

Affinity Purification of Photosystem I from *Chlamydomonas reinhardtii* using a Polyhistidine Tag

Jonathan A. Brain

Galina Gulis, Ph.D.¹

Kevin E. Redding, Ph.D.²

Associate Professor of Chemistry

Adjunct Associate Professor of Biological Sciences

Abstract

Traditional methods of Photosystem I (PSI) purification of Chlamydomonas reinhardtii require many hours of ultracentrifugation and lab work. Using a genetically inserted polyhistidine tag, PSI was isolated using affinity purification, a much quicker process. The purification eluate was analyzed for PSI using chlorophyll a over chlorophyll b ratios by UV-visible spectrophotometry.

Introduction

Isolation and purification of Photosystem I (PSI) has traditionally been done using sucrose-density gradients on solubilized thylakoid components (Hippler et al. 1997; Takahashi et al. 1991). This purification method can be very time-consuming, requiring many hours of ultracentrifugation, and sometimes may result in surprisingly low yields. Affinity purification, on the other hand, has experimentally been much faster, as shown with the genetically engineered photoreaction center of purple bacteria (Goldsmith et al. 1996).

¹ Ph.D. candidate in the Redding laboratory at the time of the research.

² Presently: Associate Professor of Biochemistry at Arizona State University, Kevin.Redding@asu.edu

Affinity purification exploits reversibly binding interactions of a specific protein to isolate it from others in a mixture. The separation process occurs by pouring the mixed solubilized proteins into a column with a solid phase matrix which binds specifically to the desired protein. The solid phase is then washed of the undesired proteins. The column, which now has only the desired protein, is rinsed with an elution buffer. This buffer binds to the solid phase much more strongly than the protein does, thus removing the protein from the solid phase matrix and putting it back into solution (Kaplan et al. 1974).

PS1 does not naturally have a feasible selective interaction over other protein complexes in the thylakoid membranes. We resolved this problem using a genetically inserted polyhistidine-tag. This sequence of amino acids, also known as a His₆-tag, binds reversibly to Ni²⁺. This approach had been successfully employed in purification of *Chlamydomonas reinhardtii* PS2 (Sugiura et al. 1998).

Using a *C. reinhardtii* mutant with a His₆-tag on PS1 and Ni-NTA as the solid phase matrix, solubilized thylakoid components were separated and PS1 isolated. Thylakoid membranes and purified PS1 were analyzed using UV-visible spectroscopy.

Materials and Methods

Materials

Ni-NTA agarose was obtained from Invitrogen. The detergent β -D-dodecylmaltoside (β -DM) was obtained from Dojindo Laboratories. All chemicals were reagent grade. For the affinity purification the solubilization buffer was 25 mM HEPES-KOH (pH 8.0), 100 mM NaCl, 5 mM MgSO₄, and 10% glycerol. The column preparation buffer was solubilization buffer plus 0.03% β -DM. The wash buffer was column buffer plus 2 mM imidazole. The elution buffer was column buffer plus 200 mM imidazole and 40 mM MES-NaOH (pH 6.0) instead of 25 mM HEPES-KOH (pH 8.0).

Spectrophotometry

Extractions were made before and after affinity purification using 80% acetone. These were analyzed using UV-visible spectrometry. Determination of chlorophyll *a* and *b* concentrations were made according to Porra et al. (1989) using the recommended absorbances at 663.6 nm and 646.6 nm with a background at 750 nm. Spectra for both extractions were taken to qualitatively ensure that the three calculated absorbances were due to chlorophyll.

Thylakoid Preparation

Chlamydomonas reinhardtii mutant JVD1-1B[pGG1-46] with His₆-tag was obtained from prior research conducted by Gulis et al. (2008). The strain was grown in well-aerated liquid TAP (Tris-acetate-phosphate) at room temperature (Harris 1989). Cells were harvested at about 9×10^6 cells per ml. Thylakoid membranes were prepared using protocol from Fischer et al. (1997). These membranes were then diluted to 0.8 mg_{Chl} per ml with Millipore water and solubilized with 1/10 by volume of 10% β -DM. After being homogenized the solution was then spun at 24,000g for 15 min under refrigeration using a 70Ti rotor. The supernatant was loaded onto a sucrose gradient of layered 2 ml of 2 M sucrose, 10 ml of 0.9 M, and 10 ml of 0.5 M, each having 5mM Tricine-KOH (pH 8.0) and 0.05% β -DM. The gradient was spun at 150,000g for 12 hours in a SW-55 rotor under refrigeration. The three green bands at the interface of the gradients were collected. These were then diluted with 5mM Tricine-NaOH (pH 8.0), homogenized, and spun with a SW-55 rotor for 3 hours at 250,000g while under refrigeration. Membranes were quickly frozen in liquid N₂ and stored at -70°C. All procedures were done at 4°C.

Affinity Purification of His₆-tagged PS1

The Ni-NTA column (12 cm x 1.5 cm diameter) was pre-equilibrated with column buffer. Membranes from the thylakoid preparation were re-suspended to .8 mg_{Chl} per ml using solubilization buffer and were put into solution by adding 1/10 by volume of 10% β -DM and gentle mixing at 4°C and in the dark. The solution was then spun at 13,000g for 15 min under refrigeration. The supernatant was then loaded into the column. The column saturates after 8 ml of supernatant; therefore, each 8 ml had to be purified separately. The column was then washed with 130 ml of wash buffer which was dripped through the column to insure that the Ni-NTA was not flushed. The PS1 was then eluted using elution buffer and the fractions were collected. The fractions were dialyzed 3 times using 2 L of solubilization buffer in order to minimize the imidazole in the fractions. The final fractions were then concentrated by 3 hours of centrifugation at 250,000g in a SW-55 rotor. All procedures were done at 4°C and in the dark.

