Isolation and Partial Characterization of an L-Amino Acid Oxidase and of Photosystem II Complexes from the Cyanobacterium *Synechococcus* PCC 7942

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An L-amino acid oxidase with high specifity for basic L-amino acids was isolated from the cyanobacterium *Synechococcus* PCC 7942, and the enzyme was partially characterized. This enzyme was compared to the previously described L-amino acid oxidase from *Synechococcus* PCC 6301 (G. Wälzlein, A. E. Gau, and E. K. Pistorius, Z. Naturforsch. **43c,** 545–553, 1988). In addition, photosystem II complexes were isolated from *Synechococcus* PCC 7942, and it could be shown that a 36 kDa polypeptide which crossreacts with the antiserum raised against the L-amino acid oxidase (50 kDa) is present in isolated PS II complexes from *Synechococcus* PCC 7942 as already shown to be the case for *Synechococcus* PCC 6301 (A. E. Gau, G. Wälzlein, S. Gärtner, M. Kuhlmann, S. Specht, and E. K. Pistorius, Z. Naturforsch. **44c,** 971–975, 1989). These results clearly show that in isolated photosystem II complexes from *Synechococcus* PCC 6301 as well as PCC 7942 a fourth polypeptide (besides D1, D2 and the manganese stabilizing protein) is present in the 30 kDa region and support our hypothesis suggesting that the water oxidizing enzyme is a separate protein (distinct from D1 and D2).

Introduction

Previously we have shown that in the cyanobacterium *Synechococcus* PCC 6301 an L-AOX with specifity for basic L-amino acids is present [1]. The L-AOX activity of this enzyme is inhibited by cations as well as anions [2]. When the cells are broken by French press treatment, a substantial amount of the enzyme (on activity basis) is found in the soluble fraction. However, part of this enzyme is tightly associated with the thylakoid membranes, and the protein can still be detected in purified PS II complexes of *Synechococcus* PCC 6301 [3]. Our initial reason to suspect that possibly a connection might exist between L-AOX activity in thylakoid membranes and photosynthetic water oxidation was based on the observation that $CaCl_2$ has an antagonistic effect on these two reactions: $CaCl_2$ stimulates photosynthetic water oxidation but inhibits the L-AOX activity [4]. This observation together with a number of additional results has led to our hypothesis that the water oxidizing enzyme might have evolved from an L-arginine dehydrogenase/oxidase which originally mediated electron flow from arginine to the plastoquinone pool of the electron transport chain in cyanobacteria. Our hypothesis further predicts that this flavoenzyme became modified with the inorganic cofactors Mn, Ca^{2+} and Cl^-, and that this modified flavoenzyme in combination with the D1/D2/cytochrome b 559 complex became the present-day water-plastoquinone oxidoreductase. Although we suggest that Mn is bound to this protein, our hypothesis does not exclude that other polypeptides of PS II (such as D1 and/or D2) contribute ligands for stabilizing Mn at the water oxidizing side [5].

We hope by applying genetic methods to eventually prove or disprove our hypothesis of the water oxidizing enzyme. Therefore, we thought that it would be essential to show that those results obtained with *Synechococcus* PCC 6301 could be confirmed by corresponding experiments with *Synechococcus* PCC 7942. These two *Synechococcus* strains are closely related, but *Synechococcus* PCC 7942 has superior transformation efficiencies [6].

Abbreviations: L-AOX, L-amino acid oxidase; Chl, chlorophyll; MSP, manganese stabilizing protein; LDAO, lauryldimethylamine *N*-oxide; PS II, photosystem II.

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Materials and Methods

Growth of cells and cell extracts

Synechococcus PCC 7942 was obtained from Collection Nationale de Cultures de Microorgan-

ismes, Institute Pasteur, Paris, France and *Synechococcus* PCC 6301 (SAUG 1402-1) was obtained from the Sammlung von Algenkulturen, Universität Göttingen, Göttingen, F.R.G. *Synechococcus* PCC 7942 cells were grown in BG 11 medium [7] and *Synechococcus* PCC 6301 cells were grown in a Kratz and Myers medium modified according to Pistorius *et al.* [8].

