

Molecular Ionic Probes: A New Class of Hill Reagents and Their Potential for Nanofabrication and Biometallocalysis

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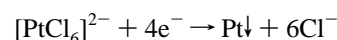
We report the results of an extensive experimental survey and characterization of over 20 species of transition-metal compounds for their activity in the Hill reaction and photoprecipitation of metal at the lipid–water interface of photosynthetic thylakoid membranes. Four new Hill reagents were identified: PtCl_4 , OsCl_3 , $[\text{RuCl}_6]^{2-}$, and $[\text{RuCl}_6]^{3-}$. Each can be photosynthetically reduced to form a metallic catalyst at the reducing site of photosystem I (PSI) in the thylakoid membranes. Negative charge of the metal-compound species is apparently essential for their interaction with the reducing site of PSI. When PtCl_4 and OsCl_3 are dissolved in water, they combine with water molecules to form $\text{H}_2[\text{PtCl}_4(\text{OH})_2]$ and $\text{H}_3[\text{OsCl}_3(\text{OH})_3]$, which can dissociate to negatively charged species $[\text{PtCl}_4(\text{OH})_2]^{2-}$ and $[\text{OsCl}_3(\text{OH})_3]^{3-}$ at neutral pH. Metallic ruthenium can also catalyze reduction of protons for H_2 production, in addition to its known catalytic ability to fix CO_2 . These findings could have important implications for nanofabrication and biometallocalysis to harness the power of photosynthetic systems.

Introduction

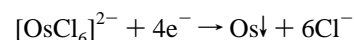
Photosynthesis is the key biological process that converts the electromagnetic energy of sunlight to useful chemical energy, thereby supporting essentially all life on earth. The primary events of photosynthesis occur in photosystem I (PSI) and photosystem II (PSII) reaction centers. Photosynthetic reaction centers such as PSI are small (6 nm), extremely fast (picosecond response), and efficient (quantum yield close to 100%) photovoltaic devices.¹ The architecture and functions of PSI reaction centers arguably surpass those of any semiconductor devices. They can generate photoinduced charge separation in 10–30 ps, a speed that is a hundred times faster than that of a silicon photodiode.² 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 PSI particles have been demonstrated at nanometer scales through the use of a chemical platinization reaction.^{3–5}

With advances in nanofabrication, it is also possible to transform photosynthetic reaction centers into molecularly engineered devices to produce fuels and chemicals by biometallocalysis.^{1,6} By use of a chemical platinization reaction, such a transformation has been demonstrated for photosynthetic membranes (thylakoids) that contain both PSII and PSI reaction centers.⁷ 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 H_2 and O_2 by water splitting and biometallocalysis. By use of 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.^{8–10}

Subsequently, a second technique^{11,12} was developed specifically to 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,¹³ 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, presumably by converting $[\text{PtCl}_6]^{2-}$ and $[\text{OsCl}_6]^{2-}$ anions to zero valent metallic platinum (Pt) and osmium (Os) at the reducing site of PSI, according to the following reactions:^{11,12}



and



In the present work, an extensive survey and characterization of more than 20 species of transition-metal compounds were conducted in search for their potential ability to act as Hill reagents and as metallocalysts. New Hill reagents and species for active metallocalysis were identified. In addition to $[\text{OsCl}_6]^{2-}$ and $[\text{PtCl}_6]^{2-}$, PtCl_4 and OsCl_3 were found to be active in the Hill reaction. The species PtCl_4 and OsCl_3 can also be photoprecipitated to catalyze simultaneous photoproduction of H_2 and O_2 by water splitting in thylakoids. Furthermore, $[\text{RuCl}_6]^{2-}$ and $[\text{RuCl}_6]^{3-}$ were also found to be active in the Hill reaction and photoprecipitation. The photoreduction products of these two species can also catalyze H_2 production. These findings have potentially important implications for both nanofabrication and biometallocalysis using photosynthetic reaction centers.

Materials and Methods

Sources of Chemicals. Three chemicals were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI): potassium

