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Solar water splitting Pt-nanoparticle photosystem I thylakoid systems: Catalyst identification, location and oligomeric structure

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Abstract

Photosynthetic conversion of light energy into chemical energy occurs in sheet-like membrane-bound compartments called thylakoids and is mediated by large integral membrane protein-pigment complexes called reaction centers (RCs). Oxygenic photosynthesis of higher plants, cyanobacteria and algae requires the symbiotic linking of two RCs, photosystem II (PSII) and photosystem I (PSI), to split water and assimilate carbon dioxide. Worldwide there is a large research investment in developing RC-based hybrids that utilize the highly evolved solar energy conversion capabilities of RCs to power catalytic reactions for solar fuel generation. Of particular interest is the solar-powered production of H_2, a clean and renewable energy source that can replace carbon-based fossil fuels and help provide for ever-increasing global energy demands. Recently, we developed thylakoid membrane hybrids with abiotic catalysts and demonstrated that photosynthetic *Z*-scheme electron flow from the light-driven water oxidation at PSII can drive H_2 production from PSI. One of these hybrid systems was created by self-assembling Pt-nanoparticles (PtNPs) with the stromal subunits of PSI that extend beyond the membrane plane in both spinach and cyanobacterial thylakoids. Using PtNPs as site-specific probe molecules, we report the electron microscopic (EM) imaging of oligomeric structure, location and organization of PSI in thylakoid membranes and provide the first direct visualization of photosynthetic *Z*-scheme solar water-splitting biohybrids for clean H_2 production.

Introduction

Sunlight is an abundant and environmentally clean resource that, if harnessed for practical use, can address long-term global energy demand [1], [2]. One means to accomplish this is to store the sun's energy in the high-energy chemical bonds of molecules as a reduced fuel, such as hydrogen, ethanol and methane. Such “solar fuels” could provide an abundant supply of sustainable and transportable energy. Nature's photosynthetic mechanisms that convert light energy into chemical energy inspire many current solar fuel strategies. In photosynthesis, the energy from the sun is used to form oxygen from water and power carbon fixation. Similarly, artificial photosynthetic strategies employ synthetic chemical systems that use solar energy to power the transformation of abundant chemicals such as water and CO_2 into fuels [3], [4], [5], [6]. Other approaches introduce photocatalytic hydrogen production capabilities into photosynthetic protein frameworks, including the creation of photosynthetic hybrids with hydrogenase [7], [8], [9], [10], platinum nanoparticle catalysts [11], [12], [13], [14], [15], [16], [17], and synthetic molecular catalysts [18], [19]. These photosynthetic hybrids take advantage of the evolved photochemistry of the integral membrane Photosystem I (PSI) reaction center (RC) protein (Fig. 1).

In higher plants, green algae, and cyanobacteria, the Photosystem II (PSII) and PSI RCs tandemly work in a coupled electron transport *Z*-scheme: PSII carries out light-driven oxidation of water whereas PSI catalyzes transmembrane light-driven electron transfer across the thylakoid membrane from plastocyanin in the lumen to ferredoxin (Fd) or flavodoxin (Fld) in the stroma. Fd and Fld mediate the transfer of reducing equivalents from PSI to the enzyme ferredoxin $NADP^+$-reductase (FNR) to produce NADPH, the source of reducing equivalents for Calvin cycle CO_2 fixation and final chemical energy storage. Several features of PSI make it a powerful photochemical driver of H_2 production in hybrid systems. These include: a long-lived charge separated state of ~60 ms formed between the oxidized primary donor $P700^+$ and reduced terminal [4Fe-4S] cluster F_B^-; a quantum yield that approaches unity; and a favorable electrochemical potential of −580 mV (vs NHE) for the F_B cluster that provides sufficient driving force to reduce protons to H_2 at neutral pH (Fig. 1A). Most photocatalytic H_2 producing PSI-hybrids to date use purified PSI protein and require a sacrificial electron donor (SED) to reduce oxidized P700.