For the purification of the L-AOX, *Synechococcus* PCC 7942 was grown in 10 l bottles (totally: 620 l) at room temperature in a stream of filtered air enriched with 2% CO_2. The bottles were illuminated from two sides with eight fluorescent tubes (Osram L 36 W/19 Daylight 5000) at a distance of 10 cm on each side. The inoculum to give a cell density of 0.24 µl cells/ml increased to about 1.5 µl cells/ml in three days. The cells were then harvested by a continuous flow centrifuge (Contifuge 17 S with a type 8575 titan rotor, Heraeus Sepatech) at 14000 rpm, resuspended in 0.02 M potassium phosphate buffer, pH 7, to a concentration of 100 µl cells/ml and passed twice through a chilled French press at 138 MPa. For isolation of thylakoid membranes and PS II complexes both *Synechococcus* PCC 6301 and *Synechococcus* PCC 7942 were grown in 250 ml gas wash-bottles at 30 °C and sparged with the same gas mixture as described above. Illumination was from the top with four Philips lamps (120 W, PAR 38 EC cool spot) at a distance of 35 cm from the water bath surface (40 cm × 60 cm). The cultures were inoculated as described above and grown for two days resulting in a cell density of about 2.5 µl cells/ml (PCC 6301) and 2.0 µl cells/ml (PCC 7942), respectively. After two days the cells were harvested, washed and broken up as reported previously [9].

Purification of the L-AOX

The purification of the L-AOX was basically the same as described in [10] under purification A with the following modifications: The heating step was performed at 55 °C instead of 60 °C, and a chromatography step on a Mono Q column coupled to an FPLC system (Pharmacia) was added. For this procedure the fractions with the highest specific activity obtained from the hydroxylapatite column chromatography were combined, dialyzed once against 0.01 M potassium phosphate buffer containing 0.001 M EDTA, pH 7, and twice against 0.01 M potassium phosphate buffer, pH 7, and then subjected to chromatography on a Mono Q column. The column was equilibrated with 0.01 M potassium buffer, pH 7. Elution was carried out with a linear gradient of equal volumes of 0.01 M and 0.2 M potassium phosphate buffer, pH 7 (20 ml total).

Preparation of thylakoid membranes and purification of PS II complexes from Synechococcus PCC 6301 and PCC 7942

Thylakoid membranes were isolated as described in [9] and PS II complexes were isolated and purified according to purification b given in [10]. The PS II complexes on top of the 30% sucrose layer (obtained after aggregation and centrifugation) were used for characterization. For measurements of the antagonistic $CaCl_2$ effect on Hill reaction and L-AOX activity of thylakoids from *Synechococcus* PCC 7942, the membrane preparations were washed twice with 0.05 M Hepes-NaOH, containing 400 mM sucrose, and resuspended in the same medium.

Activity assays and analytical methods

O_2 uptake (L-AOX activity) and photosynthetic O_2 evolution were measured in a Clark type electrode (Rank Brothers) at 20 °C in a total volume of 3 ml under conditions as described previously [9]. The activity of the L-AOX during the different purification steps was measured in a reaction mixture containing 53 mM Hepes-NaOH (pH 7), 10 mM EDTA (pH 7), 33 mM L-arginine, 40 µg beef liver catalase (Boehringer) and the enzyme. 1 unit of enzyme was defined as 1 µmol O_2 taken up/min. Further measurements were done with this assay modified in the following ways: For determination of the substrate specifity 33 mM L-lysine, L-ornithine, L-histidine, L-alanine or D-arginine were substituted for L-arginine. The assays for determination of the pH dependence each contained one of the following reaction buffers: 53 mM Mes-NaOH (pH 5.5–7.0), 53 mM Hepes-NaOH (pH 7–7.5), 53 mM Tricine-NaOH (pH 7–9) or 53 mM Ches-NaOH (pH 9–9.5). The same buffers were used to adjust L-arginine to the corresponding pH values. In assays with cation additions EDTA was omitted.

