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Stoichiometry and turnover of photosystem II polypeptides

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Using Triton X-100 solubilization and sucrose gradient fractionation, a photosystem II core complex has been isolated from the green alga *Dunaliella salina*. The complex was similar to that previously isolated from spinach [(1985) FEBS Lett. 188, 68–72] being composed of five main polypeptides of apparent molecular masses, 47, 40, 32, 30 and 10 kDa. Studies using ^{14}C indicate that with either dark or illuminated cells the stoichiometry of these polypeptides is 1:1:1:1:1 with about 20 chlorophyll *a* molecules per monomeric unit. According to ^{35}S labelling only the 32 kDa polypeptide, identified as the D1 or Q_B binding protein by immunoblotting, is rapidly turning over at a rate greater than the growth rate. The level of labelled sulphur associated with each polypeptide is reasonably consistent with the amino acid content derived from gene sequences and identifies the 47, 40, 32, 30 and 10 kDa apoproteins as products of the *psbB*, *psbC*, *psbA*, *psbD* and *psbE* genes.

Photosystem II; Detergent fractionation; Photosynthesis; Polypeptide; Stoichiometry; (*Dunaliella salina*)

1. INTRODUCTION

By combining detergent treatment with sucrose density centrifugation we were able to isolate from spinach chloroplasts a lipoprotein complex containing the reaction centre of photosystem (PS) II [1]. This complex had a composition similar to those isolated by other workers (e.g. [2–4]), being composed of five major polypeptides having molecular masses of 10 kDa and above. Here, we report the application of a similar procedure to isolate a PS II core complex from the unicellular halotolerant green alga, *Dunaliella salina*. The development of this procedure has allowed radiotracers to be used conveniently to investigate the stoichiometry and turnover of the polypeptides of the PS II core.

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Abbreviations: SDS-PAGE, SDS-polyacrylamide gel electrophoresis; Mes, (2-(*N*-morpholino)ethanesulphonic acid

2. MATERIALS AND METHODS

D. salina was grown with continuous illumination in a medium as described by Ben-Amotz et al. [5] containing 1 M NaCl. Thylakoids were prepared by osmotic lysis of cells and centrifugation. Prior to lysis, the cells were thoroughly washed with 1 M NaCl followed by a wash with a medium consisting of 20 mM Na-Tricine (pH 8.0) and 15% (v/v) glycerol. For the osmotic shock the glycerol-containing wash medium was diluted 5-fold with Na-Tricine (pH 8.0) to which 1 mM benzamidine and 5 mM α-aminocaproic acid were also added. The final chlorophyll level as determined by the method of Arnon [6] was 0.1–0.5 mg/ml and the incubation was carried out

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for 15 min on ice. The thylakoids were collected by centrifugation at 12000 × *g* for 10 min and washed once in a buffer consisting of 20 mM Na-Mes (pH 6.0), 15 mM NaCl and 5 mM $MgCl_2$. Using the same medium containing 4% (w/v) Triton X-100 the thylakoids were finally resuspended to a concentration of 2 mg Chl/ml and subjected to 30 min incubation on ice. The unsolubilized PS II was collected by centrifugation (30 min at 40000 × *g*), washed once in 20 mM Na-Mes buffer (pH 6.0) and then resuspended at 0.8 mg Chl/ml in the same buffer containing 0.64% (w/v) Triton X-100 and incubated for 1 h in the dark on ice. Samples containing 1 mg Chl were then applied to a 0.1–1 M linear sucrose density gradient containing 0.1% Triton X-100 and centrifuged at 180000 × *g* for 16 h at 4°C. The PS II core complex migrated to about 0.7 M sucrose while the light-harvesting chlorophyll *a/b* (LHC) complex occurred as a broad band at 0.3–0.4 M sucrose. This procedure is almost identical to that used for spinach chloroplasts [1] except that 0.64% Triton was used for the second solubilization step instead of 1%. SDS-PAGE was performed on a 7–17% acrylamide gel essentially according to Laemmli [7]. Autoradiography of dried gels was carried out at −70°C using Fuji NIH X-ray film and high-speed intensifying screens. Levels of radioactivity were determined by liquid scintillation counting. Transfer of proteins to nitrocellulose paper was carried out as in [8] and the antibodies were visualized by the use of peroxidase conjugate. All radiochemicals were obtained from Amersham International (England); chemicals were purchased from Sigma and detergents from Boehringer Mannheim.

3. RESULTS AND DISCUSSION

As fig.1 (lane 3) shows, the PS II core complex of *D. salina* was found to be composed of five polypeptides as resolved on the SDS-PAGE system used. These five polypeptides had apparent molecular masses similar to those found in the PS II core complex of spinach (fig.1, lane 1) isolated according to [1].