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hexachlororuthenate(III), K_3RuCl_6 ; potassium ferricyanide, $\text{K}_3\text{Fe}(\text{CN})_6$; sodium hexachloroiridate(IV), Na_2IrCl_6 . The following 19 chemicals were purchased from Johnson Matthey Company (Ward Hill, MA): ammonium hexachloroiridate(III), $(\text{NH}_4)_3\text{IrCl}_6$; ammonium hexachlororhodate(III), $(\text{NH}_4)_3\text{RhCl}_6$; ammonium hexachlororuthenate(IV), $(\text{NH}_4)_2\text{RuCl}_6$; ammonium molybdenum oxide, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$; 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, $\text{Pt}(\text{NH}_3)_4\text{Cl}_2$; tetraamminepalladium(II) chloride, $\text{Pd}(\text{NH}_3)_4\text{Cl}_2$; ammonium tetrachloropalladate(II), $(\text{NH}_4)_2\text{PdCl}_4$. 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 (1980).¹⁴ The thylakoids were suspended in Walker's assay medium¹⁵ and adjusted to a final chlorophyll (chl) concentration of 40 or 600 $\mu\text{g}/\text{mL}$ for Hill reaction assays with a Clark oxygen electrode or photoprecipitation of metallocal catalysts for production of H_2 and O_2 with a reactor-flow detection system, respectively. The chl content was determined in 90% acetone extracts.¹⁶

Hill Reaction Assayed by Clark Oxygen Electrode. Hill reactions of the compounds were assayed by measuring O_2 evolution in thylakoids using a Clark oxygen electrode system (YSI model 53). Calibration of the oxygen electrode system was accomplished by using water deoxygenated with $\text{Na}_2\text{S}_2\text{O}_4$ as a zero baseline and air-saturated water as a standard (0.0310 L $\text{O}_2/\text{L H}_2\text{O}$ at 20 °C).¹⁷ After calibration, a 1.8-mL sample was placed in the water-jacketed reaction chamber at 20 °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 $\mu\text{g chl}/\text{mL}$ 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 the oxygen level was stabilized in darkness, the reaction chamber was illuminated at about 100 $\mu\text{E m}^{-2} \text{ s}^{-1}$ ($\text{E} = \text{einstein}$) 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 metallocal catalysts for production of H_2 and O_2 was performed in a helium atmosphere using a reactor-flow detection system as described previously.^{6,12} Ten milliliters of thylakoid suspension (600 $\mu\text{g chl}/\text{mL}$) was placed into the reactor. The prospective Hill reagent was added to the thylakoid suspension for a final concentration of 2 mM. The reaction vessel was water-jacketed and held at 20 °C with a temperature-controlled water bath (Lauda RM6, Brinkmann Instruments, Germany). The reaction medium (thylakoid suspension plus a Hill reagent) was gently stirred by a magnetic stirring bar and purged by 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). Photopre-

cipitation of metallocal catalysts was then initiated by actinic illumination (50 $\mu\text{E s}^{-1} \text{ m}^{-2}$) that was 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 a tin oxide hydrogen sensor and galvanic cell.

Identification of Metallic Species by X-ray Fluorescence. Metallic products in phototreated thylakoids were identified by energy-dispersive X-ray fluorescence using a 100-keV electron beam as the actinic source. Prior to X-ray fluorescence analysis, the postexperiment thylakoids from the H_2 and O_2 production assays were washed four times with 40 mL of distilled water to remove any soluble metal compounds. After each wash, the thylakoids were pelleted with centrifugation at 1300g, and the soluble metal was removed by discarding the supernatant. A thylakoid suspension incubated without illumination in 2 mM metal compounds such as PtCl_4 , OsCl_3 , or $[\text{RuCl}_6]^{3-}$ was used as the control. After the incubation, the control samples were washed according to the same procedure that was used for phototreated thylakoids.

Results and Discussion

New Hill Reagents Identified by Clark Oxygen Electrode Assay. Among the over 20 compounds of heavy transition metals tested, 7 species— PtCl_4 , OsCl_3 , $\text{Na}_2[\text{PtCl}_6]$, $\text{Na}_2[\text{OsCl}_6]$, $(\text{NH}_4)_2\text{RuCl}_6$, $(\text{NH}_4)_3\text{RuCl}_6$ and Na_2IrCl_6 —were found to be active in the Hill reaction (Table 1). The rate of Hill reaction can be measured as the initial rate of oxygen evolution in thylakoids using the Clark oxygen electrode (Figure 1). The Hill reaction rates of PtCl_4 , $\text{Na}_2[\text{PtCl}_6]$, $\text{Na}_2[\text{OsCl}_6]$, $(\text{NH}_4)_2\text{RuCl}_6$, and $(\text{NH}_4)_3\text{RuCl}_6$ were 140–260% that of the classic Hill reagent $\text{K}_3\text{Fe}(\text{CN})_6$ (Table 1). The results indicated that these transition-metal compounds, like ferricyanide, are Hill reagents that readily accept electrons from photosynthetic water splitting in thylakoids.

