Biochimica et Biophysica Acta 849 (1986) 203–210
Elsevier

BBA 41988

Presence of a flavoprotein in O_2-evolving Photosystem II preparations from the cyanobacterium *Anacystis nidulans*

Elfriede K. Pistorius and Achim E. Gau

Universität Bielefeld, Fakultät für Biologie, Lehrstuhl Zellphysiologie, 4800 Bielefeld 1 (F.R.G.)

(Received November 19th, 1985)

Key words: Photosystem II; L-Amino acid oxidase; Flavin; Oxygen evolution; Flavoprotein; (*A. nidulans*)

A highly active O_2-evolving Photosystem II complex which was greatly depleted of phycobiliproteins was isolated from the cyanobacterium *Anacystis nidulans*. This complex contained the flavoprotein with L-amino acid oxidase activity which we have previously shown to be present in thylakoid preparations of this cyanobacterium (Pistorius, E.K. and Voss, H. (1982) Eur. J. Biochem. 126, 203–209). One of the most prominent polypeptides in this O_2-evolving Photosystem II complex had a molecular weight of 49 kDa. This polypeptide co-chromatographed on SDS-polyacrylamide gels with the purified L-amino acid oxidase which consists of two subunits of 49 kDa. The antagonistic effect of $CaCl_2$ on the two examined reactions could also be demonstrated with this O_2-evolving Photosystem II complex: $CaCl_2$ stimulated photosynthetic O_2 evolution, but inhibited the L-amino acid oxidase activity. Both reactions were inhibited by *o*-phenanthroline. These results further support a functional relationship between the flavoprotein with L-amino acid oxidase activity and Photosystem II activities in *A. nidulans*. However, we only found 1 mol FAD per 350–650 mol chlorophyll, although 1 gatom Mn per 5–10 mol chlorophyll was present. When we assume a photosynthetic unit of about 40 chlorophylls, then in most preparations the FAD values were more than a factor of 10 too low. Results which we obtained with the purified L-amino acid oxidase showed that the FAD values were in most enzyme samples lower than the theoretically expected value of 2 mol FAD per mol enzyme. Moreover, in some cases the absorption spectrum of the enzyme showed substantial deviations from the spectrum of oxidized FAD. These experiments indicated that the flavin in the enzyme could partly exist in a form which was different from 'authentic oxidized FAD'. We do not yet know the chemical nature of this 'modified flavin'.

Introduction

Highly active O_2-evolving PS II preparations have been obtained from plants, algae and cyanobacteria using various detergents [1–4]. These preparations have greatly contributed to the recent advance in our understanding of the photosynthetic O_2 evolution. We have previously shown that French press preparations as well as PS II preparations (none-O_2-evolving) from the cyanobacterium *Anacystis nidulans* contain a flavoprotein with L-amino acid oxidase activity, and we have suggested that this protein, which consists of two subunits of 49 kDa, is the reaction center polypeptide of PS II in *A. nidulans* [5]. The reason to suspect that a connection existed between the L-amino acid oxidase activity of this flavoprotein and PS II activities was based on the observation that the L-amino acid oxidase activity responded to many reagents which also affected PS II activi-

Abbreviations: PS, Photosystem; Chl, chlorophyll; LDAO, lauryldimethylamine *N*-oxide; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

0005-2728/86/$03.50 © 1986 Elsevier Science Publishers B.V. (Biomedical Division)

ties [5–7]. Since procedures for the isolation of O_2-evolving PS II complexes from *A. nidulans* have recently been worked out [8,9], we were interested to see whether the L-amino acid oxidase was present in such O_2-evolving PS II preparations from *A. nidulans*.

Materials and Methods

Anacystis nidulans (Synechococcus leopoliensis) B-1402-1 was obtained from the Sammlung von Algenkulturen (Universität Göttingen, Göttingen, F.R.G.). The growth of the cells was the same as reported previously [5]. RNAase from bovine pancreas (40 units/mg) and DNAase from bovine pancreas (1000 units/mg) were purchased from Boehringer, Mannheim, and LDAO from Fluka, Neu-Ulm, F.R.G.