For measurements of O_2 evolution activity of thylakoid membranes and PS II preparations in

the light the reaction mixture contained in a total volume of 3 ml 33 mM Hepes-NaOH (pH 7), 50 mM $CaCl_2$, 1.7 mM potassium ferricyanide and the particles containing 2.5 µg Chl. The L-AOX activity (as O_2 uptake with catalase inhibited) of these preparations was assayed in 33 mM Hepes-NaOH (pH 7), 10 mM EDTA (pH 7), 0.4 mM KCN, 10 mM L-arginine and the particle sample in a total volume of 3 ml. All measured O_2 uptake rates with L-arginine as substrate were completely inhibited by addition of 50 mM $CaCl_2$.

Manometrical measurements and assays for determination of the L-AOX reaction products were basically carried out as described previously [11]. Additional keto-arginine determinations were performed according to Nagata *et al.* [12]. Ketoarginine as standard was obtained after incubation of L-arginine with excess of snake venom L-AOX (from *Crotalus terrificus terrificus*).

Protein was determined according to Bradford [13].

Molecular weight determination of the purified L-AOX protein

The molecular weight of the native enzyme was examined with a Superose 12 column coupled to an FPLC system (Pharmacia). The column was washed with 0.02 M Hepes-NaOH containing 0.1 M NaCl (pH 7), and the determination was performed with 15 µg of the L-AOX protein (22.3 units/mg) applied to the column.

Absorption spectra

The spectra of the purified L-AOX were recorded with a Perkin Elmer spectrophotometer lambda 3.

Gel electrophoresis and immunoblots

SDS polyacrylamide gel electrophoresis were carried out as previously described [3] with modified denaturation for the PS II samples (60 min room temperature followed by 15 min at 37 °C). Immunoblots with anti-L-AOX, anti-D 1 and anti-MSP were basically done as described in [14] by transfering the proteins to nitrocellulose membranes by pressure, while electroblotting to polyvinyl difluoride membranes was used for the immunoblots with anti-CP 47 and anti-D 2. The antisera were the same as in [3].

Mn content of PS II particles

The Mn content of the PS II complexes was determined with ICP/MS (Plasmaquad 2, VG Instruments).

Results and Discussion

Comparison of the L-AOX activities in Synechococcus PCC 7942 and PCC 6301

As shown for *Synechococcus* PCC 6301, it could also be demonstrated for *Synechococcus* PCC 7942 that the L-AOX activity is partly present in the soluble fraction and partly present in thylakoid membranes after cell breakage. The activity values given in Table I were obtained from *Synechococcus* PCC 7942 grown in BG 11 medium [7] and *Synechococcus* PCC 6301 grown in a Kratz and Myers medium modified according to Pistorius *et al.* [8], since we found that under these conditions optimal growth rates were obtained for each strain.

Table I. L-AOX activity in total French press extracts and thylakoid membranes of *Synechococcus* PCC 7942 and PCC 6301.

	PCC 6301	PCC 7942
	[µmol O_2 taken up/mg Chl × h]	
French press extract	103	30
Thylakoid preparation	54	6

Purification and partial characterization of the L-AOX from Synechococcus PCC 7942

The L-AOX from PCC 7942 was basically purified according to the procedure described for the L-AOX from PCC 6301 [10] except that an additional chromatography step on a Mono Q column was added (details are given under Materials and Methods). A 16000-fold increase in specific activity was necessary (Table II), before the enzyme was homogenous based on SDS-PAGE (not shown). The high increase in specific activity which had to be achieved, before a homogenous protein was obtained from the soluble fraction of French press extracts, indicates that the actual amount of L-AOX protein present in the soluble fraction of *Synechococcus* PCC 7942 is less than 0.01% of the total protein. We believe that most of the L-AOX protein is associated with the thylakoid mem-

Table II. Purification of the L-AOX from *Synechococcus* PCC 7942. Details of the purification are given under Materials and Methods. 1 unit of the enzyme corresponds to 1 μmol O_2 taken up/min (in the presence of catalase, in air and at 20 °C).