These newly identified Hill reagents, however, have distinct characteristics. Na_2PtCl_6 and PtCl_4 support steady net oxygen production (Figure 1A), while Na_2OsCl_6 , OsCl_3 , $(\text{NH}_4)_2\text{RuCl}_6$, and K_3RuCl_6 support net oxygen production only at the initial phase of the Hill reaction (parts B and C of Figure 1). With the last four Hill reagents (Na_2OsCl_6 , OsCl_3 , $(\text{NH}_4)_2\text{RuCl}_6$, and K_3RuCl_6), oxygen production rose to a peak and then declined dramatically. The decline of the oxygen concentration in these four samples did not stop when actinic light was turned off (indicated by the downward arrows in parts B and C of Figure 1). These observations indicate that the reduction products of Na_2OsCl_6 , OsCl_3 , $(\text{NH}_4)_2\text{RuCl}_6$, and K_3RuCl_6 —presumably metallic osmium and ruthenium—react with molecular oxygen in a Mehler-like oxygen-uptake reaction.

The reduction product of Na_2PtCl_6 , presumably metallic platinum, is quite inert to molecular oxygen. No consumption of oxygen was observed when the actinic light was turned off after >10 min of Hill activity (Figure 1A). It has been previously demonstrated that reduction of $[\text{PtCl}_6]^{2-}$ by the Hill reaction results in photoprecipitation of metallic platinum at the reducing site of PSI.^{11,12} Complete reduction of $[\text{PtCl}_6]^{2-}$ to metallic platinum requires four electrons for each $[\text{PtCl}_6]^{2-}$, while conversion of the well-known Hill reagent $[\text{Fe}(\text{CN})_6]^{3-}$ to $[\text{Fe}(\text{CN})_6]^{4-}$ requires only one electron. Accordingly, one would predict that complete photoreduction of $[\text{PtCl}_6]^{2-}$ should produce an amount of molecular oxygen that is 4 times that of the $[\text{Fe}(\text{CN})_6]^{3-}$ Hill reaction on a per mole basis. The experimental data show that the maximal amount of oxygen produced by the NaPtCl_6 Hill reaction was only a little more than that of $\text{K}_3\text{Fe}(\text{CN})_6$ (Figure 1A). This result indicated that

TABLE 1: Test Results for 24 Metal Compounds Surveyed by Measuring Their Ability to Support Oxygen and Hydrogen Evolution in Thylakoids^a

initial species	% rate of control Hill reaction	H ₂	redox system	E ₀ (V)
PtCl ₄	138 ± 10	yes	PtCl ₄ + 4e ⁻ ⇌ Pt↓ + 4Cl ⁻	+0.68
OsCl ₃	88.3	yes	OsCl ₃ + 3e ⁻ ⇌ Os↓ + 3Cl ⁻	
Na ₂ [PtCl ₆]	163 ± 3	yes	[PtCl ₆] ²⁻ + 4e ⁻ ⇌ Pt↓ + 6Cl ⁻	
Na ₂ [OsCl ₆]	155 ± 8	yes	[OsCl ₆] ²⁻ + 4e ⁻ ⇌ Os↓ + 6Cl ⁻	
(NH ₄) ₂ RuCl ₆	259 ± 1	yes	[RuCl ₆] ²⁻ + 4e ⁻ ⇌ Ru↓ + 6Cl ⁻	
K ₃ RuCl ₆	189	yes	[RuCl ₆] ³⁻ + 3e ⁻ ⇌ Ru↓ + 6Cl ⁻	+0.86
Na ₂ PdCl ₆	0	no	[PdCl ₆] ²⁻ + 4e ⁻ ⇌ Pd↓ + 6Cl ⁻	
Na ₂ IrCl ₆	19.5	no	[IrCl ₆] ²⁻ + 4e ⁻ ⇌ Ir↓ + 6Cl ⁻	
(NH ₄) ₃ IrCl ₆	0	no	[IrCl ₆] ³⁻ + 3e ⁻ ⇌ Ir↓ + 6Cl ⁻	
(NH ₄) ₃ RhCl ₆	0	no	[RhCl ₆] ³⁻ + 3e ⁻ ⇌ Rh↓ + 6Cl ⁻	
K ₂ PdCl ₄	0	no	[PdCl ₄] ²⁻ + 2e ⁻ ⇌ Pd↓ + 4Cl ⁻	+0.62
(NH ₄) ₂ PdCl ₄	0	no	[PdCl ₄] ²⁻ + 2e ⁻ ⇌ Pd↓ + 4Cl ⁻	+0.62
Pd(NH ₃) ₄ Cl ₂	0	no		
ReCl ₃	0	no		
NiCl ₂	0	no		
CoCl ₂	0	no		
PtO ₂	0	no		
PtCl ₂	0	no		
Pt(NH ₃) ₄ Cl ₂	0	no		
(NH ₄) ₆ Mo ₇ O ₂₄	0	no		
NaAuCl ₄	0	no		
K ₂ [PtCl ₄] ₆	0	no	[PtCl ₄] ²⁻ + 2e ⁻ ⇌ Pt↓ + 4Cl ⁻	+0.73
K ₃ Fe(CN) ₆	100 ± 7	no	[Fe(CN) ₆] ³⁻ + e ⁻ ⇌ [Fe(CN) ₆] ⁴⁻	+0.36