Preparations of PS II complex. The *Anacystis* cells were harvested after 2 days (cell density is about 2 μl cells $\cdot$ ml^{-1}) by centrifugation for 20 min at 2200 $\times$ *g*. The cells (about 5000 μl cells) were washed with 150 ml 0.05 M Hepes-NaOH (pH 6.5), containing 50 mM $CaCl_2$ and 400 mM sucrose, and suspended in the same medium to give a cell density of 125 μl cell $\cdot$ ml^{-1} (40 ml). All subsequent preparation steps were performed at 0–5°C. 4 μg DNAase and 40 μg RNAase were added. The resuspended cells were passed twice through a French pressure cell at 137.8 MPa. The French press extract was centrifuged for 5 min at 4300 $\times$ *g*, and the supernatant was then centrifuged for 30 min at 27100 $\times$ *g*. The pellet was suspended in 40 ml 0.05 M Hepes-NaOH (pH 6.5), containing 50 mM $CaCl_2$ and 400 mM sucrose (thylakoids in Table I). 80 mg sodium cholate (final concentration, 0.2%) were added. After 10 min incubation the preparation was centrifuged for 30 min at 27100 $\times$ *g*. The pellet was then suspended in 0.05 M Hepes-NaOH (pH 6.5)/30 mM $CaCl_2$/25% glycerol to give a chlorophyll concentration of 0.6–0.75 mg chlorophyll $\cdot$ ml^{-1} (thylakoids after sodium cholate in Table I). 0.3% LDAO were added, and the suspension was stirred in ice for 1 h. After centrifugation for 30 min at 27100 $\times$ *g*, the supernatant (PS II complex in Table I) was layered on a stepwise sucrose gradient consisting of 30%, 20% and 10% sucrose in 0.05 M Hepes-NaOH (pH 6.5) containing 30 mM $CaCl_2$ and 10% glycerol. The gradient was centrifugfed for 90 min at 80000 $\times$ *g* (at r_{max}). We obtained on top of the gradient a homogeneous green band which was enriched in PS II activity (PS II complex after gradient in Table I) and a pellet and sometimes a green band at the boundary between 20% and 30% sucrose.

Activity assay. O_2 uptake or O_2 evolution was measured in a Gilson Oxygraph (model IC-OXY) fitted with a Clark type electrode. The solutions were saturated with air, and the reaction temperature was 20°C. For the Hill reaction the reaction chamber was illuminated with a Halogen lamp (24 V, 250 W) from Spindler and Hoyer, Göttingen. The light was filtered through a 2% $CuSO_4$ solu-

TABLE I

ISOLATION OF A PS II COMPLEX FROM *A. NIDULANS*

Detials of the purification are given under Materials and Methods.

	Chlorophyll total (mg)	Photosynthetic O_2 evolution (μmol O_2 per mg Chl per h)	Photosynthetic O_2 evolution total	L-Amino acid oxidase activity (O_2 uptake) (μmol O_2 per mg Chl per h)	L-Amino acid oxidase activity (O_2 uptake) total
French press extract	11.7	149	1743	78	913
Thylakoid membranes	8.0	135	1080	12	96
Thylakoid membranes after sodium cholate	6.5	137	891	8	52
PS II complex after LDAO	0.79	865	683	27	21
PS II complex after gradient centrifugation	0.29	1213	352	44	13

tion and a red glass filter RG 1 (610) from Schott, Mainz.

For the Hill reaction in the light, the reaction mixture contained in a total volume of 1.85 ml 54 mM Hepes-NaOH (pH 6.5), 54 mM $CaCl_2$, 1.6 mM potassium ferricyanide, 27 mM sucrose, and *Anacystis* particles containing 1–20 μg chlorophyll. For the L-arginine oxidation in the dark, the reaction mixture contained in a total volume of 1.85 ml 54 mM Hepes-NaOH (pH 7), 32 mM L-arginine (adjusted with Hepes to pH 7 [7]), and 11 mM EDTA (pH 7), and *Anacystis* particles containing 2–40 μg chlorophyll.

