Volume 262, number 1, 45-48 FEBS 08202 March 1990

The Photosystem I 5.5 kDa subunit (the *psa*K gene product)

An intrinsic subunit of the PSI reaction center complex

R. Max Wynn* and Richard Malkin

Department of Plant Biology, 111 Genetics-Plant Biology Building, University of California, Berkeley, CA 94720 USA

Received 29 December 1989

Previous studies of the Photosystem I reaction center complex, the chlorophyll protein 1 complex (CPI), have shown it is a heterodimer of two high-molecular-mass subunits of 83000 and 82000, the *psa*A and *psa*B gene products. The present work has identified another intrinsic low-molecular-mass subunit of 5500 (as determined by SDS-PAGE) that is tightly associated with the CPI complex. This subunit can be separated from CPI after treatment with thiol agents. CPI complexes prepared either in the presence or absence of thiols show no differences in photochemical activity under steady-state actinic illumination. A comparison of the N-terminal amino acid sequence of the 5.5 kDa subunit with the sequence of a *Chlamydomonas* PSI subunit, P37, identifies this low-molecular-mass subunit as the PSI *psa*K gene product.

Photosystem I; Chlorophyll protein 1 complex; P700; Sodium dodecyl sulfate-polyacrylamide gel electrophoresis; *psa*K gene

1. INTRODUCTION

The native Photosystem I (PSI) holocomplex from higher plants has been shown to contain some 14–15 individual polypeptides, including the reaction center core and light-harvesting chlorophyll a/b proteins (LHCI) [1,2]. Several preparations of PSI have been isolated to date. These include the detergent-solubilized 'native' PSI, termed PSI-200 since it contains approximately 200 chlorophylls per P700 [3], the antenna-depleted complex PSI-100 which contains 100 chlorophylls per P700 [4] and the PSI reaction center complex, CPI (the chlorophyll protein 1 complex), which contains only two subunits of molecular mass 83000 and 82000, the *psa*A and *psa*B gene products [5,6]. These subunits show anomalous behavior on SDS-PAGE, migrating with apparent molecular masses of 62000 and 58000, respectively (see [5,7]). Recently a new CPI preparation containing P700 and the electron acceptors A_0, A_1 and F_X has been isolated from higher plants and cyanobacteria [8]. This preparation has allowed the study of the early electron acceptors in PSI, specifically measurements of the acceptor F_X without the influence of other secondary electron acceptors, such as F_A and F_B. These studies also indicate that the Fe-S center F_X is bound to the *psa*A and *psa*B gene products and based upon sequence information [9], subunit stoichiometry [7,10] and EXAFS measurements [11], this cluster is presumed to be shared between the two high-molecular-mass subunits. Since Fe-S centers F_A and F_B are known to be bound to a 9 kDa subunit (the *psa*C gene product see [2]), the minimal PSI complex which is capable of charge separation is believed to be the CPI complex that contains only the two high-molecular-mass subunits. However, more recent work with resolved PSI complexes and high-resolution SDS-PAGE techniques has identified several low-molecular-mass subunits in PSI [12]. In the present study, a 5.5 kDa subunit is shown to be tightly associated with the CPI reaction center complex. This subunit can only be dissociated after treatment with thiols. The N-terminal amino sequence of the spinach subunit identified it as the *psa*K gene product recently characterized in *Chlamydomonas* [13].

Correspondence address: R. Malkin, Department of Plant Biology, 111 Genetics-Plant Biology Building, University of California, Berkeley, CA 94720 USA

* *Present address:* University of Texas, Southwestern Medical School, Department of Biochemistry, 5323 Harry Hines Blvd., Dallas, TX 75235, USA

2. MATERIALS AND METHODS

Spinach (*Spinacea oleracea*) was grown hydroponically in a greenhouse and mature leaves were used to isolate chloroplasts. The isolation procedure for the PSI-200 complex was similar to that described by Bruce and Malkin [7] except that no protease inhibitors were used. The native PSI complex, PSI-200, was isolated from cytochrome b_6-f-depleted thylakoids essentially as described by Bruce and Malkin [7]. To prepare the CPI complex from PSI-200, PSI-200 was diluted with 5 vol. of 20 mM Tris-HCl buffer (pH 7.5) and centrifuged at 250000 × *g* for 1 h. The pellet was resuspended in the same buffer to a chlorophyll (Chl) concentration of 0.5 mg/ml and solid SDS was added to give a 5–100:1 SDS:Chl (w/w) solution. This solution was immediately overlaid on a 0.15–0.75 M sucrose density gradient containing 50 mM Tris-HCl (pH 7.8) and 0.1% Triton X-100. The gradients were centrifuged overnight at 280000 × *g*. The lower green band contained the CPI complex. Thiol-treated CPI was

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

prepared as above except that 200 mM dithiothreitol (DTT) was added to the SDS solubilizing mixture prior to running the sucrose gradients.