^a The initial O₂ production rates by Hill reactions of 0.5 mM test compounds were measured with a Clark oxygen electrode and normalized to the non-light-saturated standard (21.4 μmol O₂ mg ch⁻¹ h⁻¹) using 0.5 mM [Fe(CN)₆]³⁻. Their catalytic activities for H₂ production were monitored with a reactor-flow detection system. Values of midpoint redox potentials are from Charlot et al. (1971)²³ and Milazzo and Caroli (1978).²⁴

the Hill reaction consumed slightly more than 25% of the total [PtCl₆]²⁻ added, assuming a stoichiometric Pt:O₂ ratio of 1:1. It is likely that precipitated metallic platinum modifies the reducing site of PSI, which finally limits further consumption of the substrate.

Note that the oxygen-production curve of the classic Hill reagent [Fe(CN)₆]³⁻ rose quite linearly with the actinic illumination and then had a sharp turn at the point of maximum oxygen production (Figure 1). This sharp turning point marks complete conversion of [Fe(CN)₆]³⁻ into [Fe(CN)₆]⁴⁻, which cannot be further reduced by PSI, and the beginning of oxygen consumption by the Mehler reaction, in which emergent electrons from PSI reduce molecular oxygen. The Mehler reaction is a light-dependent process. When the actinic illumination was off (indicated by a downward arrow), the oxygen level remained constant (Figure 1). The oxygen production curves of both PtCl₄ and [PtCl₆]²⁻ exhibited greater curvature but showed no sharp turning point (Figure 1A). As suggested in our previous studies,¹² oxygen production was limited primarily by the accumulation of metallic platinum at the reducing site(s) of PSI rather than by the amount of PtCl₄ or [PtCl₆]²⁻. On the basis of the stoichiometric ratio (1:1) of Pt to O₂, the maximum utilization efficiencies were about 20% and 30% for the added PtCl₄ and [PtCl₆]²⁻, respectively.

Perhaps the most remarkable aspect of our observations is that platinum chloride (PtCl₄) and osmium chloride (OsCl₃) were able to accept photosynthetically generated electrons in thylakoids (parts A and B of Figure 1). This finding is important, since PtCl₄ and OsCl₃ are structurally quite different from [PtCl₆]²⁻, [OsCl₆]²⁻, and [Fe(CN)₆]³⁻. Comparative studies with [Pt(NH₃)₄]²⁺ and [PtCl₆]²⁻ has previously suggested that the negative charge of [PtCl₆]²⁻ is required for its interaction with the reducing site of PSI and that the positively charged species, [Pt(NH₃)₄]²⁺, is not photoactive.⁷ Apparently, PtCl₄ and OsCl₃ satisfy this requirement by reacting with water. When they are dissolved in water, they combine with water molecules to form H₂[PtCl₄(OH)₂] and H₃[OsCl₃(OH)₃],^{18,19} which can dissociate to negatively charged species [PtCl₄(OH)₂]²⁻

and [OsCl₃(OH)₃]³⁻ at neutral pH. These results are consistent with the fact that the reducing side of the PSI complex possesses positively charged domain(s). In natural photosynthesis, the positively charged domain(s) such as the subunit II of the PSI protein complex serves as the docking site for negatively charged ferredoxin. According to Colvert and Davis' experimental determination,²⁰ the net charge on ferredoxin is -13, whereas the thylakoid membrane in the region of the ferredoxin binding site (PSI) has a surface charge density of +0.91 μC/cm². Believably, it is this electrostatic interaction that brings the ferredoxin into proximity with the reducing site of PSI for electron transfer. This may also explain the requirement of the negative charge for the transition-metal compounds to achieve electron transfer from PSI. That is, the negatively charged Hill reagents may achieve their contact with PSI also by electrostatic interaction.

However, electrostatic interaction may not be the only factor that determines the electron transport from PSI. The ability of an ionic species to act as a Hill reagent is also determined by the redox potential and by interfacial reactions with the photosynthetic membranes. Our results show that 15 of the tested metal compounds are not active (Table 1). It is interesting to note that [PdCl₆]²⁻, [IrCl₆]³⁻, and [RhCl₆]³⁻ are completely unable to act as Hill reagents despite the fact that they have redox and structural properties similar to those of their homologous ionic species [PtCl₆]²⁻ and [RuCl₆]³⁻, which are excellent 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 level and molecular-orbital interactions with the reducing site of PSI. This finding merits a theoretical investigation.

