

Chapter 2

Isolation of Photosystem I Submembrane Fractions

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Abstract

In this chapter, we describe a method to prepare photosystem I (PSI) submembrane fractions derived from the chloroplast stroma lamellae of spinach chloroplasts. These preparations retain the cytochrome b_6/f complex and a pool of about 11 plastoquinones per P700. The PSI submembrane fractions are thus able to perform both cyclic and linear electron transport reactions from various artificial electron donors to oxygen or methylviologen. They are useful to study both PSI and cytochrome b_6/f complex activities in a nearly native form without interference from photosystem II.

Key words: Photosystem I, Cytochrome b_6/f complex, Digitonin, Stroma lamellae

1. Introduction

In this chapter, a simple method is described to prepare photosystem I (PSI) submembrane fractions from spinach leaves. Stable and well coupled PSI-enriched vesicles, mainly derived from the chloroplast stroma lamellae, are obtained by mild digitonin treatment of spinach chloroplasts using the method first described by Peters et al. (1) with some modifications (2, 3). Optimal conditions for chloroplast solubilization were established at a digitonin/chlorophyll ratio of 1 (w/w) and a chlorophyll concentration of 0.2 mM, resulting in a little loss of native components (1). In particular, plastocyanin is easily released at a higher digitonin/chlorophyll ratio. The PSI submembrane fractions retain plastocyanin, the cytochrome b_6/f complex and a pool of about 11 plastoquinones per P700 (1). However, these membranes are devoid of PSII activity and proteins (3). They are able to sustain strong electron transport reactions from various artificial electron donors to oxygen or methylviologen (2, 4, 5) and to maintain cyclic electron transport with the photoinduced reduction and oxidation

of the cyt b_6/f complex (1, 5). The endogenous pool of plastoquinones that is retained also takes part in PSI photochemistry (6, 7). Hence, these preparations are useful not only to study PSI reactions in the absence of PSII, but should also be appropriate to study the function of the cyt b_6/f complex in a nearly native form.

2. Materials

1. Fresh spinach leaves from the local market.
2. Homogenization buffer A: 50 mM Tricine-KOH, pH 7.8, 10 mM KCl, 10 mM NaCl, 5 mM MgCl_2 , 400 mM sorbitol, and 1 mM phenylmethyl sulfonyl fluoride (PMSF) (see Notes 1–5).
3. Hypotonic buffer B: 20 mM Tricine-KOH, pH 7.8, and 10 mM MgCl_2 .
4. Isotonic buffer C: 20 mM Tricine-KOH, pH 7.8, 20 mM KCl, 20 mM NaCl, and 500 mM sorbitol (see Notes 1–3).
5. Incubation buffer D: 20 mM Tricine-KOH, pH 7.8, 10 mM KCl, 10 mM NaCl, 5 mM MgCl_2 , and 250 mM sorbitol (see Notes 1–3).
6. Resuspension buffer E: 20 mM Tricine-KOH, pH 7.8, 10 mM KCl, 10 mM NaCl, and 5 mM MgCl_2 .
7. Digitonin.
8. 80% (v/v) acetone in distilled water.
9. Miracloth (Calbiochem).
10. Wheaton tissue grinder (55 mL).
11. Waring blender with sharp blades.
12. Vortex mixer.
13. Bench-top centrifuge.

3. Methods

3.1. Photosystem I Submembrane Fractions Preparation

1. Weigh 200 g of deveined spinach leaves.
2. Clean the spinach leaves in cold distilled water and dry on absorbent paper.
3. Cut the leaves in small pieces and place them with 300 mL of homogenization buffer A in the Waring blender (see Note 6).
4. Add 1 mM PMSF.
5. Homogenize for about 1 min using the pulse mode.
6. Filter the slurry through two layers of Miracloth.
7. Centrifuge the filtrate 5 min at $3,500\times g$ at 4°C .

8. Resuspend the pellets in 50 mL of hypotonic buffer B using a paintbrush and homogenized with a 55 mL Wheaton tissue grinder.
9. Incubate the solution for 2–3 min on ice in the dark.
10. Add 50 mL of isotonic buffer C (see Note 7).
11. Centrifuge for 5 min at $3,500\times g$ at 4°C .
12. Resuspend the new pellets in the incubation buffer D to obtain a chlorophyll concentration of 2 mg/mL after the further addition of 0.2% digitonin (see Note 8) and [Subheading 3.2](#).
13. Incubate the solution for 30 min in the dark at 4°C with gentle stirring.
14. Add more incubation buffer D to obtain a threefold dilution.
15. Centrifuge for 30 min at $42,000\times g$ at 4°C .
16. Pool the supernatants and centrifuge for 1 h at $150,000\times g$ at 4°C .
17. Resuspend the final pellets (PSI preparation) in a small volume of the resuspension buffer E, and then dilute to a chlorophyll concentration of 2–3 mg/mL.
18. Chlorophyll is determined in 80% acetone as described in [Subheading 3.2](#).

3.2. Determination of Chlorophyll Concentration

1. Add 10 μL of photosynthetic membrane to 5 mL acetone 80% in a conical tube and mix carefully using a vortex mixer (see Note 9).
2. Centrifuge in the bench-top centrifuge for a few minutes to remove precipitated proteins.
3. Verify the exact volume (5 mL) and adjust if necessary to compensate for evaporation.
4. Measure the absorbance at 647 and 664 nm.
5. Taking the dilution of the membranes in the acetone solution into account, the chlorophyll concentration (mg/mL) in the membrane preparation is calculated from the following equation: $0.5 [17.76 (A_{647}) + 7.38 (A_{664})]$, in which A_{647} and A_{664} represent the absorbancies at the respective wavelengths (see Note 10).

4. Notes

1. This buffer should be prepared just before use.
2. Sorbitol is used to keep the medium isotonic; sugars are also known to help in the protection of biological membranes against denaturation.

3. Sorbitol can be prepared in advance as a concentrated solution (2 M) and kept at -20°C . It is diluted to the required concentration during the preparation of the buffer.
4. PMSF is used as an inhibitor of proteases. It should be prepared as a concentrated solution (1 M) in dioxane and diluted to the proper concentration.
5. The PMSF should be added directly in the blender just before grinding the leaves.
6. The chamber of the blender and all the solutions used must be ice cold when used. During the preparation, care must be applied to always keep the material in a cold (near 4°C) environment.
7. Add isotonic buffer at the same of the hypotonic buffer.
8. The membranes should be first resuspended in a small volume for chlorophyll determination. Then, the final volume required to obtain 2 mg chlorophyll/mL is calculated. The volume of incubation buffer D to be added to adjust the final concentration is used to prepare a digitonin solution containing the amount of detergent required to obtain 0.2% in the final volume. It may be necessary to heat this solution for about 5 min to improve the solubility of digitonin. Cool down the solution, and add it progressively to the membrane suspension with gentle stirring on ice.
9. The method of Arnon (8) was used for many years and is now used with the corrections of Porra et al. (9). The sample of photosynthetic membranes should be added to the acetone while mixing. This is necessary to minimize the amount of chlorophyll that remains bound to the precipitated proteins.
10. Several replicates should be done to obtain a better estimation of the chlorophyll concentration. If the concentration is above 6 mg/mL, it is better to prepare a first dilution just above the required final concentration and to determine the chlorophyll concentration in this predilution before final dilution.

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