Denaturing SDS-PAGE was done using a Laemmli system [14], with a 10–20% gradient-resolving gel and a 4% stacking gel. Samples were solubilized prior to electrophoresis by heating at 55°C for 20 min in a solution containing 10 mM Tris-HCl (pH 8.3), 100 mM DTT, 10% glycerol, 2% SDS and 0.01% Bromphenol blue. Electrophoresis was done for 10–12 h at a constant current of 10 mA. Gels were stained with Coomassie brilliant blue. The low-molecular-weight calibration kit (Biorad) was used to determine relative molecular weights. For quantitation of stained gels, gels were scanned with a Hoefer scanning densitometer and the integrated peak areas used to estimate subunit stoichiometries.

Samples for amino acid composition were electroeluted out of the gel and hydrolyzed with 6 N HCl at 110°C in sealed evacuated tubes for 24 h and the hydrolyzate analyzed using a Durrum D-500 amino acid analyzer. Cysteine was determined following performic acid oxidation. Amino acid sequence analysis was done using a vapor phase microprotein sequencer (Applied Biosystems model 470A) with online, microbore PTH-amino acid analyzer (Applied Systems model 120A).

Chlorophyll concentrations were estimated in 80% acetone using the extinction coefficients of Arnon [15]. P700 measurements were made optically using an Aminco DW-2A spectrophotometer and the concentration calculated using the extinction coefficients of Hiyama and Ke [16].

3. RESULTS AND DISCUSSION

The effect of increasing concentrations of SDS on the native PSI-200 complex was used to document the association of the 5.5 kDa subunit with the high-molecular-mass reaction center subunits of PSI. The results of this study are shown in fig.1 where the subunit composition of the active complexes is described in detail. Lanes 2–4 show the subunit composition of the lower green band from a sucrose gradient after treatment with SDS:Chl ratios of 5, 10 and 25. This lower band is photochemically active in that it shows light-induced P700 photooxidation while the upper green band on each respective gradient has no activity. At these SDS:Chl ratios, there is a progressive loss of various subunits from the active PSI complex but these preparations all retain a single low-molecular-mass subunit of 5.5 kDa. Lanes 5, 7 and 9 show the subunits lost from the respective complexes at high SDS concentrations and demonstrate that all subunits are depleted except for the 5.5 kDa subunit. At the highest concentration of SDS used (SDS:Chl = 100, lane 10), a complex can be isolated which contains only the two high-molecular-mass subunits (62 and 58 kDa but unresolved under these gel conditions) and the 5.5 kDa subunit. A preliminary measurement of the stoichiometry between the high-molecular-mass subunits and the 5.5 kDa subunit was made on the basis of staining intensity after SDS-PAGE, and it was found that there was one low-molecular-mass subunit per each CPI subunit in the complex. These results show that the 5.5 kDa subunit is present in a stoichiometric amount in the PSI reaction center complex and is tightly associated with the two high-molecular-mass subunits. SDS treatment of PSI is