Purification step	Protein, total [mg]	L-Amino acid oxidase activity	
		Total units	Specific activity [units/mg]
French-press extract	82400	820	0.010
Ammoniumsulfate prec. 30–50%	45672	278	0.006
Heating to 55 °C	32321	220	0.007
CM-Sephadex column	1353	191	0.141
DEAE-Sephadex column	6.8	189	27.7
Hydroxylapatite column	0.9	115	121
Mono-Q column	0.8	128	160

branes. However, in thylakoid membranes the L-AOX activity is greatly masked [15]. The isolated L-AOX from PCC 7942 is – as expected – immunologically related to the L-AOX from PCC 6301, since the antiserum raised against the L-AOX from PCC 6301 [10] recognizes the L-AOX from PCC 7942 in immunoblot experiments and inhibits its activity to about 60–70% (not shown).

The native enzyme has a molecular weight of 100 kDa determined by chromatography of the protein on a Superose 12 column, and SDS-PAGE showed that the enzyme consists of two subunits of equal molecular weight of 52 kDa. The absorbance spectra of two representative enzyme samples are given in Fig. 1. As shown for the L-AOX from PCC 6301 and as can be seen from these spectra, the enzyme from PCC 7942 also contains variable amounts of authentic oxidized FAD (absorption bands with maxima at 375 and 465 nm) and of a modified flavin of yet unknown structure (absorption bands with a maximum at 400–405 nm and variable bands in the 500 to 600 nm region). The unmodified flavin present in the enzyme seems to be authentic oxidized FAD based on reconstitution assays with the apoprotein prepared from kidney D-amino acid oxidase which is known to be FAD specific (data not shown). The amount of oxidized FAD relative to the modified FAD present in different enzyme samples was variable as can be seen from the absorption spectra. As already concluded for the enzyme from PCC 6301, the L-AOX activity correlates with the amount of oxidized FAD present in the enzyme sample [10].

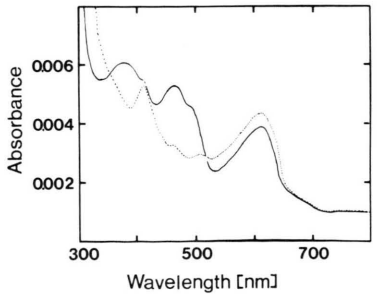

Fig. 1. Absorbance spectra of two representative L-AOX samples from *Synechococcus* PCC 7942. The absorbance spectra were recorded with two L-AOX samples obtained after chromatography of the enzyme on the hydroxylapatite column. L-AOX fraction with a specific activity of 137 (0.04 mg protein/ml 0.02 M potassium phosphate buffer, pH 7) (——); L-AOX fraction with specific activity of 3.4 (0.03 mg protein/ml 0.02 M potassium phosphate buffer, pH 7) (·····).

As the L-AOX from PCC 6301 [1, 2] the enzyme has activity from pH 5.5 to 9.5 and shows a bell-shaped pH curve with optimum rates at pH 7.5 (not shown). It has a high specifity for basic L-amino acids (L-Arg > L-Lys > L-Orn > L-His, relative rates are 100, 82, 28 and 3%, respectively). D-arginine is not oxidized (not shown). The K_M value of 3 mM for L-arginine is comparable to the value determined for the enzyme from PCC 6301 [1]. As shown in Table III the enzyme converts L-arginine to ketoarginine and NH_4^+ in the presence of catalase. This reaction can be classified as a regular L-AOX reaction in which the reduced enzyme becomes reoxidized by molecular O_2 under H_2O_2 formation. In the absence of catalase most of the ketoarginine seems to be non-enzymatically degraded to 4-guanidinobutyrate under utilization of the initially formed H_2O_2. The activity of the enzyme is approximately twice as high in 100% O_2 than in 20% O_2 and is very low in 5% O_2.

Table III. Determination of products formed from L-arginine in the L-AOX reaction in the presence or absence of catalase in air and the influence of O_2 concentration on the L-AOX activity.
A: For these experiments the reactions were carried out in Warburg vessels connected to manometers. The products were determined after incubating the L-AOX with L-arginine as substrate in Warburg vessels in an air saturated atmosphere for 90 min. The products were determined as described under Materials and Methods. Guanidinobutyric acid nonenzymatically formed from ketoarginine in the presence of H_2O_2 was not determined.
B: The conditions were the same as under A except that prior to starting the reaction by tipping the substrate, the Warburg vessels were gassed with 100%, 20% or 5% O_2 in N_2.

