Photocatalysis Hot Paper

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Photocatalytic Hydrogen Evolution with a Hydrogenase in a Mediator-Free System under High Levels of Oxygen**

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The development of solar water-splitting systems provides a route to renewable H_2.[1] A prerequisite for an efficient water-splitting process is the availability of highly efficient, inexpensive, and stable catalysts for H_2 and O_2 production. Despite much progress by synthetic and materials chemists,[2] it is still the natural enzymes that set the benchmark efficiency in the reduction and oxidation of water.[3] [FeFe] and [NiFe] hydrogenases convert protons and electrons into H_2 with remarkably high rates at a low overpotential.[4] Photocatalytic H_2 generation has previously been reported with hydrogenases in a heterogeneous scheme with the enzyme attached directly to semiconducting particles or electrodes.[5] Homogeneous systems with a dye and hydrogenase in solution are well established, but these multicomponent systems require a soluble redox mediator to transport the electron from the light absorber to the catalyst.[6]

An important requirement for water splitting is that the catalyst for H_2 evolution tolerates at least small levels of O_2, which will enter the system either through leakage of atmospheric O_2 into the photoreactor and/or from the in situ formation of O_2 during the water-splitting process.[7] Although many hydrogenases, in particular [FeFe] hydrogenase, are highly sensitive to O_2, much progress has recently been reported in identifying and understanding the factors leading to O_2 tolerance in [NiFe] hydrogenases.[8] In addition, a photocatalytic H_2 evolution system without a redox mediator is desirable, because a chemically reduced mediator is easily quenched by O_2, which can result in drastically reduced photoactivity for the system in the presence of O_2.

Herein we report on a photocatalytic H_2 evolution system consisting of a *Desulfomicrobium baculatum* (*Db*) [NiFeSe] hydrogenase and an organic dye, Eosin Y (EY), which evolves H_2 photocatalytically under high levels of O_2 (Figure 1). *Db* [NiFeSe] hydrogenase was selected as the H_2 evolution catalyst because it displays unique properties among hydrogenases.[9] This enzyme has been reported to be biased towards H_2 evolution, showing electrocatalytic H_2 production activity in the presence of as much as 1% O_2 and displaying little product inhibition.[10] This hydrogenase has previously been adsorbed on ruthenium-dye-sensitized TiO_2 nanoparticles for the photocatalytic generation of H_2, but the formation of reactive oxygen species (ROS) on TiO_2 during irradiation prevented its use in the presence of O_2.[5a,11] In this study, we removed the necessity for TiO_2 by replacing dye-sensitized TiO_2 with soluble EY, which allows for photo-induced direct electron transfer to a catalyst.[7,12] Elimination of radical-forming TiO_2 allowed us to produce H_2 photocatalytically in our hydrogenase-based system under remarkably high levels of O_2.

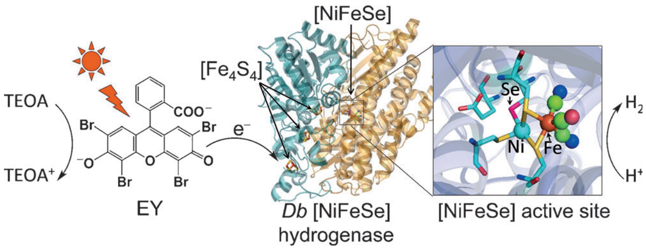

Figure 1. Schematic representation of photocatalytic H_2 generation with EY and an O_2-tolerant *Db* [NiFeSe] hydrogenase (EY–hydrogenase) system in the presence of TEOA in pH-neutral aqueous solution. Photo-induced electron transfer occurs directly from the EY to the [Fe_4S_4] cluster relay[3a,6c,8g] and then to the H_2-evolving [NiFeSe]-active site of the enzyme (see text).

First, the photocatalytic activity was studied of a homogeneous aqueous solution of *Db* [NiFeSe] hydrogenase and EY in the presence of the electron donor triethanolamine (TEOA) under an inert atmosphere. Optimized conditions were obtained by varying the amount of hydrogenase, EY, and TEOA and the pH of the solution (Figures S1–S3). The EY–hydrogenase system worked efficiently in the absence of any soluble redox mediator when a stirred solution of hydrogenase (10 pmol) and EY (disodium salt, 1 μmol) in TEOA (2.25 mL, 150 mM) at pH 7 and 25 °C was exposed to visible light (solar light simulator; AM 1.5 G, 100 mW cm^{-2}, $\lambda > 420$ nm). The photoreactor was purged prior to the experiment with N_2 containing 2% CH_4 (internal standard for gas chromatography (GC) measurements, see the Supporting Information).