Recently, we removed the need for SED by building hybrid systems in thylakoid membranes and using the built-in *Z*-scheme electron transport chain to power H_2 production [21]. PSI has three stromal subunits that provide a basic patch for docking of the anionic acceptor proteins Fd and Fld (Fig. 1B). Pt nanoparticles (PtNPs) with a similar charge and size as the acceptor proteins to PSI can readily self-assemble with the acceptor end of isolated PSI from cyanobacteria to form stoichiometric (1 PtNP: 1 PSI monomer) complexes [14]. The resultant noncovalent, electrostatically associated PSI-PtNP hybrids photocatalytically produce H_2, confirming EPR spectroscopic results that definitively show PtNP mimics Fld docking to PSI [14]. This PSI-PtNP hybrid system remains one of the most effective photocatalytic Pt-based PSI systems to date [20]. The electrostatic PtNP binding strategy for isolated PSI translated to in situ hybrid assembly in spinach and cyanobacteria membranes [21]. PtNPs were found to readily self-assemble to the solvent accessible stromal end of PSI within photosynthetic membranes to create a complete water-splitting system [21]. In addition, first-row transition metal molecular catalysts were found to perform photocatalysis when bound in situ to cyanobacterial thylakoid membranes, creating a more sustainable system [21]. In these systems, electrons originating from PSII photoexcitation are coupled to reduction of $P700^+$, removing the need for sacrificial redox reagents. Light-driven water oxidation at PSII drives H_2 production at PSI catalyst sites via the *Z*-scheme electron transport chain provided by the membrane environment (Fig. 2).

Photocatalysis in these hybrids provides evidence that PtNPs act as a site-specific probe of PSI in membranes and a mimic of acceptor protein binding. Additional evidence is provided by EPR spectroscopy of light-induced electron transfer in thylakoid-PtNP hybrids [21]. EPR results showed that PtNP blocks docking of Fld to the stromal end of PSI or, if both PtNP and Fld bind to PSI, electron transfer to PtNP is preferential to Fld, consistent with PtNP mimicking acceptor protein binding [21]. Thus, further investigation of PtNP interactions in thylakoids will not only inform future solar fuel hybrid development but also provide a unique opportunity to examine both PSI location and PSI-acceptor protein interactions in membrane environments. Herein, we report the first visualization of PSI-PtNP complexes in photosynthetic thylakoid hybrid complete water-splitting systems using electron microscopy (EM). Hyperspectral elemental imaging of Mg, S, Fe, and Pt facilitates co-localization and aids in the identification of Pt sites at intrinsic membrane PSI complexes. These imaging studies provide a structural framework for the functional studies of solar water splitting in biohybrid thylakoid systems and offer key insight for optimizing thylakoid solar fuel production systems for sustainable H_2 production.

Section snippets

Thylakoid preparation

Spinach thylakoids were prepared in a dark laboratory with green light as previously described [21]. Starting material was organic baby spinach (16 oz, store bought). The final thylakoid pellet was resuspended in 20 mM MES pH 6.0, 15 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 1 mg ml^{-1} BSA, and 15% glycerol to a final concentration of 2.3 mg ml^{-1} Chl. Chl content was measured in 80% cold acetone [22].

Cyanobacterial thylakoids were prepared from *Synechococcus leopoliensis* (UTEX625) grown in AC medium at…

Pt-nanoparticles

STEM HAADF imaging measurements of the thylakoid-PtNP hybrids revealed that PtNP shapes were variable ranging from slightly elliptical to spheroidal and occasionally faceted spheroids. The PtNPs were confirmed to be crystalline by the observation of diffraction contrast during sample tilting as well as by high resolution electron imaging by the visualization of the Pt crystalline lattice as illustrated in Fig. 3A.

NP elemental composition was confirmed as Pt by X-ray Energy Dispersive…

Discussion

In 1985 photocatalytic H_2 evolution from platinized chloroplasts was first reported [11]. This system involved the photochemical precipitation of metallic Pt from hexachloroplatinate salts onto the acceptor end of PSI [32], [33] and recently has been optimized for extreme durability [13]. This photoprecipitated Pt system has been imaged with scanning electron microscopy (SEM) and scanning tunneling microscopy (STM) [34], [35]. Another important biohybrid system tethered PtNPs to PSI with a…

Conclusion

Photosynthetic *Z*-scheme solar water-splitting biohybrids provide an opportunity to sustainably generate hydrogen, a renewable alternative fuel to fossil fuels. AEM/HAADF-STEM results presented here provide the first direct structural information of electrostatically-associated thylakoid-PtNP hybrids for clean H_2. These studies reveal important details about PtNP structure, size and crystallinity, co-localization of PtNP at PSI sites in photosynthetic thylakoids, and the relative inter-PtNP…

CRediT authorship contribution statement

D. M. T and L. M. U. conceived the project. L. M. U. prepared the thylakoids and hybrids. T.M. and N. S. P. interpreted the data. N. J. Z. performed the EM imaging and analysis. T. M. conducted the negative staining experiments. L. M. U., N. J. Z. and T. M. wrote the manuscript. All authors discussed the results and contributed to manuscript editing.…

Statement of informed consent, human/animal rights

No conflicts, informed consent, human or animal rights are applicable.…

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.…

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