2-2003

Oxygen-Evolving Reaction Center Core Preparation
(Modified from Ghanotakis et. al., 1987)

[chl] assay first.

1. Thaw out frozen OEP on ice, spin 35,000 x g for 30 min in the SS-34 head. Resuspend pellet to 2.5 mg/ml chl in SMN (or use fresh OEP finally suspended in SMN to 2.5 mg/ml chl). Measure volume. Also thaw out the stock 10% β-D-dodecyl maltoside (DM).

2. Measure an equal volume of Solution B - OGP. Add solid octyl glucopyranoside (OGP) to 2.06% (w/v), dissolve on rocker. Cool Solution B + OGP and add to OEP suspension. Incubate 10 min on ice. (in large flask to accomodate increasing volume). 2.06 g/100 ml ~70mM

3. To the total volume of solubilized membranes add two volumes of Solution C. Incubate 5 min, spin at 35,000 x g for 90 min in the SS-34 head. This will precipitate the LHC from the solution. Collect the supernatant, this contains the reaction center complex. 20ml x

4. To the total volume of crude reaction center complex, add two volumes of Solution D and incubate for 5 min. Spin at 25,500 x g for 60 min in the GSA rotor. Carefully remove the bottles and CAREFULLY decant, and discard, the supernatant. The reaction center complex is found in the pellet. Remove as much supernant as possible with a Pasture pipette. Resuspend the pellets in a small amount of Solution E (1-2 ml). Measure chl and bring to 1.0 mg/ml chl. Measure the volume using an ice cold 10 ml graduated cylinder. stopper for

5. Transfer to a cold SS-34 centrifuge tube and add DM from a 10% stock solution to a final concentration of 0.5%. Incubate 60 min. Spin for 5 min at 35,000 x g in the SS-34 rotor. Carefully remove the supernatant and load onto a Sepharose 6B column previously equilibrated with 10 mM Mes-NaOH, pH 6.0, 5 mM NaCl, 5 mM $CaCl_2$, 0.025% DM. Elute column at 1.5 ml/min.

6. As the green band approaches the bottom of the column, connect the fraction collector and collect 3.33 min fractions (5 ml). After the green band has eluted measure the absorbance of the fractions at 674 nm after diluting 100 µl of fraction with 1 ml of elution buffer. Plot data as absorbance vs. volume. Discard the leading shoulder and pool the main band.

7. Fully concentrate in centricon 100. This is the Reaction Center Core Complex.

$\frac{2.06}{100} \cdot 3 \quad \frac{6}{100}$

$\frac{2}{100} \cdot 3 \quad \frac{6}{100}$

The Liu Laboratory protocol — PSII-core Spinach
Arts & Sci Washington University in St. Louis

Solutions

1. SMN

2. Solution B - OGP
1 M sucrose
50 mM Mes-NaOH, pH 6.0
0.8 M NaCl
10 mM $CaCl_2$

3. Solution C
1 M sucrose
50 mM Mes-NaOH, pH 6.0
0.4 M NaCl
5 mM $CaCl_2$

4. Solution D
50 mM Mes-NaOH, pH 6.0
10 mM NaCl
5 mM $CaCl_2$

5. Solution E
0.4 M sucrose
50 mM Mes-NaOH, pH 6.0
15 mM NaCl
5 mM $CaCl_2$

6. Column Elution Buffer
10 mM Mes-NaOH, pH 6.0
5 mM NaCl
5 mM $CaCl_2$
0.025% DM

SMN 400 mM Sucrose
50mM MES NaOH 6.0
15mM NaCl
0.5 · 0.1 = 0.5

Solution # Vol needed
#2 100 ml
#3 200 ml
#4 600 ml
#5 50 ml

0.1 x 1 x 342. = 34.2

5 · x = 0.8 · 0.1
= 0.08 / 5 =

1 · x = 0.1 · 0.01
x = 0.01

0.2 · 1 0.2

0.4 · 0.2 = 5 · x
0.08 / 5

0.6 · 0.01 = 5 · x
0.006 / 5

0.6 · 0.005 = 1 x
0.0030

0.05 · 0.4 0.02

0.005 · 0.05 = 1 · x
0.00025

0.015 · 0.05 = 5 · x
1.01

0.00015

Solution B:
100ml
Sucrose 34.2 g
0.5 MES 6.0 10 ml
5 M NaCl 16 ml
1 M $CaCl_2$ 1ml

Solution C:
200ml
Sucrose 68.4 g
0.5 MES 6.0 20 ml
5 M NaCl 16ml
1M $CaCl_2$ 1ml

Solution D:
600 ml
0.5 M MES 60 ml
5 M NaCl 1.2 ml
1 M $CaCl_2$ 3 ml

Solution E:
50ml
Sucrose : 6.8 g
0.5 M MES. 5 ml
5 M NaCl 150 µl
1 M $CaCl_2$ 25 µl