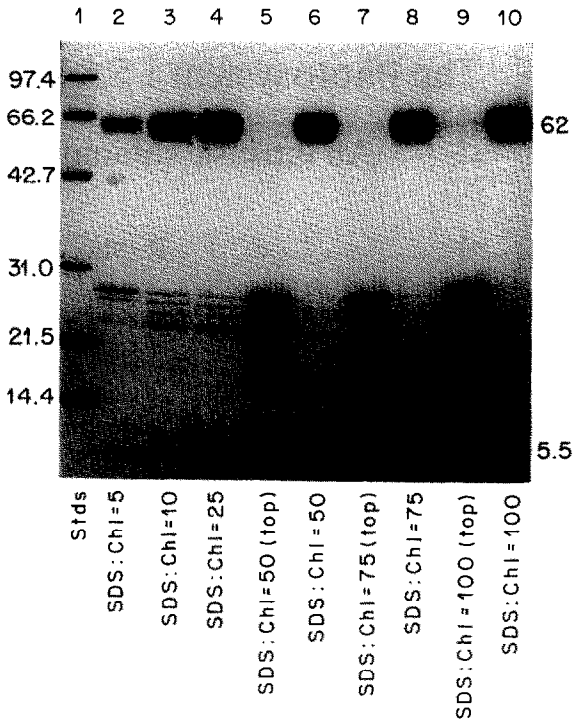

Fig.1. SDS-PAGE analysis of PSI fractions after SDS treatment of PSI-200. Lane 1, molecular weight markers. Lane 2, SDS:Chl = 5. Lane 3, SDS:Chl = 10. Lane 4, SDS:CHl = 25. Lane 5, SDS:Chl = 50, top band from gradient. Lane 6, SDS:Chl = 50, bottom band from gradient. Lane 7, SDS:Chl = 75, top of gradient. Lane 8, SDS:Chl = 75, bottom band from gradient. Lane 9, SDS:Chl = 100, top band from gradient. Lane 10, SDS:Chl = 100, bottom band from gradient. Chlorophyll concentration was 15 μg per lane.

not sufficient to release this subunit. In fact, it is likely that this subunit may have been present in all CPI complexes previously prepared, but that its presence may have been overlooked due to the poor resolution of small subunits by the gel systems.

A CPI complex which contains only the two high-molecular-mass subunits and is devoid of the 5.5 kDa subunit can be isolated by treating PSI-200 with a thiol, such as DTT or mercaptoethanol, in the presence of SDS. The results of this study are shown in fig.2 where the sample which had not been treated with DTT (lane 1) retains the 5.5 kDa subunit while the DTT-treated sample (lane 2) has lost this subunit. The observation that thiol treatment releases the 5.5 kDa subunit from the high-molecular-mass subunits suggests that the low-molecular-mass component might be linked via a disulfide bond. Alternatively, the conformation of the high-molecular-mass heterodimer may be changed by thiol treatment to allow for release of the tightly bound 5.5 kDa subunit.

The CPI complexes prepared either in the presence or the absence of DTT showed similar kinetics of P700 photooxidation in strong actinic light and both contained approximately 100 chlorophyll molecules per P700.

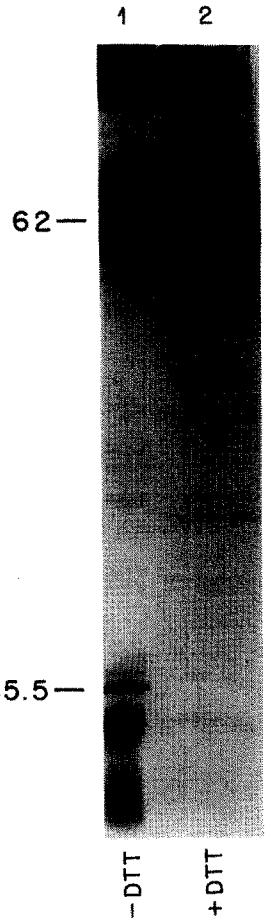

Fig.2. Treatment of PSI-200 with SDS in the presence or absence of DTT. Samples were treated with an SDS:Chl ratio of 100, as described in section 2, except that 200 mM DTT was added with the SDS to one sample. Samples were then fractionated on a sucrose gradient and the lower green band collected and analyzed by denaturing SDS-PAGE. Lane 1, no added DTT. Lane 2, plus DTT.

The absorbance spectra of the two preparations were also identical.

The 5.5 kDa subunit was isolated from a sample of PSI prepared with a high concentration of SDS by electroelution after SDS-PAGE. The N-terminal amino acid sequence of the first 9 amino acids is shown in fig.3 and is compared with the sequence of a protein, P37, from *Chlamydomonas*. The latter sequence was derived from a cDNA sequence published by Franzen et al. [13]. The two sequences are identical except for a single amino acid. The gene for this subunit has been named *psa*K by Golbeck and Bryant [2] in their recent compilation of the PSI genes in plants and cyanobacteria, and on the basis of this sequence comparison, we would conclude that the 5.5 kDa spinach PSI subunit is encoded by the *psa*K gene. Although Franzen et al. [13] reported a molecular mass of P37 based on SDS-PAGE of approximately 3 kDa, the deduced amino acid sequence of the mature protein indicates a molecular mass of approximately 8.4 kDa. The migration of the

	1				5					10
C. reinhardtii	G	F	I	G	S	S	T	N	L	I.....
Spinach	G	D	I	G	S	S	T	N	L	L.....