Standard markers gave apparent molecular masses of about 47, 40, 32, 30 and 10 kDa for the five polypeptides and immunoblotting with antibody raised to the Q_B binding protein (kindly supplied by Dr J. Hirschberg via Dr J. Marder) identified the location of this protein at 32 kDa in both core complexes. This polypeptide is the product of the *psbA* gene and is often called the D1 protein in order to distinguish it from the *psbD* product known as D2. The D2 protein has recently been identified by immunoblotting, using spinach PS II core complexes, to be the band at 30 kDa [9]. The origin of a lower molecular mass band at about 29 kDa observed in the immunoblots is unknown and was also detected previously [9]. The remaining bands at 47, 40 and 10 kDa can be identified with the products of the *psbB*, *psbC* and *psbE* genes [10] and represent two chlorophyll-binding proteins (47 and 40 kDa) and one of the polypeptides of cytochrome *b*-559 (10 kDa) with the lower molecular mass component of this cytochrome (4 kDa) not being resolved in our gel system. For convenience we denote the five clearly resolved polypeptides 47, 40, 32, 30 and 10 kDa as subunits I–V.

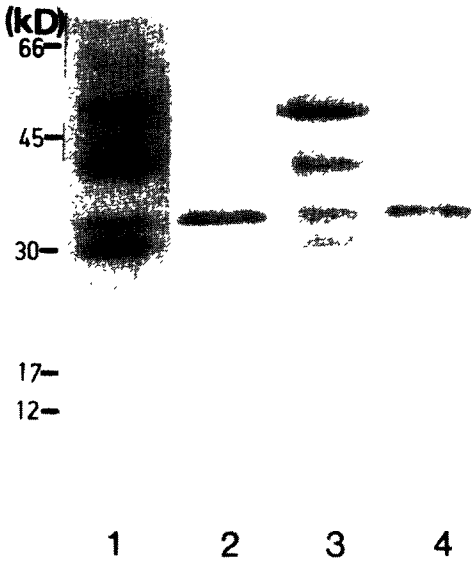

Fig.1. SDS-PAGE of the PS II core complex of spinach (lane 1) and *D. salina* (lane 3). The cross-reaction with an antibody raised against the Q_B binding (D1) protein is also shown for spinach (lane 2) and *D. salina* (lane 4) as obtained by Western blot analysis.

In order to obtain the stoichiometric relationship between the five subunits, *D. salina* cells were cultured for 7 days in the presence of [^{14}C]bicar-

Table 1

Subunit stoichiometry and sulphur content of PS II core complex from *D. salina*

Subunit	Apparent molecular mass (kDa)	^{14}C/apparent molecular mass		Sulphur equivalents	
		Light	Dark-adapted	Measured	Predicted
I	47	0.99 ± 0.05	0.93 ± 0.08	10.8 ± 0.5	14
II	40	1.00 ± 0.04	1.00 ± 0.09	13.2 ± 0.8	12
III	32	0.95 ± 0.06	1.02 ± 0.06	13.0 ± 0.5	13
IV	30	0.90 ± 0.13	0.97 ± 0.09	7.9 ± 0.7	13
V	10	0.96 ± 0.06	0.98 ± 0.11	0.05 ± 0.02	1

D. salina were cultured in the light in the presence of either [^{14}C]bicarbonate or [^{35}S]sulphate. Columns 3 and 4 are relative ^{14}C contents obtained by dividing averaged ^{14}C contents by apparent molecular mass of each subunit. Calculation of the sulphur equivalents (column 5) was done from the ^{35}S content of each band and the known specific activity of [^{35}S]sulphate. The predicted values (column 6) are taken from [11–15] for unprocessed polypeptides. Dark-adapted was a 48 h dark treatment following 7 days growth in the radioactive media

bonate until uniform labelling was obtained. The ^{14}C content of the individual subunits was then estimated after electrophoresis of the PS II core preparation under denaturing conditions. As table 1 shows, the ^{14}C content of each of the five subunits was essentially proportional to their apparent molecular masses giving a stoichiometry of 1:1:1:1:1. It was found that this same ratio was maintained when cultures were subjected to 48 h of dark treatment.

The sulphur content of each subunit was also measured after incorporation of ^{35}S from inorganic sulphate. As shown in table 1, the calculated levels of sulphur were found to be in reasonably good agreement with the sulphur content value predicted from gene sequencing for the unprocessed products, assuming the gene origins given above. A significant discrepancy was found in the case of subunit IV but whether this reflects loss of sulphur equivalents during processing or a species difference is not known. Worthy of particular note is that subunit V was found to contain