The EY–hydrogenase system photogenerated (0.50 ± 0.03) μmol of H_2 per hour and almost linear H_2 evolution rates up to 15 hours (Figure S4). This result corresponds to

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a hydrogenase-based turnover frequency ($TOF_{hydrogenase}$) of (13.9 ± 0.7) (mol H_2)(mol hydrogenase)$^{-1}$ s^{-1}. The photoactivity of the system is lost after 24 h, whereupon (5.0 ± 0.3) μmol of H_2 had accumulated, corresponding to a $TON_{hydrogenase}$ of $(5.0 \pm 0.3) \times 10^5$ and an EY-based TON (TON_{EY}) of (5.0 ± 0.3) (mol H_2)(mol EY)$^{-1}$. No H_2 was detected in the dark or in the absence of EY or hydrogenase. At 5 °C and 45 °C $TOF_{hydrogenase}$ values of (7.5 ± 0.5) s^{-1} and (19 ± 2) s^{-1} were obtained, respectively. The system exhibits an optimum activity at pH 7.0 and its performance is decreased by more than 50% at pH 6.0 and 8.0 (Figure S2). This can be explained by the lower intrinsic activity of the hydrogenase under basic conditions[10] and an increased amount of protonated TEOA donor under an acidic environment.[5a,12a] The EY–hydrogenase system operates with high photoactivity in the absence of any soluble redox mediator and electrons are transfered directly from the dye to the hydrogenase. The per-active-site performance of the EY–hydrogenase system is on the same order of magnitude as that of a previously reported system with *Db* [NiFeSe] hydrogenase on ruthenium-dye-sensitized TiO_2 ($TOF_{hydrogenase}$ up to 50 s^{-1})[5a] and much higher than a photocatalytic system with EY and a synthetic Co catalyst ($TOF_{Co} = 0.02$ s^{-1})[7] in an aqueous pH-neutral TEOA solution.

Variation of the light intensity of monochromatic LED light (525 nm; pH 7.0 and 25 °C) from 1.5 to 5 and finally 18 mW cm^{-2} resulted in external quantum efficiencies (EQE) of (1.50 ± 0.08), (0.49 ± 0.03), and (0.18 ± 0.01)% with a corresponding $TOF_{hydrogenase}$ of (16 ± 1), (18 ± 1), and (24 ± 1) (mol H_2)(mol hydrogenase)$^{-1}$ s^{-1}, respectively. The EQE increases with decreasing light intensity, whereas the $TOF_{hydrogenase}$ changes only marginally. The $TOF_{hydrogenase}$ also remained almost constant when the light intensity of visible light was increased from 50 to 100 mW cm^{-2} (Figure S5). At 100 mW cm^{-2} visible-light irradiation, an increasing amount of EY from 1 to 3 μmol did not result in the photogeneration of higher amounts of H_2. Furthermore, when the amount of hydrogenase was increased from 10 to 50 pmol the amount of H_2 photogenerated in the system more than doubled (Table S1, Figures S1 and S3). These experiments demonstrate that the hydrogenase limits the EY–hydrogenase system, which is an important requirement for studying the effect of inhibitors on the enzyme in the photocatalytic system.

Subsequently, the photocatalytic H_2 production activity of the EY–hydrogenase system was investigated in the presence of varying concentrations of O_2. Previously, protein film electrochemistry with *Db* [NiFeSe] hydrogenase on a pyrolytic graphite edge electrode demonstrated that this enzyme evolves H_2 in the presence of 1% O_2 at an applied potential of −0.45 V versus the normal hydrogen electrode (NHE) in an aqueous electrolyte solution at pH 6.0.[10] Thus, H_2 evolution under O_2 should be possible if the photoexcited EY dye can efficiently transfer electrons directly to the hydrogenase.

To test this hypothesis, the EY–hydrogenase system (10 pmol of hydrogenase and 1 μmol of EY in 2.25 mL of aqueous 150 mM TEOA solution at pH 7 and 25 °C) was irradiated for one hour under N_2 atmosphere (with 2% CH_4) to verify its activity under inert atmosphere. The photoreactor was purged with 2% CH_4/N_2 and different amounts of O_2 were injected into the headspace after 1 hour with subsequent irradiation (Table 1 and Figure 2). In all cases, the photoactivity of the EY–hydrogenase system decreased with an increasing O_2 concentration in the headspace. Remarkably, in the presence of 21% O_2 some photoactivity still remained ((11 ± 3)% of the photoactivity under anaerobic conditions; Figure 2, insert).