Fig.3. N-terminal amino acid sequence of the spinach 5.5 kDa subunit and the P37 subunit from *Chlamydomonas* (from [13]).

protein in our SDS-PAGE system would then be anomalous.

Franzen et al. [13] showed the *psa*K gene product had a transit sequence characteristic of nuclear-encoded proteins that are transported into the chloroplast. This would agree with our search of the chloroplast gene sequences from tobacco and liverwort for a sequence which corresponds to the N-terminus of the protein since this search did not reveal any positive matches. It is also of interest that although the amino acid composition of the spinach subunit indicates the presence of one cysteine residue, the complete sequence of the *Chlamydomonas* subunit does not show any cysteine residues. It is therefore unlikely that this subunit is covalently bound to the high-molecular-mass subunits of PSI via a disulfide bridge.

The *Chlamydomonas psa*K gene product has been predicted to be an intrinsic protein with one membrane-spanning region near the C-terminus. The hydrophobic nature of the subunit is consistent with Triton X-114 phase fractionation studies in which this subunit and the two high-molecular-mass subunits of PSI were found to fractionate into the detergent phase (Wynn and Webber, unpublished). The hydrophobic nature of the protein is also consistent with its tight association with the hydrophobic CPI subunits.

The function of the *psa*K gene product in the PSI complex is unknown. The close relationship of this subunit to the reaction center binding subunits suggests a possible role in the primary photochemical charge separation, but our preliminary measurements of photochemical activity under steady-state illumination conditions have not indicated a requirement for the subunit for stable charge separation. A more detailed time-resolved kinetic analysis is required in order to document a role for the *psa*K product in the PSI reaction center.

Acknowledgements: This work was supported in part by a grant from the National Science Foundation. We would like to thank A. Zilber for her helpful comments on the manuscript.

REFERENCES

[1] Golbeck, J.H. (1987) Biochim. Biophys. Acta 895, 167–204.
[2] Golbeck, J.H. and Bryant, D.A., Curr. Top. Bioenerg., in press.
[3] Lam, E., Ortiz, W., Mayfield, S. and Malkin, R. (1984) Plant Physiol. 74, 650–655.
[4] Haworth, P., Watson, J.L. and Arntzen, C.J. (1983) Biochim. Biophys. Acta 724, 151–158.

[5] Vierling, E. and Alberte, R.G. (1983) Plant Physiol. 72, 625–633.
[6] Fish, L.E., Kuck, U. and Bogorad, L. (1985) J. Biol. Chem. 260, 1413–1421.
[7] Bruce, B.D. and Malkin, R. (1988). J. Biol. Chem. 263, 7302–7308.
[8] Golbeck, J.H., Parrett, K. and McDermott, A.E. (1987) Biochim. Biophys. Acta 893, 149–160.
[9] Hoj, P.B., Svendsen, I., Scheller, H.V. and Moller, B.L. (1987) J. Biol. Chem. 264, 6929–6934.
[10] Bruce, B.D. and Malkin, R. (1988) Plant Physiol. 88, 1201–1206.
[11] McDermott, A.E., Yachandra, V.K., Guiles, R.D., Britt, R.D., Dexheimer, S.L., Sauer, K. and Klein, M.P. (1988) Biochemistry 27, 4013–4020.
[12] Scheller, H.V., Okkels, J.S., Hoj, P.B., Svendsen, I., Roepstorff, P. and Moller, B.L. (1989) J. Biol. Chem. 264, 18402–18406.
[13] Franzen, L.-G., Frank, G., Zuber, H. and Rochaix, J.-D., in: Proceedings of the 8th International Congress on Photosynthesis, Stockholm, in press.
[14] Laemmli, U.K. (1970) Nature 227, 680–685.
[15] Arnon, D.I. (1949) Plant Physiol. 14, 1–14.
[16] Hiyama, T. and Ke, B. (1972) Biochim. Biophys. Acta 276, 160–171.