>2</sub>], which can dissociate to a negatively charged species [PtCl<sub>4</sub>(OH)<sub>2</sub>]<sup>2-</sup> at neutral pH.<sup>22,23</sup>

However, electrostatic interaction may not be the only factor that determines electron transfer from PSII. The ability of an ionic species to act as a Hill reagent is also determined by redox potential and by interfacial reactions with the PSII membranes.

Our results show that 18 of the tested metal compounds are not active (Table 1). It is interesting to note that  $[\text{PdCl}_6]^{2-}$ ,  $[\text{IrCl}_6]^{3-}$ , and  $[\text{RhCl}_6]^{3-}$  are unable to act as Hill reagents despite the fact that they have redox and structural properties similar to the homologous ionic species,  $[\text{PtCl}_6]^{2-}$ ,  $[\text{OsCl}_6]^{2-}$ , and  $[\text{RuCl}_6]^{3-}$ , which are active Hill reagents (Table 1). The differences in the ability of these homologous species to act as Hill reagents are probably due to differences in electronic energy levels and molecular orbital interactions with the reducing site of PSII. This phenomenon merits a theoretical investigation.

**In Situ Photoprecipitation of Metallocatalysts for Simultaneous  $\text{H}_2$  and  $\text{O}_2$  Production by Photosynthetic Water Splitting.** We have previously demonstrated that hexachloroplatinate ( $[\text{PtCl}_6]^{2-}$ ) and hexachloroosmate ( $[\text{OsCl}_6]^{2-}$ ) can be photoconverted to metallic platinum and osmium at the reducing site of PSI in thylakoid membranes via the Hill reaction.<sup>11,12,21</sup> PSII is another class of nanometer photosynthetic material that could have significant potential applications if it can be properly interfaced through chemical design. It is well-known that PSII reaction centers can naturally oxidize water to molecular oxygen and protons. As illustrated in Figure 1, the electrons acquired via water oxidation are vectorially transported from P680 to the reduction site ( $\text{Q}_\text{B}$ ) via the intermediate carriers Phe and  $\text{Q}_\text{A}$ . If a nanometer metallocatalyst could be deposited at the reducing end of PSII for  $\text{H}_2$  production as we have successfully demonstrated at the reducing end of PSI in thylakoid membranes and isolated PSI preparations,<sup>24</sup> it would be theoretically possible to achieve simultaneous photoevolution of  $\text{O}_2$  and  $\text{H}_2$  through a single PSII reaction center.

Thermodynamically, the energy captured by the PSII light reaction ( $\lambda = 680 \text{ nm} \leftrightarrow 1.8 \text{ eV}$ ) should be sufficient to drive this process:



As shown in this reaction, this process has a significantly large negative value of  $\Delta G^\circ$ . It should occur spontaneously if a proper catalyst can be provided. In this study, therefore, we also explored the possibility of photodeposition of metallocatalysts at the reducing site of PSII using the Hill reactions. We assayed the 23 compounds listed in Table 1 for the possibility of photodeposition of metallocatalysts through PSII Hill reactions by simultaneously measuring  $\text{H}_2$  and  $\text{O}_2$  production in a helium atmosphere using a reactor-flow detection system. In addition to confirmation of our earlier findings<sup>11,12</sup> with thylakoids where photodeposition of metallocatalysts at the reducing site of PSI was demonstrated, we found that of the 23 compounds studied, including the newly identified PSII Hill reagents  $[\text{PtCl}_6]^{2-}$ ,  $[\text{OsCl}_6]^{2-}$ ,  $\text{PtCl}_4$ , and  $(\text{NH}_4)_2\text{RuCl}_6$ , hydrogen evolution, if it occurred at all, was below the level of detection for our measuring system [about 2 nmol/mg chl/h].

This is a significant result because it indicates that either the electrons emergent from PSII are not able to reduce any of the 23 metal compounds including  $[\text{PtCl}_6]^{2-}$ ,  $[\text{OsCl}_6]^{2-}$ ,  $\text{PtCl}_4$ , and  $[\text{RuCl}_6]^{2-}$  to their metallic state or the metallocatalysts (if formed at PSII) are inactive for  $\text{H}_2$  evolution. According to our analysis, the former is more likely. The data that support this hypothesis are in Figure 5 and Table 1, where it is demonstrated that PSII can reduce  $[\text{RuCl}_6]^{2-}$  but not  $[\text{RuCl}_6]^{3-}$ . The data indicate that the PSII Hill reaction of  $[\text{RuCl}_6]^{2-}$  is only a one-electron-reduction event to form  $[\text{RuCl}_6]^{3-}$ , which cannot be further reduced by PSII. Further studies are needed to overcome this challenge, probably through biomolecular re-engineering of the

PSII reducing site, so that nanometer metallocatalysts can be deposited in situ by photosynthetic reduction.

## Conclusions

A new class of PSII Hill reagents has been identified. The results indicated that the ability of a species to act as a Hill reagent is determined not only by its redox potential but also by its molecular interaction with PSII membranes. In addition to the hexachloro species  $[\text{PtCl}_6]^{2-}$ ,  $[\text{OsCl}_6]^{2-}$ , and  $[\text{RuCl}_6]^{2-}$ , platinum tetrachloride ( $\text{PtCl}_4$ ) is also capable of interacting with PSII membranes and acting as a Hill reagent with a slower rate. These findings may have potential applications in biometallocatalysis for the production of fuels and chemicals and in nanofabrication for the construction of biomolecular optoelectronic devices.

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