In Situ Photoprecipitation of Metallocatalysts for Simultaneous H₂ and O₂ Production by Water Splitting in Thylakoids. We have previously demonstrated that hexachloroplatinate ([PtCl₆]²⁻) and hexachloroosmate ([OsCl₆]²⁻) can be photoconverted to metallic platinum and osmium at the reducing site of PSI in thylakoid membranes through the Hill reaction.^{11,12} In the search for additional compounds that can be used for photodeposition of metallocatalysts at the reducing

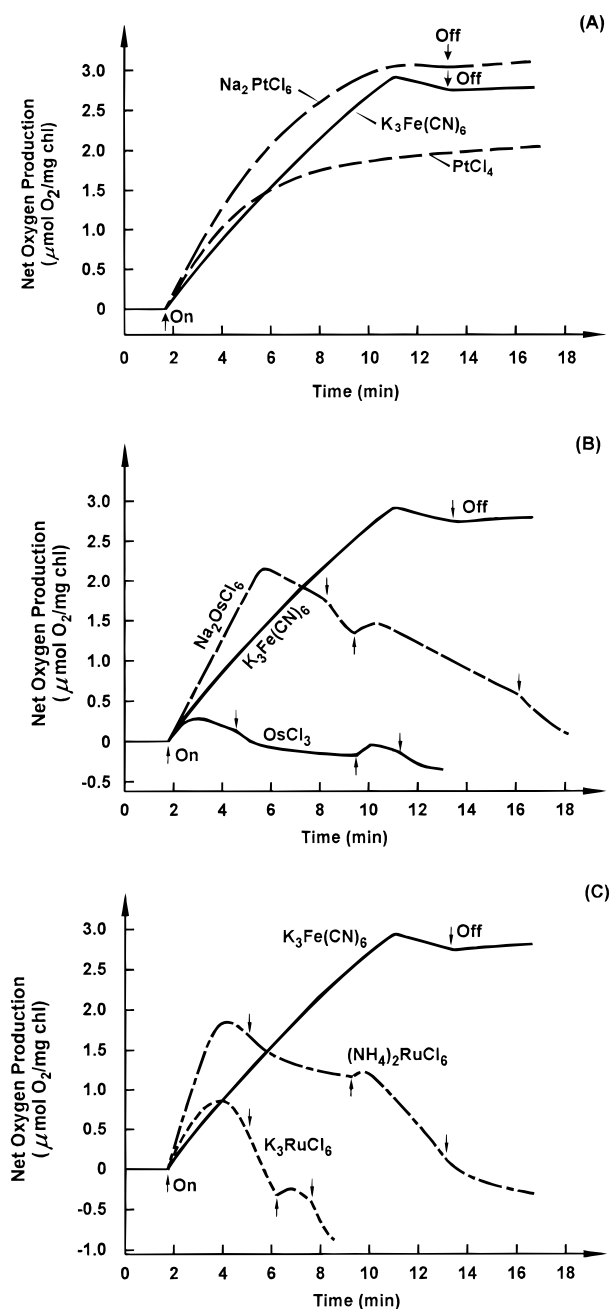


Figure 1. Activity of a new class of Hill reagents with thylakoids as detected by a Clark oxygen electrode: (A) Hill reactions of PtCl_4 , Na_2PtCl_6 , and $\text{K}_3\text{Fe}(\text{CN})_6$; (B) Hill reactions of Na_2OsCl_6 and OsCl_3 in comparison with the control $\text{K}_3\text{Fe}(\text{CN})_6$; (C) Hill reactions of $(\text{NH}_4)_2\text{RuCl}_6$ and K_3RuCl_6 in comparison with the control. The initial concentration of the Hill reagents was 0.50 mM. The chlorophyll concentration of the thylakoid suspension was 40 $\mu\text{g}/\text{mL}$. The upward and downward arrows indicate the time at which actinic illumination (PAR, 100 $\mu\text{E m}^{-2} \text{ s}^{-1}$) was turned on and off, respectively.

site of PSI in thylakoid membranes, we assayed the 23 compounds listed in Table 1 by simultaneously measuring H_2 and O_2 production in a helium atmosphere using a reactor-flow detection system. In addition to confirmation of our earlier findings^{11,12} with $[\text{PtCl}_6]^{2-}$ and $[\text{OsCl}_6]^{2-}$, we found that PtCl_4 , OsCl_3 , $(\text{NH}_4)_2\text{RuCl}_6$, and K_3RuCl_6 can also support simultaneous H_2 and O_2 photoevolution after photoprecipitation (Table 1).