FAD determination. FAD was determined fluorometrically as described previously [10] or enzymatically. Reconstitution of the apoprotein from kidney D-amino acid oxidase can be used for quantitative FAD determination, and provides a very sensitive and a very specific test for FAD [11]. The apoprotein from kidney D-amino acid oxidase was prepared according to Ref. 12. The degree of reconstitution of the apoprotein by FAD was measured as O_2 uptake at 25°C in a Gilson oxygraph. The reaction mixture contained in a total volume of 1.85 ml 54 mM Tricine-NaOH (pH 8.5), 16 mM D-alanine and the apoprotein of the D-amino acid oxidase, containing 10–15 μg protein. Under these conditions the measured O_2 uptake was proportional to the added FAD in the range of 0.002–0.1 nmol FAD added. The samples of the L-amino acid oxidase or of the PS II complex were denatured by heat or by acid treatment. For heat denaturation the sample was placed for 5 min in boiling water. After centrifugation the supernatant was used for FAD determination. For the acid treatment 5 μmol EDTA and 50 μmol H_2SO_4 were added to 0.5 ml sample. After incubation for 5 min in ice and centrifugation, the supernatant was neutralized with NaOH and used for FAD determination. Most samples of the PS II complex would give higher FAD values (about 2 times higher) after acid denaturation as compared to heat denaturation.

Gel electrophoresis. SDS-polyacrylamide gel electrophoresis was performed according to Ref. 13, but a Tris-glycine buffer (pH 8.6) was used instead of the phosphate buffer. Samples were incubated with 2% SDS and 2% 2-mercaptoethanol for 2 h at 37°C, and then run on 10% slab gels with a 5% stacking gel (1.5 cm) on top. The gel contained 0.1% SDS. After staining with Coomassie blue, the gels were scanned with a Joyce Loebl Chromoscan 3 at 626 nm. As protein standards were used: bovine serum albumin (68 kDa), glutamate dehydrogenase (53 kDa), ovalbumin (45 kDa), D-amino acid oxidase (37 kDa), trypsin (23.3 kDa) and RNAase (13.7 kDa). Absorption spectra were recorded with a Perkin Elmer spectrophotometer λ 3 and Mn was determined with a Perkin Elmer atomic absorption spectrophotometer, model 380, with a flameless graphite furnace HGA 500. L-Amino acid oxidase was purified as previously described [10].

Results and Discussion

Isolation of O_2-evolving PS II membranes

PS II membranes were isolated with the detergent LDAO essentially according to the procedure described by England and Evans [8], but we washed the thylakoid membranes with Hepes buffer containing sodium cholate before we solubilized the PS II complex with LDAO (for details, see Materials and Methods). This washing procedure reduced the bound phycobili proteins significantly. Moreover, French press treatment of *Anacystis* cells resulted in solubilization of part of the L-amino acid oxidase. Therefore, an additional washing of the membranes was necessary to remove the more loosely bound L-amino acid oxidase. The results of such a purification are given in Table I.

Unfortunately, it is difficult to assay the L-amino acid oxidase activity in a quantitative way, when the enzyme is still bound to the thylakoid membrane. Therefore, a strict quantitative correlation between L-amino acid oxidase activity (measured as L-arginine oxidation) and PS II activities could not be expected. The 'detectable' L-amino acid oxidase activity is probably dependent on the degree to which this activity is suppressed in favor of the PS II activity by cations, like Ca^{2+} and Mn^{2+}, and by anions, like Cl^-, and this is likely to change during the various isolation steps. Despite these difficulties the results in Table I show that a relatively good correlation was obtained between the two activities when the 'loosely bound L-amino acid oxidase' was removed by washing the

thylakoid membranes. The PS II complex isolated from the sodium cholate washed thylakoid membranes showed a (5–9)-fold increase in specific activity for photosynthetic O_2 evolution and a (3–7)-fold increase for the L-amino acid oxidase activity. The increase in the O_2 evolving activity was comparable to the increase observed in Ref. 9. Since cyanobacteria are known to have only about 16% of their total chlorophyll associated with PS II, an approx. 6-fold enrichment could be expected on that basis [14].