Table III A

O_2 uptake product formation	− Catalase [µmol]	+ Catalase [µmol]
O_2 uptake	9.4	5.3
Ketoarginine formation	2.4	7.4
NH_4^+ formation	9.6	11.4
H_2O_2 formation	6.8	0

Table III B

O_2 concentration	L-AOX activity [µmol O_2 taken up]
5% O_2	0.7
20% O_2	3.6
100% O_2	7.8

The L-AOX is strongly inhibited by cations ($M^{3+} > M^{2+} > M^+$) and less strongly by anions (Table IV). In the group of divalent cations, transient metal ions, such as Mn^{2+}, inhibit more strongly than alkali earth metals, such as Ca^{2+}. 50% inhibition is obtained at 0.7 mM, 1.1 mM and 103 mM with La^{3+}, Ca^{2+} and Na^+, respectively. These values agree well with those published for the L-AOX from PCC 6301 [2]. The inhibition by cations, such as Ca^{2+}, decreases with increasing L-arginine concentrations. However, evaluation of Lineweaver Burk graphics indicates that the Ca^{2+}-inhibition is only partly competitive (and partly noncompetitive). The enzyme is also inhibited by o-phenanthroline as described for *Synechococcus* PCC 6301 [1].

Isolation of PS II complexes from Synechococcus PCC 7942 and immunological identification of PS II polypeptides in the 30 kDa region

The antagonistic effect of $CaCl_2$ on the L-AOX activity detectable in thylakoid membranes and on photosynthetic O_2 evolution [4] could also be demonstrated in *Synechococcus* PCC 7942 (Fig. 2). $CaCl_2$ inhibited the L-AOX activity to approximately the same extent as it stimulated O_2 evolution suggesting that the L-AOX activity has to become suppressed before water oxidation can pro-

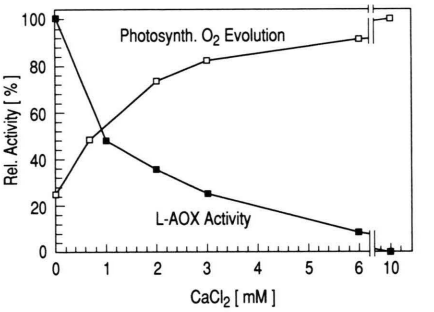

Fig. 2. Antagonistic effect of $CaCl_2$ on photosynthetic O_2 evolution (ferricyanide as electron acceptor) and on the L-AOX reaction (measured as oxygen uptake with L-arginine as substrate in the dark) of thylaloid membrane preparations from *Synechococcus* PCC 7942.

Table IV. Inhibition of the L-AOX from *Synechococcus* PCC 7942 by various cations and anions.

Additions	50% Inhibition [mM]	Additions	50% Inhibition [mM]
KCl	108	NaSCN	16
NaCl	103	$NaNO_3$	44
$CaCl_2$	1.1	NaBr	70
$MgCl_2$	0.8	NaCl	85
$ZnCl_2$	0.12	$Na(OOCCH_3)$	225
$MnCl_2$	0.12		
$LaCl_3$	0.66		

ceed. From such thylakoid membranes PS II complexes were solubilized with the detergent LDAO and further purified by sucrose cushion and sucrose gradient centrifugation (details are given under Materials and Methods). These PS II preparations contained 1 gatom Mn per 19 mol Chl after sucrose cushion and 1 gatom Mn per 28 mol Chl after gradient centrifugation, respectively, indicating a partial loss of Mn during the aggregation step. The Mn content of the PS II preparation after sucrose cushion agrees well with values obtained with other PS II preparations from cyanobacteria [16, 17]. The activity measurements for O_2 evolution and L-AOX activity are summarized in Table V. After the aggregation step during the purification of PS II complexes, the L-AOX activity was not or hardly detectable in PS II complexes indicating that the enzyme is not accessible for the hydrophilic substrate L-arginine in such aggregated PS II complexes.