A typical set of data are presented in Figures 2 and 3 for PtCl_4 and $(\text{NH}_4)_2\text{RuCl}_6$. The photoprecipitation process was characterized as a burst of O_2 evolution upon onset of actinic illumination during the first light cycle (Figure 2). During the

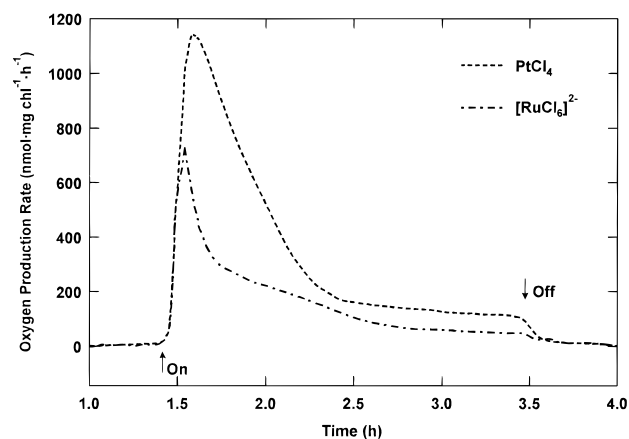


Figure 2. Initial burst of oxygen production from the Hill reaction of 2 mM PtCl_4 and $[\text{RuCl}_6]^{2-}$ in 10 mL of thylakoid suspension under helium atmosphere.

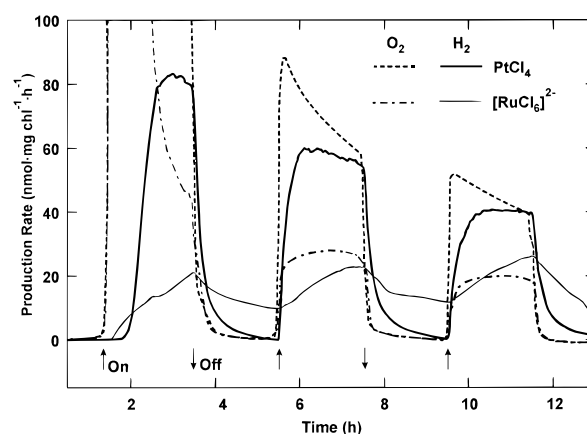


Figure 3. Comparison of simultaneous hydrogen and oxygen production from photosynthetically metallized thylakoids using 2 mM PtCl_4 and $[\text{RuCl}_6]^{2-}$ under multiple light and dark cycles. The upward and downward arrows mark on and off phases of the actinic light.

first 2 h of illumination, integrated yields of 710.8 and 340.0 nmol $\text{O}_2/\text{mg chl}$ (i.e., 4265 and 2046 nmol O_2 in a 10-mL sample) were produced in thylakoid samples treated with 2 mM PtCl_4 and $(\text{NH}_4)_2\text{RuCl}_6$, respectively. These large amounts of O_2 were generated almost entirely by the Hill reaction of PtCl_4 and $(\text{NH}_4)_2\text{RuCl}_6$, since the contribution to O_2 production from the oxidized plastoquinone pool was negligibly small (only about 0.8 nmol $\text{O}_2/\text{mg chl}$), as determined in control thylakoids without any Hill oxidants. On the basis of the amount of O_2 produced by the end of the first 2 h of illumination, it can be estimated that about 420 atoms of metallic platinum were produced per PSI on the thylakoid membrane and that the Hill reaction consumed about 21% of the total PtCl_4 added, assuming a stoichiometric $\text{Pt}:\text{O}_2$ ratio of 1:1 and 600 chls per PSI and PSII photosynthetic unit in the thylakoids. Similarly, it can be estimated that about 200 atoms of metallic ruthenium were produced per PSI and that the Hill reaction consumed about 10% of the total $(\text{NH}_4)_2\text{RuCl}_6$ added in the thylakoid suspension.

Photoprecipitation of the metallocatalysts (metallic platinum and ruthenium) was followed by an onset of H_2 production, resulting in simultaneous evolution of both O_2 from water splitting by PSII and H_2 from the metallocatalytic reduction of protons at PSI by linearly transported electrons from water splitting (Figure 3). There was a characteristic lag (10 and 20 min for $(\text{NH}_4)_2[\text{RuCl}_6]$ and PtCl_4 , respectively), which occurred from the initial burst of O_2 evolution to the onset of H_2 evolution. During this lag, colloidal platinum (or ruthenium)

