Isolation and Deposition of Photosystem II onto Gold Electrodes

Danna Sharp¹, Gabriel LeBlanc², Gongping Chen², David Cliffel²

¹University of Tennessee Knoxville, ²Vanderbilt University

ABSTRACT

Photosystem II (PSII) is a protein found in the thylakoid membranes of photophosphorylation. 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-mercaptopentanol attached to a gold coated silicon wafer. This formed an immobilized film of functional protein for use in future applications.

APPLICATIONS

Photosystem II (PSII) films have been used as biosensors and for energy conversion.

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 to 5 mg/ml 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 48000 g
- Incubate on a shaker rack for 1 hr
- Centrifuge at 48000 g for 10 min
- Change buffer solutions and centrifuge at 150,000 g for 1 hr to precipitate LHII
- 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 air-lit 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 photocronoamperometry.

APPLICATIONS

的独特功能，使PSII具有在未来的应用中使用该蛋白质的能力。

APPLICATIONS

PSII薄膜已被用作生物传感器和能量转换。

PHASE 1

- 研磨菠菜并过滤残留物
- 在4,000 g离心去除破碎的叶绿体
- 在40,000 g离心30 min
- 加入5 mg/ml Triton X-5到叶绿素中
- 在40,000 g离心30 min
- 收获：~240 ml BBY (chl): 0.124 mg/ml

PHASE 2

- 在48,000 g离心BBY 10 min
- 在1 hr摇拌机中孵育
- 在48,000 g离心10 min
- 改变缓冲溶液并离心150,000 g 1 hr以沉淀LHII
- 重复上一步
- 每25 mM n-十二烷基-β-麦芽糖醇
- 在3.5 hrs以90,000 g离心
- 收获：100-200 µl每 sucrose梯度，几乎1 ml的PSII核心复合物

CHARACTERIZATION RESULTS

PSII溶液的活性已被用于催化氧气的产生，使用铂超微电极和Ag/AgCl参考在空气照明的电化学细胞中。不同的媒介被检查以优化氧气气体的产生。最后，通过真空沉积（A）和48hr浸渍（B）将PSII蛋白质在金电极上用SAM技术进行固定。这些薄膜被用于分析椭圆度、IR光谱和光光电位计。