The specific activity for photosynthetic O_2 evolution which we obtained, was about 2–4-times higher than the activity reported in Ref. 8. The increase in specific activity which was observed upon dilution of the sample in that paper was not seen in our PS II preparation. We obtained fairly linear rates, when samples of the PS II complex containing 1–10 μg chlorophyll were used in the assay. A PS II complex from *A. nidulans* which had a higher specific activity than the one here reported was isolated by Pakrasi and Sherman [9]. This complex still contained the phycobilisomes attached to PS II, and the O_2-evolution activity was measured with ferricyanide plus 2,6-dichloro-*p*-benzoquinone. The numbers given in Table I were obtained with ferricyanide alone as acceptor. The combination of ferricyanide plus phenyl-*p*-benzoquinone resulted in inactivation of the O_2-evolving activity of the PS II complex here described.

Preliminary molecular weight determinations on a Sephacryl S-200 column indicated that the PS II complex was heterogenous in size. However, O_2-evolving activity could be detected in fractions which seemed to have a molecular weight below 250 kDa. Similar observations have been made with a PS II complex from *Phormidium laminosum* [2].

Characteristics of the PS II complex

Absorption spectrum. The room temperature absorption spectrum of the PS II preparation is shown in Fig. 1. The preparation had a red absorption peak of chlorophyll a at 673 nm and still contained a small amount of phycocyanin indicated by the band at 625 nm. However, the phycocyanin content was substantially reduced in these PS II preparations compared to preparations

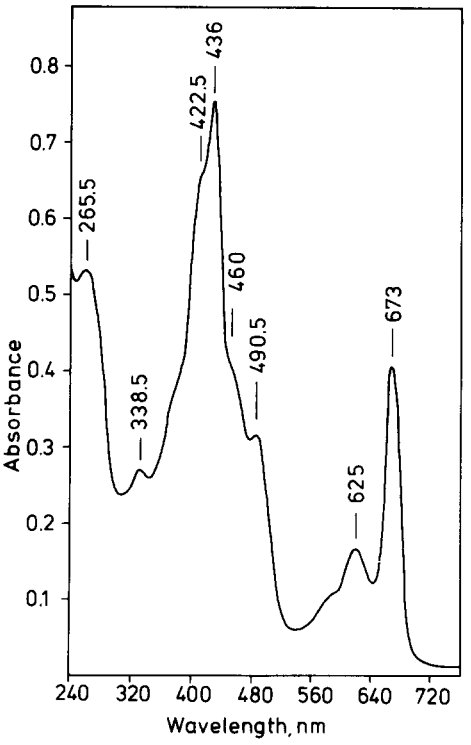

Fig. 1. Absorption spectrum of the PS II complex from *A. nidulans*.

which were isolated without treatment of the thylakoid membranes with sodium cholate (not shown, but see also Refs. 8 and 9). The bands at about 490 and 460 nm indicated the presence of carotenoids, although it is quite possible that the flavin contributed to the 460 nm band. This band was always more prominent in PS II preparations with higher specific activity for O_2 evolution than in those with lower activity.