Table 1: Visible-light-driven (100 mW cm^{-2}, AM 1.5 G, $\lambda > 420$ nm) H_2 production with EY–hydrogenase in an aqueous TEOA solution (2.25 mL, 150 mM) at pH 7 and 25 °C.

Conditions	$TOF_{hydrogenase}$[a] [s^{-1}]	H_2[a] [μmol H_2 h^{-1}]
EY (1 μmol) and hydrogenase (10 pmol)		
0% O_2	13.9 ± 0.7	0.50 ± 0.03
5% O_2	11.5 ± 1.0	0.41 ± 0.04
10% O_2	7.3 ± 0.4	0.26 ± 0.01
15% O_2	4.5 ± 0.5	0.16 ± 0.02
21% O_2	1.5 ± 0.3	0.05 ± 0.01
2% CO	< 0.4	< 0.02
$[Ru(bipy)_3]^{2+}$ (1 μmol), hydrogenase (10 pmol), and MV (1 μmol)		
0% O_2	27 ± 2	0.98 ± 0.08
5% O_2	3.3 ± 0.5	0.12 ± 0.02

[a] Calculated based on the amount of H_2 produced in the first 0.5 h of irradiation; standard deviation for at least three experiments.

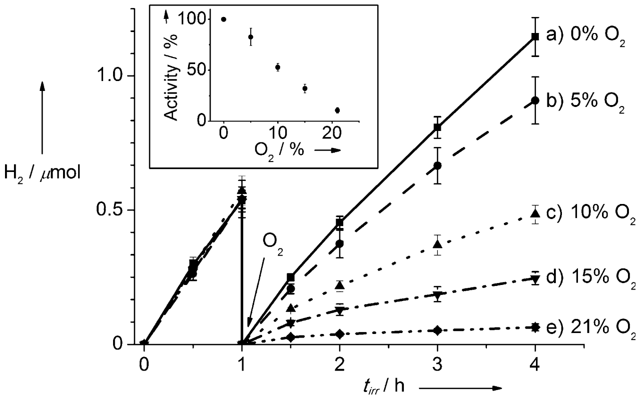

Figure 2. Amount of H_2 generated with the EY–hydrogenase system in an aqueous TEOA solution (150 mM, pH 7.0) during visible-light irradiation (t_{irr}, 100 mW cm^{-2}, AM 1.5 G, $\lambda > 420$ nm) at 25 °C. The EY–hydrogenase system was exposed to different O_2 headspace concentrations after 1 h of irradiation under 2% CH_4/N_2. Insert: Relative photocatalytic H_2 evolution activity compared to that under anaerobic conditions (based on photoactivity within the first 0.5 h of irradiation).

The concentration of O_2 was also measured in the solution for experiments with various concentrations of O_2 in the headspace (Table S2). Irradiation of the EY–hydrogenase system under an atmosphere of 21% O_2 resulted in decrease of the concentration of dissolved O_2 from (6.0 ± 1.0) ppm to below 0.2 ppm within 1 min of irradiation, thereby creating conditions conducive for the O_2-tolerant hydrogenase. The same behavior was observed in the absence of hydrogenase and can be ascribed to the known reaction of photo-excited

EY with O_2, resulting in the formation of singlet O_2.[13] EY was used in excess in the system and the quenching of photoexcited EY with O_2 explains the rapid decrease in the amount of dissolved O_2 and the slow depletion of headspace O_2 after several hours (Figure S6). Irradiation of the solution under a high concentration of O_2 in the headspace presumably also resulted in the formation of increased amounts of singlet O_2, causing the decreased lifetime of the photo-H_2 evolution system (Figure 2).

The EY–hydrogenase system is fully photoactive after three hours under anaerobic conditions, but it is inactivated after three hours of irradiation under a 21% O_2 atmosphere. This endurance is remarkable when one considers the complete photo-decomposition of the same hydrogenase under air on dye-sensitized TiO_2 within 2 min, which is presumably due to the decomposition of the enzyme by ROS formed upon reduction of O_2 by conduction band electrons.[5a,11] Notable differences between the dye–TiO_2–hydrogenase and EY–hydrogenase systems are the generation of radical species in close proximity to the hydrogenase in the former case, whereas singlet O_2 is mainly produced remote from the enzyme in the latter system.