The polypeptide pattern of the purified PS II complexes is given in Fig. 3. With the corresponding antisera the polypeptides of 47, 36, 31 and 28 kDa could be identified as being CP47, L-AOX, D1 and MSP, respectively. Peptides below 10 kDa were not examined in this work. Previously, we failed to detect the D2 polypeptide in PS II com-

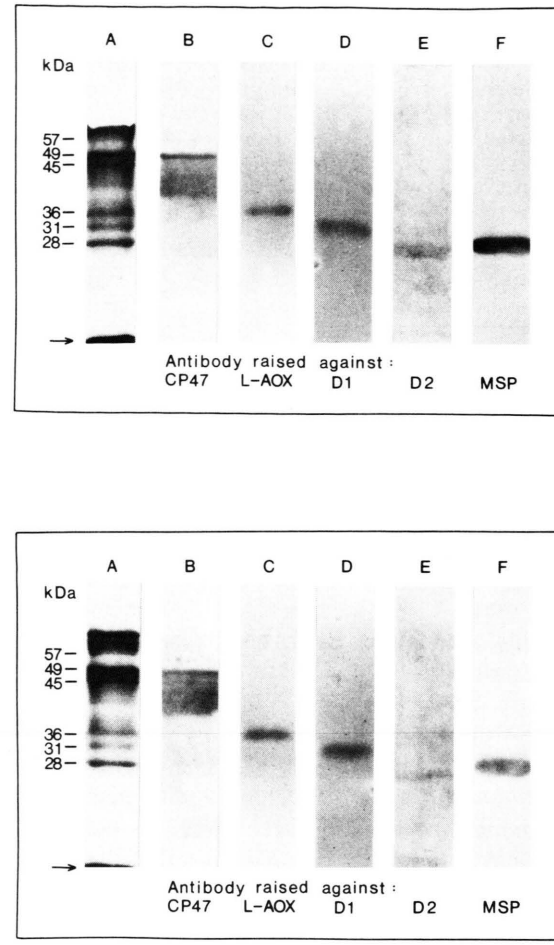

Fig. 3. Immunological identification of polypeptides in purified PS II complexes from *Synechococcus* PCC 7942 and PCC 6301. A. Coomassie Brilliant Blue stained SDS polyacrylamide gels of purified PS II complexes (each sample containing 6 µg Chl). B to F represent the corresponding immunoblots which were immunostained with: B: anti-CP47 (dilution 1 to 30), C: anti-L-AOX (dilution 1 to 75), D: anti-D1 (dilution 1 to 25), E: anti-D2 (dilution 1 to 25) and F: anti-MSP (dilution 1 to 40). Top: PS II complexes from *Synechococcus* PCC 7942; bottom: PS II complexes from *Synechococcus* PCC 6301.

Table V. Purification of PS II complexes from thylakoid membranes of *Synechococcus* PCC 7942. Details are given under Materials and Methods.

	Chlorophyll *a*	Photosynthetic O_2 evolution		L-Amino acid oxidase activity (O_2 uptake)	
	total [mg]	[µmol O_2/ mg Chl × h]	total [µmol O_2/h]	[µmol O_2/ mg Chl × h]	total [µmol O_2/h]
Thylakoid membranes	14.8	591	8723	5.88	86.7
Thylakoid membranes after sodium cholate	11.8	609	7197	2.81	33.3
Supernatant after LDAO treatment	2.91	991	2882	4.92	14.3
LDAO extract after sucrose cushion	2.33	1080	2516	6.75	15.7
PS II complexes after aggregation and gradient centrifugation	1.27	721	913	not detectable*	–

* Enzyme most likely not anymore accessible for the hydrophilic substrate L-arginine.

plexes from PCC 6301 with the antiserum raised against the D2 peptide isolated from oat [3]. However, after improving our blot conditions, we show in this paper that it became possible to also identify the D2 peptide. The D2 peptide is mainly associated with the 28 kDa band which means that this band contains MSP as well as D2. We have tried a number of other gel and denaturation conditions (as *e.g.* gradient gels or denaturation at higher temperatures). However, no improvements were obtained, since under such conditions the L-AOX protein had a tendency to give a broad diffuse band and/or to run further down into the 30 kDa region, while the D2 peptide could be detected with the anti-D2 just above the L-AOX protein band as well as in the MSP region.