Polypeptide composition. The polypeptide composition of the PS II complex was examined by SDS polyacrylamide gel electrophoresis (Fig. 2). The major bands had molecular weights of 49 (48–50), 43 (40–44), 36, 31, 27, 13 and 10 kDa. In addition a band of approx. 57 kDa was seen, but the extent of its appearance was variable (even when the same preparation was used), and could possibly be an aggregation product, as has also been suggested in Ref. 15. The polypeptides of 49, 43, 36, 31 and 10 kDa are similar to polypeptides found in other PS II preparations [3,9]. Prominent bands at 13 and 27 kDa could possibly be sub-

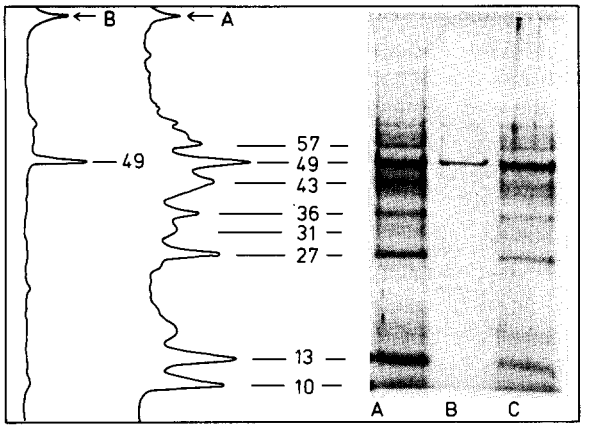

Fig. 2. SDS polyacrylamide gel electrophoresis of PS II complex and purified L-amino acid oxidase. Samples were prepared as described under Materials and Methods. The gel contained from left to right: Lane A: PS II complex (4 μg chl); lane B: purified L-amino acid oxidase (2.8 μg protein); and lane C; PS II complex (2 μg chl) and purified L-amino acid oxidase (1.4 μg protein). On the left-hand side of the figure the densitometer tracings are given for lane A and lane B of the gel. The numbers given between the gel and the densitometer tracings are the apparent molecular weights in kDa. The arrows indicate the start of the running gel.

units of the phycobili proteins [3,9,16].

The PS II complex of *A. nidulans* did not contain polypeptides corresponding to the 23 and 16 kDa polypeptides which are present in higher plant PS II complexes. These peptides were also absent in PS II preparations of *Synechococcus* sp. [3] and *Phormidium laminosum* [17]. Although polypeptides of similar size can be seen in cyanobacterial cells [17] and in thylakoid preparations of *A. nidulans* [18], immunoblotting experiments with cyanobacteria preparations showed no cross-reaction with antibodies to the plant 23 and 16 kDa polypeptides. Stewart et al. [17] concluded from the latter experiments that cyanobacteria do not have these polypeptides.

The 49 and 43 kDa polypeptides are the apoproteins of the chlorophyll proteins. The 49 kDa polypeptide has been attributed to the reaction center polypeptide while the 43 kDa polypeptide has been identified as the main antenna chlorophyll protein of the PS II reaction center [19]. The polypeptide of 49 kDa was one of the most prominent bands in the complex. The corresponding polypeptide had a molecular weight of 48 kDa in the *Anacystis* PS II preparation of Pakrasi and Sherman [9] and of 50 kDa in the Anacystis PS II preparation of Koenig and Vernon [20]. The molecular weight of this peptide is identical to the molecular weight of the purified L-amino acid oxidase (2 subunits of 49 kDa [10]). Addition of the purified L-amino acid oxidase to the PS II preparation showed that the enzyme co-migrated with the 49 kDa polypeptide present in the PS II complex.

Effect of $CaCl_2$, o-phenanthroline and DCMU. The antagonistic effect of $CaCl_2$ on the two activities could also be demonstrated with these O_2-

TABLE II

THE EFFECT OF $CaCl_2$, *o*-PHENANTHROLINE AND DCMU ON PHOTOSYNTHETIC O_2 EVOLUTION AND L-ARGININE OXIDATION IN PS II PREPARATION OF *A. NIDULANS*

Activity assays were done as described under Materials and Methods except that EDTA and $CaCl_2$ were added as indicated in the table.

