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Localization of a small chloroplast-encoded polypeptide PsbK in photosystem II core complex

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Introduction

PsbK is a small hydrophobic polypeptide of about 4 kDa. This polypeptide is encoded by the chloroplast *psbK* gene, synthesized as a precursor form with N-terminal extension, and processed to a mature form. The PsbK was first found in the O_2-evolving PSII membrane fragment but was not detected in O_2-evolving PSII core complex from spinach (Murata et al. 1988). Thus, it was concluded that PsbK is loosely associated with PSII complex and is not essential for PSII activity. However, it was later shown that PSII core complex purified from *Chlamydomonas reinhardtii* still contains substantial amount of PsbK (de Vitry et al. 1991). More recently, it was reported that a dimer form of a PSII core complex depleted in CP43 from spinach contains PsbK (Rhee et al. 1997), suggesting a rather tight association of PsbK to PSII core complex. Therefore, it is not yet clear how tightly PsbK is associated with PSII core complex. In the present study, we have generated an antibody against *Chlamydomonas* PsbK and used it to detect PsbK in various PSII preparations more sensitively and quantitatively. We have revealed that all PsbK present in thylakoid remains associated with the purified PSII core complex from *Chlamydomonas*. It is furthermore revealed that PsbK is specifically bound to one of the PSII core antenna complexes, CP43.

Materials and methods

Chlamydomonas reinhardtii wild-type 137c, psbA deletion mutant, Fud7, and *psbC* mutant, F34 were grown in Tris-acetate-phosphate (TAP) medium in the dim light at 25 ^{o}C. Thylakoid was purified according to (Chua and Bennoun 1975) and chlorophyll-protein complexes were purified by sucrose density gradient centrifugation (Takahashi et al. 1991), and subsequently by DEAE Toyopearl chromatography. To generate an antibody against PsbK, the coding sequence for mature PsbK was amplified by polymerase chain reaction with pfu polymerase (Promega), cloned into the pET-3xc vector (Novagen), and subsequently overexpressed in *E. coli*. The overexpressed PsbK polypeptide was solubilized with 2% SDS and subsequently purified by SDS-PAGE (Schäger and von Jagow 1987). The purified PsbK was injected into rabbits. Western blotting signals were visualized by enhanced chemiluminescence (ECL). Major PSII proteins were separated by urea-SDS-PAGE as described in (Takahashi et al. 1994) except PsbK. This polypeptide was more clearly separated by urea-SDS-PAGE according to (Schäger and von Jagow 1987)

Results

Although *Chlamydomonas* PSII core complex contains PsbK (de Vitry et al. 1991), it remains unclear whether PsbK present in thylakoids is fully associated with the purified PSII core complex or is partially released during solubilization of thylakoids and/or purification. To test these possibilities, the purified thylakoid was solubilized with 0.8% (w/v) n-dodecyl-β-D-maltoside (DM) and the resulting extracts were separated by sucrose density gradient centrifugation. Figure 1 shows that four green bands were separated and designated as A-1, A-2, A-3 and A-4, respectively, from top of the gradient. SDS-PAGE of proteins of each fraction revealed that A-1 contains LHCII while both A-3 and A-4 contain PSI complex as already reported (Takahashi et al. 1991). PSII core complex was detected only in A-2 and PsbK exclusively in A-2 as shown in Western blotting, suggesting a tight association of PsbK with PSII core complex and no dissociation of PsbK from the complex during the purification procedures.

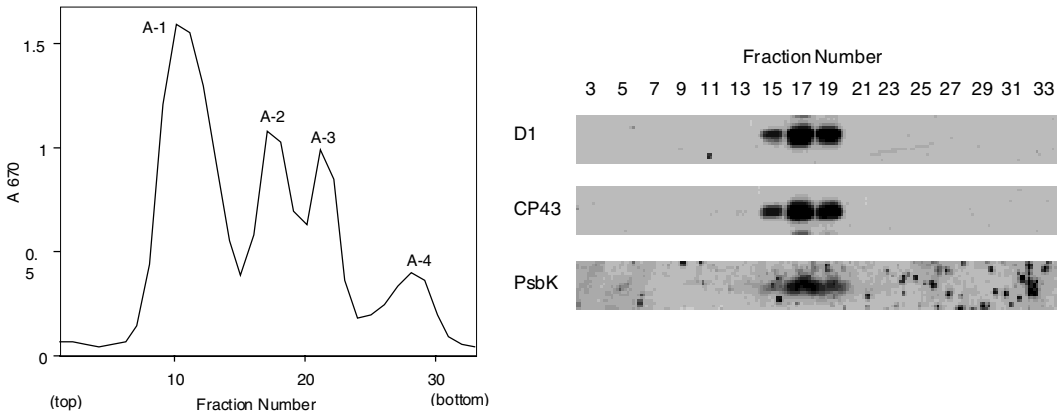

Fig. 1 Separation of chlorophyll-protein complexes by sucrose density gradient centrifugation. Elution profile was recorded by measuring absorption at 670 nm. PSII proteins in each fraction were detected by Western blotting.

Since A-2 is still contaminated by small amount of PSI complex, this fraction was subsequently fractionated by DEAE-column chromatography (data not shown). The resulting highly purified PSII core complex was used for subsequent dissociation into subcore complexes by treatment with 0.5 M KSCN. The dissociated subcore complexes were diluted with H_2O and subjected to DEAE column chromatography. The subcore complexes were separated by increasing NaCl concentration in elution buffer. The elution profile shown in Figure 2 reveals that three peaks were separated (C1, C-2 and C-3). Western blotting analyses using several antibodies raised against major PSII proteins revealed that C-1 corresponds to CP43 while C-2 is CP47. C-3 is PSII reaction center preparation because it contains D1 but completely lacks CP43 and CP47. These observations indicate that the KSCN treatment completely disintegrated the PSII core complex into chlorophyll-protein complexes. Detection of PsbK in each fraction clearly shows that PsbK is present exclusively in C-1. These results indicate that PsbK is specifically and tightly associated with CP43. The association of PsbK with CP43 was confirmed by copurification of PsbK with CP43 on gel filtration column chromatography (data not shown).

