Isolation and Deposition of Photosystem II onto Gold Electrodes

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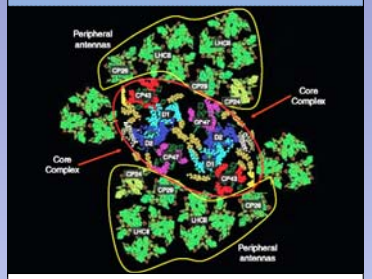
ABSTRACT

Photosystem II (PSII) is a protein found in the thylakoid membranes of phototroph's chloroplasts. It has the ability to collect energy from light and split water molecules to produce protons, electrons, and oxygen gas. To harness this unique capability, a procedure was developed to isolate PSII from spinach chloroplast membranes. This was done without the use of an ion exchange column which makes isolation procedures lengthy. The isolated protein was characterized and deposited on a self-assembled monolayer (SAM) of 6-mercaptohexanol attached to a gold coated silicon wafer. This formed an immobilized film of functional protein for use in future applications.

STRUCTURE of PSII



BBY includes the whole complex shown and cytochrome b559. The core complex is in red.

Lynn Yarris. "Berkeley Researchers Identify Photosynthetic Dimmer Switch". Research News Berkeley Lab. 2008. web. 7/29/11

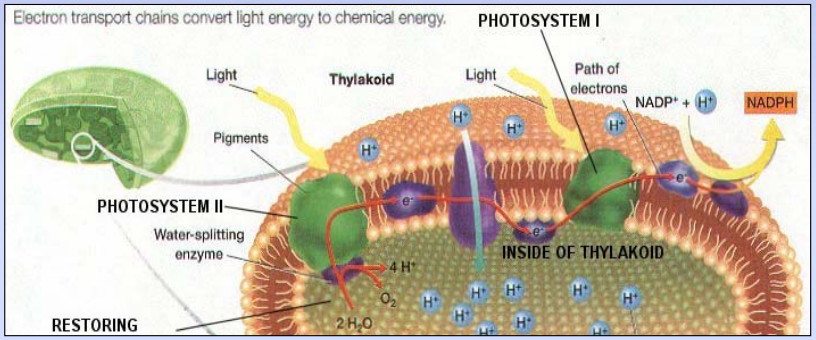
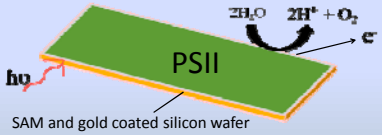


Image adapted from www.LPS.org

APPLICATIONS

PSII films have been used as biosensors and for energy conversion.



SAM and gold coated silicon wafer

ISOLATION PROCEDURE

PHASE 1

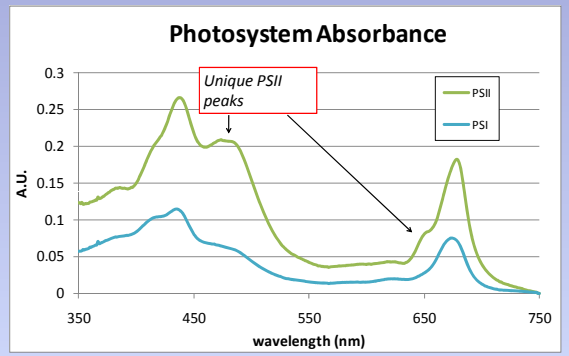
- Grind spinach and filter remnants
- Centrifuge at 4,000 g to remove broken chloroplasts
- Centrifuge at 40,000 g for 30 min
- Add Triton X 5 mg/ mg chl
- Centrifuge at 40,000 g for 30 min
- Yield: ~240 ml BBY, [chl]: 0.124 mg/ ml