Additions	Photosynthetic O_2 evolution (μmol O_2 per mg Chl per h)	L-Amino acid oxidase activity (O_2 uptake) (μmol O_2 per mg Chl per h)
11 mM EDTA	0	58.4
3 mM $CaCl_2$	221	22.1
54 mM $CaCl_2$	838	4.4
11 mM EDTA, 2 mM *o*-phenanthroline	–	30.9
11 mM EDTA, 5 mM *o*-phenanthroline	–	15.3
11 mM EDTA, 5 μM DCMU	–	57.3
54 mM $CaCl_2$, 2 mM *o*-phenanthroline	347	–
54 mM $CaCl_2$, 5 mM *o*-phenanthroline	298	–
54 mM $CaCl_2$, 5 μM DCMU	154	–

evolving PS II preparations (Table II). $CaCl_2$ was required for photosynthetic O_2 evolution, but inhibited L-arginine oxidation as shown in Table II. Both activities were inhibited by *o*-phenanthroline, while DCMU only inhibited photosynthetic O_2 evolution, but had no effect on L-arginine oxidation. The reaction center polypeptide is assumed to be the protein which is affected by Cl^- and Ca^{2+} [21,22]. Since one of the major peptides in the PS II complex here described is the flavoprotein which has a molecular weight of 49 kDa and which is strongly affected by cations and anions, such as Ca^{2+} and Cl^- [5,7], it seems highly likely that this flavoprotein actually is the reaction center of PS II. Moreover, the cation and anion effect on this protein has been shown to be strongly pH dependent, and a group with a pK of about 7 seems to be involved [7]. These results might be connected to observations made by Critchley et al. [23] who suggested that the Cl^- binding to the PS II reaction center was possibly affected by a group with a p*K* of about 7 on a yet 'unidentified' protein.

Mn and FAD content. The Mn content (1 gatom Mn per 5–10 mol chlorophyll) was slightly higher than values obtained with other PS II preparations from cyanobacteria [3]. We also determined FAD and found 1 mol FAD per 350–650 mol chlorophyll. When we assume a photosynthetic unit of about 40 chlorophylls [24], then in most preparations the found FAD corresponded to less than 0.1 mol FAD per mol reaction center, and this would be more than a factor of 10 too low. Moreover, minor impurities by other flavoproteins could not be excluded. Therefore, these results clearly show that the isolated PS II complex did not contain the expected stoichiometric amount of 'authentic oxidized FAD'. However, since all our previous experiments and the results here presented support a functional role of this flavoprotein in PS II reactions, then the low FAD values should be the result of properties of the enzyme which we do not yet understand. All our experiments so far have indicated that the flavoprotein has to be converted (by Cl^-, Ca^{2+} and possibly also by Mn) into a yet 'unknown form' which is then functional in PS II reactions. This form might behave entirely different than normal oxidized FAD does. Some unusual and unexplained observations have also been made about the flavin in the isolated L-amino acid oxidase, and these results will be briefly described in the next section.

Variations in flavin content and flavin spectrum of the isolated L-amino acid oxidase

Several large scale purifications of the L-amino acid oxidase of *A. nidulans* have been carried out by the procedure previously described [10]. Different batches of purified enzyme have shown large variation in flavin content (see legend of Fig. 3) and flavin spectrum (Fig. 3). The best preparation (based on L-amino acid oxidase activity) had a flavin content of 1.8 mol FAD per mol enzyme and a specific activity of 292 which corresponded to a turn-over number of 57200 (mol L-arginine deaminated per mol enzyme per min). However,