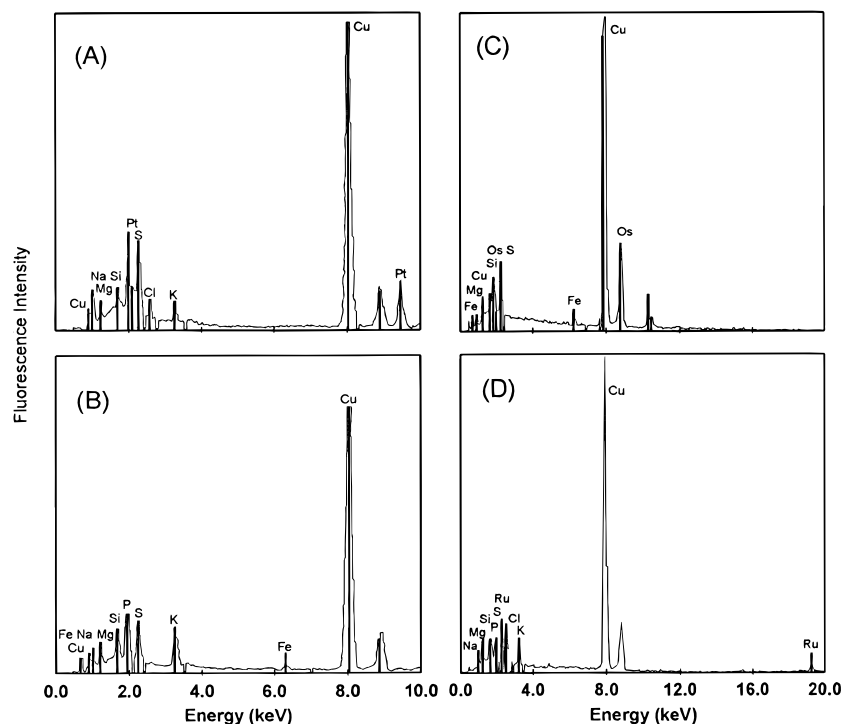


Figure 4. Identification of metallic products by X-ray fluorescence. Spectra A and B are from thylakoids incubated with PtCl_4 in the presence and absence of actinic illumination. Spectra C and D are from thylakoids photometallized with OsCl_3 and $[\text{RuCl}_6]^{3-}$. Soluble metal compounds were removed by washing the thylakoid samples four times with 40 mL of distilled water before taking the X-ray fluorescence spectra.

was formed presumably as a result of the four-step reduction of PtCl_4 (or $[\text{RuCl}_6]^{2-}$) at the reducing site of a PSI complex in the thylakoid membrane. This lag time indicated that there was a threshold platinum (or ruthenium) particle size for catalyzing H_2 production. Previous measurements indicated a size of about 50 platinum atoms, based on repetitive flash illumination and the onset of catalytic H_2 evolution.¹¹ As shown in Figure 3, the rate of H_2 production for the first cycle of illumination followed a slower but steady increase after the lag. This indicated further growth of the metallic particles. Note that after the establishment of the metallic colloids during the first period of actinic illumination, little or no lag was observed, indicating that catalyst formed during the first cycle of illumination was present for subsequent cycles. The small and constant (about 3 min) lag that appeared in the later illumination cycles (Figure 3) was due to the response time of the flow system. Simultaneous O_2 and H_2 production can be sustained for over 20 h with periodic (or continuous) illumination at room temperature. The maximal rates of H_2 production were about 80 and 20 nmol $\text{mg chl}^{-1} \text{ h}^{-1}$ for PtCl_4 - and $[\text{RuCl}_6]^{2-}$ -treated samples, respectively. The rates of H_2 and O_2 production decreased slightly during later periods, possibly owing to the natural decline of thylakoid activity (Figure 3). This pattern of H_2 and O_2 production is very similar to that of chemically platinized thylakoids reported previously.⁷ Furthermore, since the PtCl_4 and $(\text{NH}_4)_2\text{RuCl}_6$ that remained after the first cycles were still available for additional O_2 production by the Hill reaction, the $\text{O}_2:\text{H}_2$ ratio was higher than 1:2 for both samples (Figure 3). This characteristic was very similar to that observed with photosynthetically platinized thylakoids using $[\text{PtCl}_6]^{2-}$ as previously reported.¹²

Ruthenium is known to catalyze reduction of CO_2 to produce methane.²¹ The data in Figure 3 suggest that ruthenium could also act as a weak catalyst for H_2 production in a pure-helium atmosphere. The catalytic activity for H_2 production observed for ruthenium was about 25% of that of metallic platinum, as

estimated by their respective rates of H_2 photoevolution. It is interesting to note that $[\text{RuCl}_6]^{2-}$ -phototreated thylakoids were also able to produce small but persistent amounts of H_2 in darkness after the actinic light was turned off (Figure 3). This result indicates that although the catalytic activity of ruthenium for H_2 production was low, the photoprecipitated ruthenium might be accessible to reductant that could be stored during illumination in the sample (such as in the plastoquinone pool of thylakoids) for H_2 production.