In related work, a photocatalytic H_2 evolution system consisting of a ruthenium dye covalently linked to [NiFe] hydrogenase from *Thiocapsa roseopersicina* in the presence of the soluble redox mediator methyl viologen (MV) was exposed to O_2 in a closed photoreactor.[6b] Initial irradiation did not show formation of H_2, but resulted in the depletion of O_2 in the system, whereupon an anoxic environment in the system allowed for reactivation of the enzyme and formation of H_2.[6b]

Therefore, we also tested a homogeneous system comprising $[Ru(2,2'\text{-bipyridine})_3]Cl_2$ (1 μmol), [NiFeSe] hydrogenase (10 pmol), and MV (1 μmol) for comparison with the EY–hydrogenase system. The multicomponent Ru–MV–hydrogenase system is only photo-active in the presence of MV because electron transfer does not occur from photoexcited Ru directly to the hydrogenase.[5a,6a,b] Under anaerobic conditions, the Ru–MV–hydrogenase system evolves $(0.98 \pm 0.08)\ \mu mol\ H_2\ h^{-1}$ with a $TOF_{hydrogenase}$ of (27 ± 2) mol H_2 (mol hydrogenase)$^{-1}$ s^{-1} during visible-light irradiation. In the presence of 5% headspace O_2, the photoactivity decreased dramatically to $(3.3 \pm 0.5)\ s^{-1}$ and $(0.12 \pm 0.02)\ \mu mol\ H_2\ h^{-1}$ (Table 1) and no significant amounts of H_2 were observed under 21% O_2. Photoexcited $[Ru(2,2'\text{-bipyridine})_3]^{2+}$ and reduced MV react with O_2,[14] resulting in almost complete inactivation of the system.

In order to investigate the reversibility of the inhibitory effect of O_2 in the EY–hydrogenase system, reactivation of hydrogenase under inert conditions after exposure to atmospheric O_2 (21%) was also examined. The EY–hydrogenase system was exposed to aerobic conditions for different periods of time at continuous white-light irradiation. After repurging the photoreactor with 2% CH_4/N_2, we measured the photocatalytic H_2 production. The resulting TOF dropped to (81 ± 4), (65 ± 6), and (31 ± 3)% of the initial value after 30, 60, and 120 min of continuous exposure to air and light, respectively (Table S3 and Figure S7). Control experiments with EY–hydrogenase exposed to air in the dark for the same duration has led to a negligible inactivation of the hydrogenase (Table S3 and Figure S7).

Thus, the hydrogenase displays a good robustness in the presence of air and can be partially reactivated under inert conditions following light exposure with EY. Exposure of [NiFeSe] hydrogenases to O_2 results in the oxidation of sulfur and/or selenium in cysteine and selenocysteine ligands at the active site.[9b–d] These inactive states can be reactivated through reduction and recovery of the amino acids. Exposing the EY–hydrogenase system to light results in photoexcitation of EY and formation of a long-lived triplet state.[12] Low-potential electrons are transferred to the hydrogenase, thereby reactivating O_2-inactive states in the hydrogenase.[10] This fast reactivation of any O_2-inactivated hydrogenase can be explained by the negative excited state reduction potential of EY ($^*EY = -0.91$ V vs. NHE).[12a]

The effect of carbon monoxide, a well-known inhibitor of hydrogenases,[10] on the photocatalytic formation of H_2 was also tested with EY–hydrogenase. Introduction of 2% CO in N_2 resulted in complete inactivation of the EY–hydrogenase system (Figure S8) and only a negligible amount of H_2 was detectable by GC. Inhibition by CO was at least partially reversible and purging the inactive system with N_2 containing 2% CH_4 resulted in (52 ± 3)% of the initial $TOF_{hydrogenase}$ activity. The reversibility of CO inhibition is in agreement with the previously reported electrochemical studies.[10]

In conclusion, a photocatalytic H_2 evolution system with an O_2-tolerant *Db* [NiFeSe] hydrogenase and EY was assembled, which contains solely earth-abundant materials and maintains photoactivity under remarkably high levels of headspace O_2. H_2 evolution is driven efficiently by photo-induced direct electron transfer from EY to hydrogenase, making a soluble redox mediator unnecessary and thereby allowing for remarkable photostability under high O_2 levels. This work demonstrates an unprecedented robustness of a hydrogenase towards O_2 and paves the way to the exploitation of hydrogenases in full water splitting.

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