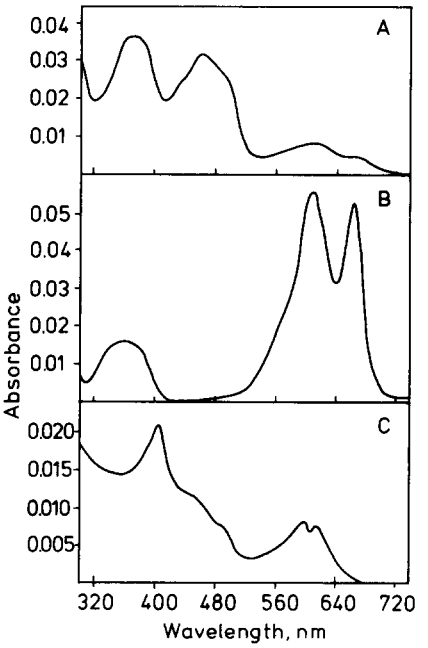

Fig. 3. Absorption spectrum of three samples of purified L-amino acid oxidase. Spectra were recorded from top fractions of three different purifications. The enzymes were in 0.02 M potassium phosphate buffer (pH 7). A: 0.14 mg protein·ml^{-1}; B: 0.08 mg protein·ml^{-1}; and C: 0.12 mg protein·ml^{-1}. The samples had a specific activity (μmol O_2 taken up per mg protein per min with L-arginine as substrate, in presence of catalase, at 20°C) of 292 (A), 142 (B), and 125 (C). The corresponding flavin values (mol FAD per mol enzyme) were 1.8, (A), 0.5 (B), and 0.8 (C).

values as low as 0.5 mol FAD per mol enzyme have also been observed in the top fractions of some purifications.

The absorption spectrum of the various purified enzymes showed considerable variations (Fig. 3). The color of the enzyme ranged from yellow over various shades of green to blue. In the better preparations (based on L-amino acid oxidase activity) the enzyme had the general characteristics of flavin (absorbance bands at 465 and 375 nm), but small deviations from the absorption spectrum of free oxidized riboflavin could also be observed (Fig. 3A).

However, we could also obtain an enzyme in which the flavin seemed to be totally reduced, since no band at 465 nm could be detected. Such samples contained substantial amounts of phycocyanin and allophycocyanin (bands at 610 and 665 nm) which remained bound to the enzyme throughout the entire purification procedure (Fig. 3B). Another enzyme sample (Fig. 3C) showed a pronounced band at about 400 nm and a double band in the 600 nm region (in addition to a shoulder at 465 nm which indicated the presence of some oxidized flavin). This spectrum (bands at about 400 and 600 nm) had similarities with 10,10[a] ring-opened intermediates which have been shown to arise in the autoxidation of dihydroflavin model compounds and which have been suggested to occur as intermediates in oxygenase reactions [25]. Such intermediates might be worthwhile to consider, since an absorption change in the 400 nm region is suggested to be associated with 'Z' [26]. If such a modified flavin would be present in the PS II complex, we would not detect it in our assay, and that could explain the low flavin values.

These fluctuations in flavin content and flavin spectrum of the isolated L-amino acid oxidase and the problem about the low flavin values in the PS II complex could probably be ascribed to a common cause, but we have not yet learned how to manipulate these variations. If flavin is functional in photosynthetic O_2 evolution, then it may be expected to go through more redox states than those recognized for a simple flavin oxidase. It is quite possible that one of these redox states is very unstable, and this instability would lead to loss or destruction of flavin during isolation or during the denaturation procedure. These problems have to be examined in more detail when larger amounts of the enzyme and of the purified PS II complex are available.

Concluding remarks

The primary events of PS II are associated with the reaction center chlorophyll a species, P-680, and its immediate donor 'Z'. In addition to 'Z' and P-680 the O_2-evolving complex requires Mn, Cl^- and Ca^{2+} [27]. The redox couple 'Z/Z^+' has been examined by absorbance difference spectra and by EPR studies. Based on these results, several groups have suggested that 'Z^+' is likely to be a semiquinone cation radical of a plastoquinone or plastoquinone-like molecule which is tightly bound to a protein of the PS II complex. Despite these results some uncertainty about the identity of 'Z' has still remained [27–29]. We have shown here that a flavoprotein with L-amino acid oxidase activity is present in O_2-evolving PS II preparations of *A. nidulans*. Based on the antagonistic effect of $CaCl_2$ on photosynthetic O_2 evolution and L-arginine oxidation and based on the inhibition of these two reactions by *o*-phenanthroline and chlorpromazine [5–7,10] a functional relationship between the L-amino acid oxidase activity of the flavoprotein and PS II activities is indicated. Although other workers in the field favor a radical of a plastoquinone-like molecule as the essential catalyst in the water-splitting reaction, we believe that a flavin radical is equally plausible and in view of our results a more reasonable cofactor than a quinone. Of course much more information is needed about the special and rather unusual properties of this flavoprotein.