Identification of Metallic Catalysts in Phototreated Thylakoids Using PtCl_4 , OsCl_3 , and $[\text{RuCl}_6]^{3-}$ with X-ray Fluorescence Spectroscopy. The formation of insoluble metalocatalysts by the Hill reaction of PtCl_4 , OsCl_3 , and $[\text{RuCl}_6]^{3-}$ in thylakoids was also demonstrated by using X-ray fluorescence spectroscopy. As illustrated in Figure 4A, a metallic (non-soluble) platinum fluorescence spectrum appeared only in PtCl_4 -phototreated thylakoids. The absence of a platinum signal in the control (PtCl_4 -incubated thylakoids without illumination) confirmed the expected requirement of actinic photons for platinization (Figure 4B). The Cl, K, S, Si, Mg, and Na peaks in parts A and B of Figure 4 were common to thylakoids, while the Cu peaks were from the copper sample holder. Similarly, the formation of metallic osmium and ruthenium was identified in the OsCl_3 - and $[\text{RuCl}_6]^{3-}$ -phototreated thylakoids (parts C and D of Figure 4). These results are in excellent agreement with the Hill reaction of PtCl_4 , OsCl_3 , and $[\text{RuCl}_6]^{3-}$ (Figure 1) and the catalytic action of platinum, osmium, and ruthenium for H_2 production in photometallized thylakoids (Table 1 and Figure 3).

This result is important, since it confirms the hypothesis that metallic catalysts can be formed by photosynthetic reduction of water-soluble species of metal compounds. It provides new opportunities to impart novel characteristics to biological systems by incorporation of metalocatalysis in conjunction with biological function. Depending on the choice of specific catalysts, photosynthetic systems could be transformed for

production not only of hydrogen but also of other chemicals. Metallic ruthenium, for example, is a known catalyst for reduction of CO_2 to CH_4 ,²¹ although it can also catalyze H_2 production by reduction of protons as demonstrated in the current experiments in a helium atmosphere.

Furthermore, the growth of metallic particles can be controlled by photosynthetic reactions. By use of single-turnover flashes, atom-by-atom growth of metallic platinum has been previously demonstrated. The size of metallic platinum particles grown at the reducing site of PSI can be determined by the number of actinic flashes.¹¹ Therefore, this photosynthetic deposition process can generate well-defined and uniform nanostructures with precision size control, a feature that could have important applications in nanofabrication. Moreover, bimetallic structures can, in principle, be produced by blending two metal-ion species in solution for coprecipitation. By use of chemical platinization, a two-dimensional nanostructural spatial array of PSI reaction centers has recently been constructed by anchoring isolated PSI particles onto a gold surface with PSI–platinum–gold bonding.³ Use of photosynthetic metal deposition may be able to provide more site-specific and precise molecular “welding” at nanometer scales. Since the structures of PtCl_4 and OsCl_3 are somewhat simpler, they may be able to penetrate structures that are impermeable to $[\text{OsCl}_6]^{2-}$ and $[\text{PtCl}_6]^{2-}$. Utilization of PtCl_4 or OsCl_3 may enable photoprecipitation of metallocatalysts in intact alga for H_2 production²² and/or make it possible to perform molecular “welding” for nanofabrication based on their interaction with the photosynthetic reaction centers.

Conclusions

A new class of 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 photosynthetic membranes. The species $[\text{RuCl}_6]^{2-}$ and $[\text{RuCl}_6]^{3-}$ can be photosynthetically precipitated at the reducing site of PSI in the thylakoid membrane. Metallic ruthenium is a known catalyst for reduction of CO_2 to produce methane. In a pure-helium atmosphere, ruthenium can also act as a weak catalyst for H_2 production by reduction of protons. In addition to the hexachloro species $[\text{PtCl}_6]^{2-}$, $[\text{OsCl}_6]^{2-}$, $[\text{RuCl}_6]^{2-}$, and $[\text{RuCl}_6]^{3-}$, platinum tetrachloride PtCl_4 and osmium trichloride OsCl_3 are also capable of interacting with thylakoid membranes and of acting as excellent Hill reagents. Both PtCl_4 and OsCl_3 can be photoreduced to metallic catalysts in thylakoids for simultaneous

production of H_2 and O_2 by water splitting. These findings may have potential applications in biometallocatalysis for production of fuels and chemicals and in nanofabrication for construction of biomolecular optoelectronic devices.

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