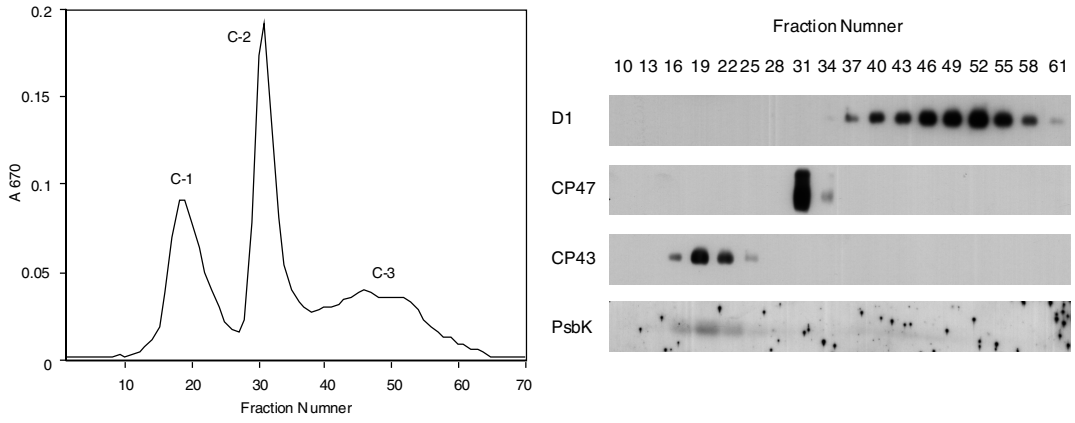

Fig. 2 Separation subcore complexes by DEAE chromatography. Elution profile was recorded by measuring absorption at 670 nm. PSII proteins in each fraction were detected by Western blotting.

We have subsequently estimated the amounts of major PSII proteins in thylakoid purified from two distinct *Chlamydomonas* PSII mutants, Fud7 that lacks D1 and F34 that is deficient in CP43 (Figure 3). D2 and CP47 in addition to D1 were deficient in thylakoid from Fud7. Probably stability of D2 and CP47 are significantly affected in the absence of D1. In contrast to D2 and CP47, CP43 accumulated to 30% of wild-type level. It is noteworthy that PsbK accumulated to a similar level as CP43 in Fud7. In contrast to Fud7, F34 accumulated D1, D2, and CP47 at 10-20% of wild-type level but no CP43 was detected as expected. Accumulation of D1, D2 and CP47 to a similar level suggests that they might form a complex. Interestingly, PsbK was not detected in F34. These observations strongly suggest that PsbK is stable only in the presence of CP43. This finding is consistent with the result that PsbK is associated with CP43. PsbK might be somehow protected by a protease digestion by its association to CP43.

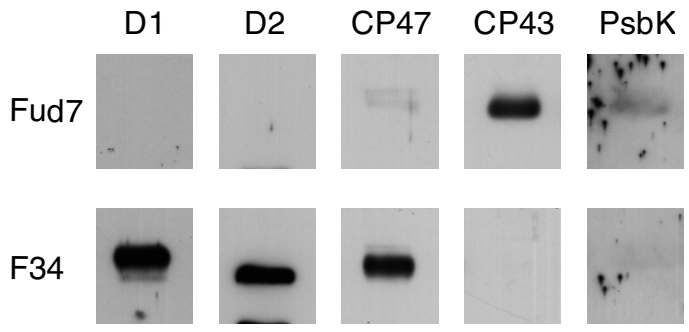

Fig. 3 Estimation of PSII proteins accumulated in PSII mutants. Proteins from purified thylakoids were separated by Urea-SDS-polyacrylamide gel electrophoresis and detected by Western blltting. The amount of PS II proteins were estimated by comparing the signal intensity with that of wild-type.

Discussion

In the present study, we have generated an antibody raised against mature PsbK overexpressed in *E. coli* and have successfully used it to localize PsbK in PSII complex quantitatively. It was shown in Figure 1 that all PsbK present in thylakoid is copurified with PSII core complex, suggesting that all PsbK is associated with PSII complex in thylakoid. We have also revealed that PsbK is tightly associated with CP43 after dissociation of PSII core complex into subcore complexes. The present study provides for the first time the evidence showing that CP43 directly associates small polypeptide, PsbK. This result is also supported independently by analysis of PSII-deficient mutants.

In contrast to PsbK, several small hydrophobic polypeptides are found to be associated with PSII RC (D1/D2 heterodimer). At least a half of cyt. b-559 (Rhee et al. 1988) and PsbW (Lorkovic et al. 1995) are found in RC preparations. Recently, it was revealed that chloroplast-encoded PsbT is also present in RC (Ohnishi and Takahashi 2001). So far, no small polypeptide was found to be associated with another core antenna complex, CP47. It was thought that other small polypeptide might be released from PSII complex by dissociation treatment and/or purification procedures. However, a current model of three-dimensional structure of PSII complex predicts topology of transmembrane helices in PSII complex. According to this model, several small polypeptide with a transmembrane helix should be present in close proximity of CP43 and CP47 as well as RC (Rhee et al. 1997). However it is not clear which small polypeptide corresponds to which helix. The present finding that PsbK has a direct association to CP43 will provide a valuable insight into localization of PsbK helix in the three dimensional structure in PSII complex.

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