The problem of not getting a perfect separation of all the polypeptides present in PS II complexes of both *Synechococcus* species under one defined denaturation condition is probably related to the fact that variable amounts of lipids and/or detergent are associated with the peptides resulting in several species of the same peptide. This would lead to variable gel patterns during SDS-PAGE due to different migration velocities of the peptides and due to variable tendencies of the peptides for aggregation. These problems have been described for other membrane proteins [18]. So far, the polypeptide pattern shown in Fig. 3 is the best separation of the peptides in the 30 kDa region which we were able to obtain with isolated PS II complexes from *Synechococcus* PCC 7942. However, despite these problems, the results of Fig. 3 clearly show that in isolated PS II complexes besides D1, D2 and MSP a fourth polypeptide with an apparent molecular weight of 36 kDa is present in the 30 kDa region, and this fourth polypeptide shows a cross-reaction with the anti-L-AOX. The reason why other groups working in this area (see for review [19]) have so far not detected this polypeptide, might be related to the above described difficulties in finding conditions for separation of all four peptides in the 30 kDa region on SDS polyacrylamide gels.

Since we were unable to identify the D2 polypeptide in the previously published paper on the polypeptide composition of PS II complexes from PCC 6301 [3], we also give the polypeptide composition of isolated PS II complexes from PCC 6301 and the corresponding immunoblots for comparison (Fig. 3). The polypeptide pattern of the PS II complexes from PCC 6301 and PCC 7942 were almost identical. Only minor differences were observed in the pattern of the higher molecular weight peptides. However, this peptide pattern in the 50 to 60 kDa region was somewhat variable when the denaturating conditions were varied or PS II complexes from different purifications were examined. Most likely the variations were caused by aggregation of various PS II peptides.

Concluding remarks

All results previously obtained with *Synechococcus* PCC 6301 with respect to the L-AOX with high specificity for basic L-amino acids [1, 2, 10] could be confirmed with *Synechococcus* PCC 7942 with only minor deviations. These differences were mainly related to the observation that the L-AOX activity, detectable in PCC 7942 cells, was lower than in cells from PCC 6301 and that the enzyme seemed to be somewhat more labile indicating that the irreversible modification of the flavin in the enzyme occurred with higher rates in crude extracts of PCC 7942 than in crude extracts of PCC 6301. This greater lability was also found with respect to the photosynthetic O_2 evolving activity. The increase in specific activity for O_2 evolution on chlorophyll basis during purification was always higher in PS II complexes isolated from PCC 6301 [9, 20] than in those isolated from PCC 7942 (Table V). Possibly, the greater lability of the water oxidizing enzyme is a consequence of the greater lability of the L-AOX in PCC 7942 – if our hypothesis, suggesting that the L-AOX enzyme and the water oxidizing enzyme represent different forms of the same protein, is correct.

The immunoblot experiments clearly show that in isolated PS II complexes from PCC 6301 as well as PCC 7942 a polypeptide of 36 kDa is present which shows a cross reaction with the antiserum raised against the isolated L-AOX protein of 50 kDa from PCC 6301 [10]. This protein is an additional PS II peptide (in addition to D1, D2 and MSP) in the 30 kDa region [19]. The differences in the apparent molecular weight of the "soluble" L-AOX protein of 50 kDa and the PS II associated polypeptide of 36 kDa recognized by the anti-L-AOX could probably be best explained by assuming that the L-AOX protein becomes modified

(processed, covalently modified or associated with lipids) when incorporated into PS II, and that the modified protein migrates differently during SDS-PAGE.

The here presented results are a further support of our hypothesis that the water oxidizing enzyme is a separate protein (distinct from D1 and D2) in PS II. The identification of the gene coding for this protein is in progress.

Acknowledgements

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