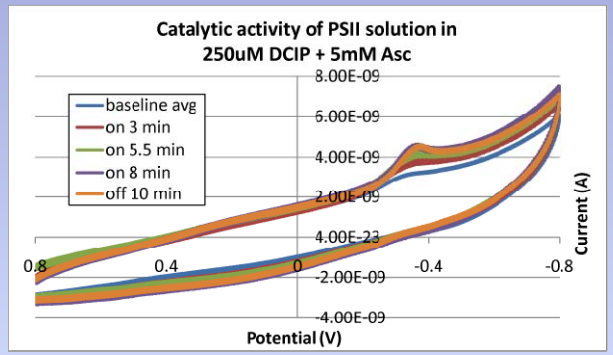
PHASE 2

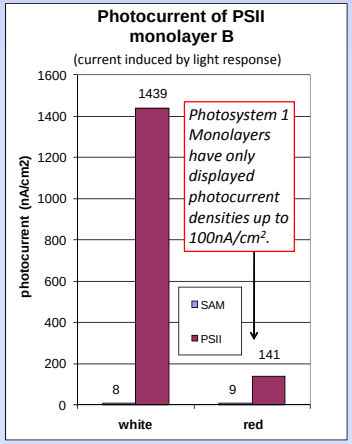
- Centrifuge BBY's for 10 min at 48400 g
- Incubate on a shaker rack for 1 hr
- Centrifuge at 48400 g for 10 min
- Change buffer solutions and centrifuge at 150,000 g for 1 hr to precipitate LHCII
- Repeat previous step
- Load onto sucrose gradient with 25 mM n-dodecyl-beta- maltoside
- Centrifuge for 3.5 hrs at 90,000 g
- Yield: 100-200 µl per sucrose gradient, almost 1 ml of PSII core complex

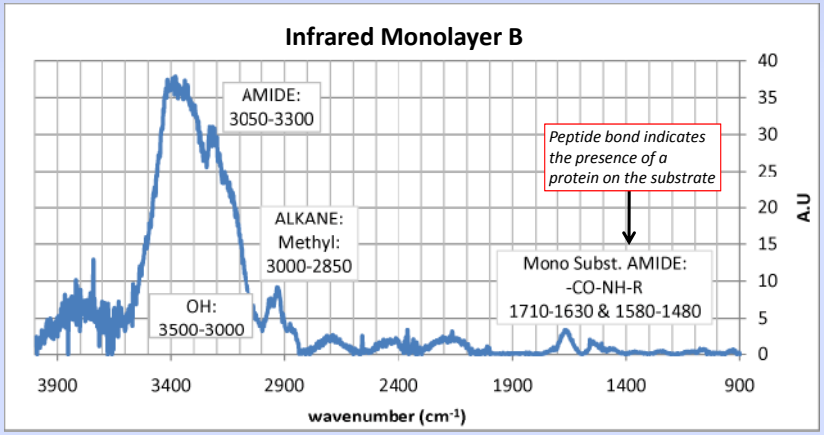
CHARACTERIZATION RESULTS

The activity of PSII solution to catalyze oxygen evolution was measured using a platinum ultramicroelectrode with a Ag/AgCl reference in an airtight electrochemical cell. Different mediators were examined to optimize oxygen gas production. Finally, the isolated PSII proteins were immobilized on planar gold electrodes using SAM techniques. One was made via vacuum deposition (A) and the other with 48hr incubation (B) . The films were analyzed with ellipsometry, IR spectroscopy, and photochronoamperometry.

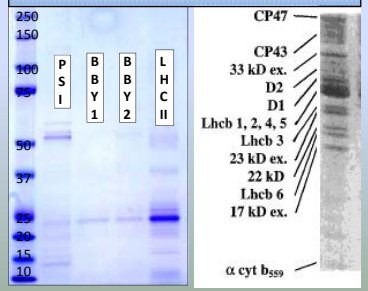








SDS PAGE CHARACTERIZATION



Hankamer et al. 1997. Eur. J. Biochem. 243, 422-429

CONCLUSIONS

Photosystem II was isolated quickly, conveniently, and deposited on a SAM coated gold surface. The photocurrent density of film B was 132 nA /cm². This value is significantly higher than any monolayer of PSI deposited on a planar gold electrode. Further research in the development of PSII films will enable the development of PSII bio-hybrid devices which utilize water as a preliminary electron source.

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