Results

The spectra for each extraction show clearly that the two calculated absorbance points are due to chlorophyll (Fig. 1; Fig. 2). UV-visible spectral analysis of extractions before and after affinity purification shows a large increase in the chlorophyll *a* over chlorophyll *b* ratio. Since PS1 has mostly chlorophyll *a* as opposed to the other light-harvesting complexes found in the thylakoids, a high chlorophyll *a* over chlorophyll *b* ratio is due to higher purity of PS1. As can be seen in Table 1, this ratio approaches 4.5, the value consistent with 100% purity of complexed PS1 (Kargul et al. 2003).

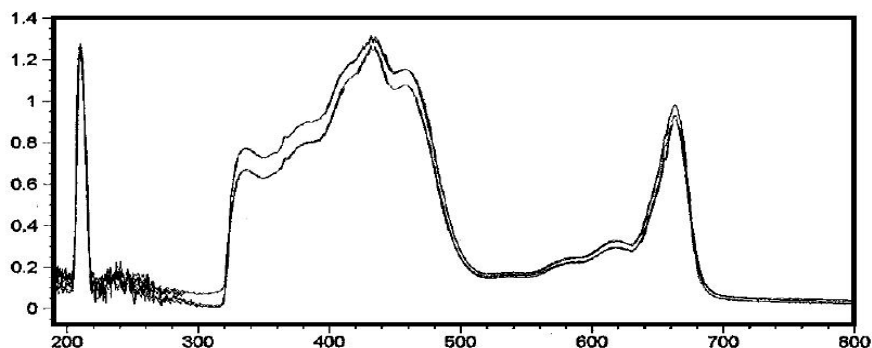


Fig. 1 UV-visible spectrum of thylakoid components before affinity purification. The two spectra are for two different samples.

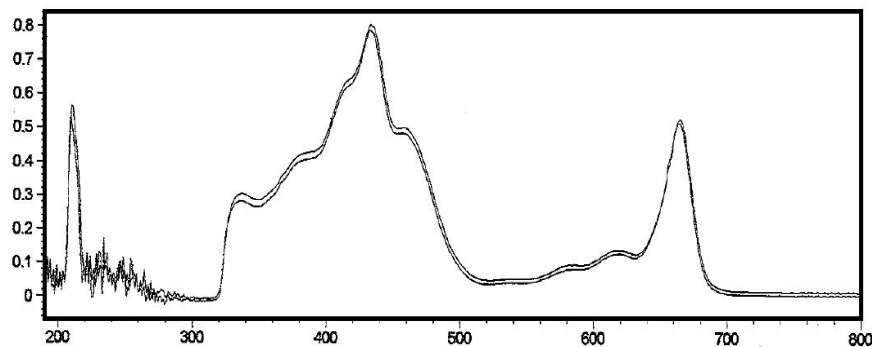


Fig. 2 UV-visible spectrum of affinity purified thylakoid components. The two spectra are for two different samples.

	Sample	Net Absorbances		<i>a/b</i> ratio
		663.6 nm	646.6 nm	
Thylakoid	1	0.974	0.516	1.90
Membranes	2	0.846	0.434	2.02
After Affinity	1	0.512	0.212	3.26
Purification	2	0.513	0.213	3.24

Table 1 Absorbance analysis of chlorophyll ratios before and after affinity purification

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References

- Fischer N, Setif P, Rochaix JD (1997) Targeted mutations in the PsaC gene of *Chlamydomonas reinhardtii*: preferential reduction of FB at low temperature is not accompanied by altered electron flow from photo system I to ferredoxin. *Biochem* 36:93-102
- Goldsmith J, Boxer S (1996) Rapid isolation of bacterial photosynthetic reaction centers with an engineered poly-histidine tag. *Biochim Biophys Acta* 1276:171-175
- Gulis G, Narasimhulu KV, Fox LN, Redding KE (2008) Purification of His₆-tagged Photosystem I from *Chlamydomonas reinhardtii*. *Photosynthesis Research* 96:51-60
- Harris EH (1989) The *Chlamydomonas* sourcebook: a comprehensive guide to biology and laboratory use. Academic Press, San Diego
- Hippler M, Drepper F, Farah J et al. (1997) Fast electron transfer from cytochrome c6 and plastocyanin to photosystem I of *Chlamydomonas reinhardtii* requires PsaF. *Biochem* 36:6343-6349
- Kaplan NO, Everse J, Dixon JE et al. (1974) Purification and separation of pyridine nucleotide-linked dehydrogenases by affinity chromatography techniques. *PNAS USA* 71:3450-3454

- Kargul J, Nield J, Barber J (2003) 3D reconstruction of a PSI-LHCI super-complex from the green alga *Chlamydomonas reinhardtii*: insights into light harvesting for PSI. *J Biol Chem* 278:16135 – 16141
- Porra R, Thompson W, Kriedemann P (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochim Biophys Acta* 975:384-394
- Sugiura M, Inoue Y, Minagawa J (1998) Rapid and discrete isolation of oxygen-evolving His-tagged photosystem II core complex from *Chlamydomonas reinhardtii* by Ni²⁺ affinity column chromatography. *FEBS Lett* 426:140-144
- Takahashi Y, Golschmidt-Clermont M, Soen S et al. (1991) Directed chloroplast transformation in *Chlamydomonas reinhardtii*: insertional inactivation of the PsaC gene encoding the iron sulfur protein destabilizes photosystem I. *Embo J* 10:2033-2040