Acknowledgement

This work was supported by the Deutsche Forschungsgemeinschaft. We are very grateful to Prof. Birgit Vennesland and Prof. Georg H. Schmid for discussions, and we thank Wolfgang Schwarze for performing the Mn analysis.

References

1 Berthold, D.A., Babcock, G.T. and Yocum, C.F. (1981) FEBS Lett. 134, 231–234

2 Stewart, A.C. and Bendall, D.S. (1979) FEBS Lett. 107, 308–312
3 Satoh, K., Ohno, T. and Katoh, S. (1985) FEBS Lett. 180, 326–330
4 De Vitry, C., Wollmann, F.-A. and Delepelaire, P. (1984) Biochim. Biophys. Acta 767, 415–422
5 Pistorius, E.K. and Voss, H. (1982) Eur. J. Biochem. 126, 203–209
6 Pistorius, E.K. (1983) Eur. J. Biochem. 135, 217–222
7 Pistorius, E.K. (1985) Z. Naturforsch. 40c, 806–813
8 England, R.R. and Evans, E.H. (1981) FEBS Lett. 134, 175–177
9 Pakrasi, H.B. and Sherman, L.A. (1984) Plant Physiol. 74, 742–745
10 Pistorius, E.K. and Voss, H. (1980) Biochim. Biophys. Acta 611, 227–240
11 Rao, N.A., Pelton, S.P. and Huennekens, F.M. (1967) Methods Enzymol. 10, 494–499
12 Warburg, O. and Christian, W. (1938) Biochem. Z. 298, 150–165
13 Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412
14 Gantt, E., Lipschultz, C.A. and Grabowsky, J. (1978) J. Phycol., Suppl. to 14, 37
15 Koike, H. and Inoue, Y. (1985) Biochim. Biophys. Acta 807, 64–73
16 Lönneborg, A., Lind, L.K., Kalla, S.R., Gustafsson, P. and Öquist, G. (1985) Plant Physiol. 78, 110–114
17 Stewart, A.C., Ljungberg, U., Åkerlund, H.-E. and Andersson, B. (1985) Biochim. Biophys. Acta 808, 353–362
18 England, R.R. and Evans, E.H. (1985) Biochim. Biophys. Acta 808, 323–327
19 Nakatani, H.Y., Ke, B., Dolan, E. and Arntzen, C.J. (1984) Biochim. Biophys. Acta 765, 347–352
20 Koenig, F. and Vernon, L.P. (1981) Z. Naturforsch. C36, 295–304
21 Nakatani, H.Y. (1984) Biochem. Biophys. Res. Commun. 121, 626–633
22 Murata, N. and Mijao, M. (1985) Trends Biochem. Sci. 10, 122–124
23 Critchley, C., Andersson, B., Ryrie, I.J. and Anderson, J.M. (1984) Biochim. Biophys. Acta 767, 532–539
24 Stewart, A.C. and Bendall, D.S. (1981) Biochem. J. 194, 877–887
25 Mager, H.I.X. and Addink, R. (1983) Tetrahedron 39, 3359–3366
26 Dekker, J.P., Brok, M. and Van Gorkom, H.J. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. I, pp. 171–174, Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, The Netherlands
27 Van Gorkom, H.J. (1985) Photosynth. Res. 6, 97–112
28 O'Malley, P.J. and Babcock, G.T. (1984) Biochim. Biophys. Acta 765, 370–379
29 Hales, B.J. and Gupta, A.D. (1981) Biochim. Biophys. Acta 637, 303–311