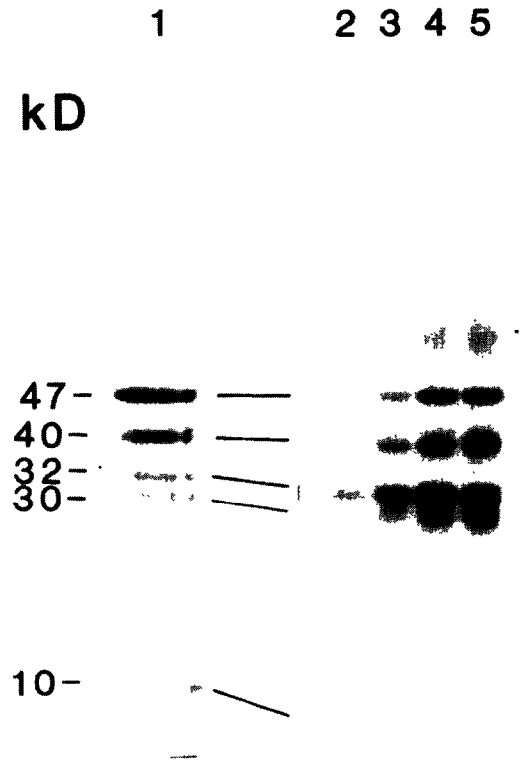

Fig.2. Coomassie blue-stained SDS-PAGE (lane 1) and autoradiograph (lanes 2–5) of PS II core polypeptides labelled with ^{35}S. [^{35}S]Sulphate was added to continuously illuminated cultures of *D. salina* and after different times of incubation the cells were harvested and the PS II core complex isolated. Each lane was loaded with equal chlorophyll levels and ^{35}S incubation times were 1 h (lane 2), 5 h (lane 3), 12 h (lane 4) and 24 h (lane 5).

no sulphur in agreement with predictions from the *psbE* sequence.

To estimate the turnover of the polypeptides which constitute the PS II core, we measured the time course of ^{35}S incorporation into the five subunits during continuous illumination. After full incorporation of ^{35}S we also followed the reduction of radioactivity during illumination but after diluting the external specific activity to a low value. Fig.2 shows that the 32 kDa polypeptide (subunit II) incorporates ^{35}S before the other subunits. The time course of ^{35}S incorporation shown in fig.3A further indicates that subunit III turns over in the light since the rate of radiolabelling of other subunits corresponded to the rate of chlorophyll synthesis and the growth rate of the cultures. This striking difference between subunit III and the remaining polypeptides of PS II is clearly seen in the data of fig.2B when isotopic dilution was induced. The calculated turnover time for subunit III is about 6 h for the experimental conditions used.

Using ^{14}C, an analysis of the PS II core complex

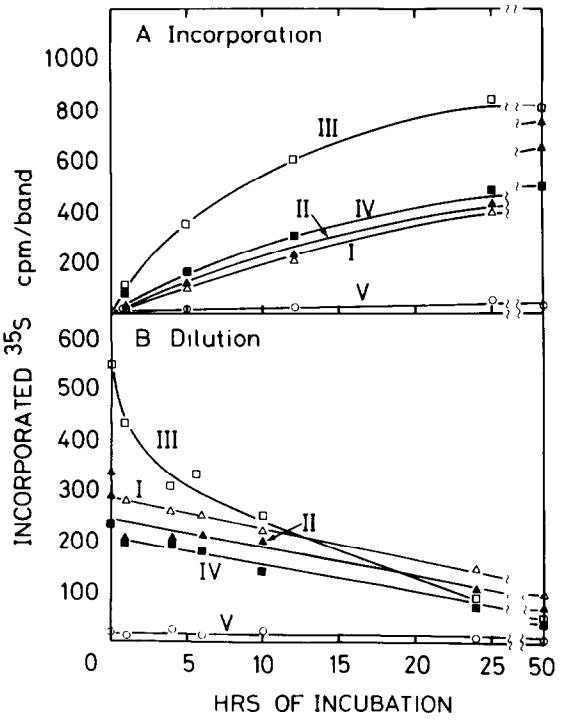

Fig.3. Time courses of incorporation and dilution of ^{35}S in the polypeptides of the PS II core complex of *D. salina*.

Table 2

Relative magnitudes of the constituents of PS II core complex from *D. salina*

Constituent	% carbon	
	Light	Dark
Protein	80.6	81.9
Chlorophyll	11.1	10.1
Lipid	8.3	8.0
Chl/protein (mol/mol)	19.7	18.0

Percentage carbon in the PS II core complex was determined after growing *D. salina* cells in [^{14}C]bicarbonate (0.5 mCi/l, 5 mM $NaHCO_3$) for 7 days. Cells were either then kept in the light for 24 h or given a dark treatment for the same time. The chl/protein (mol/mol ratio) was estimated by assuming the molecular mass for the monomeric complex to be 159 kDa and the carbon contents to be 74% for chlorophyll and 59% for protein

of *D. salina* for protein, chlorophyll and total polar lipid composition gave the values in table 2. The overall composition remained the same whether the cells were kept in continuous light or subjected to 24 h of darkness. Assuming the subunit stoichiometry of 1:1:1:1:1 and a monomeric unit we calculate the chlorophyll to protein ratio (mol/mol) to be about 20.

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