

# Which Polypeptides Are Characteristic for Photosystem II? Analysis of Active Photosystem II Particles from the Blue-Green Alga *Anacystis nidulans*\*

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Dedicated to Prof. Dr. W. Menke on the Occasion of His 70th Birthday

Photosystem II, Active Particles, Polypeptides, Thylakoid Membrane, *Anacystis*

A thylakoid membrane preparation isolated from the blue-green alga *Anacystis nidulans* was freed from carboxysomes, soluble enzymes and the pigment P750 by floating in a discontinuous sucrose density gradient. In a buffer containing sucrose and the zwitterionic detergent Miranol S2M-SF the thylakoids were loaded on a linear 10–18% sucrose density gradient which also contained Miranol. The sedimentation yielded three bands, the lower two of which were green and the upper one was orange. The light green band in the middle of the gradient was the only one to show any photosystem II activity. This was measured as light-induced electron transport from diphenylcarbazide (DPC) to dichlorophenol-indophenol (DCPIP). The activity was sensitive to dichlorophenyl-dimethylurea (DCMU).

The red absorption maximum of the particles in this middle band – henceforth called photosystem II particles – was found at 672 nm and the maximum of their low temperature fluorescence emission spectrum at 685 nm upon excitation with blue light. Cytochrome  $b_{559}$  was the only cytochrome found in these particles; it was present at an average ratio of one molecule cytochrome per 40–50 molecules chlorophyll a. C550 photoreduction with accompanying photooxidation of cytochrome  $b_{559}$  was also observed in the photosystem II particles. Good photosystem II preparations did not contain any detectable amounts of P700.

By means of sodium dodecylsulfate polyacrylamide gel electrophoresis the polypeptide composition of the photosystem II particles was studied. Dissolution of the chlorophyll protein complexes was done under strongly denaturing conditions; consequently, no green bands were observed on the gels. The polypeptide pattern of the photosystem II particles showed two strong predominant bands of protein components with apparent molecular weights (app. mol. wts.) of about 50 000 and 48 000. These two bands are unique for photosystem II. Two other weaker bands were also found characteristic for photosystem II, the band of a polypeptide with an app. mol. wt. of 38 000 and that of a polypeptide with an app. mol. wt. of 31 000. Sometimes in addition the weak band of a polypeptide with the app. mol. wt. 27 000 was observed on the gel. The polypeptide 38 000 aggregated upon boiling of the sample in the presence of the denaturing agents prior to the electrophoresis, yielding an aggregate with an app. mol. wt. of 50 000. Additional polypeptides which were often found in the photosystem II particle preparation could be identified as subunits of the coupling factor of photophosphorylation  $CF_1$ . None of the polypeptides described as characteristic for photosystem II are due to proteolytic activity.

As the observed photosystem II activity was found to be DCMU-sensitive it appears that the DCMU-binding protein is among the here described photosystem II polypeptides. Moreover, the authors have reason to believe that one of the major protein components found characteristic for photosystem II is cytochrome  $b_{559}$ .

## Introduction

Although we have gained rather detailed knowledge about the polypeptides involved in photosystem I activity [1–7] much less information is

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*Abbreviations:* DPC, diphenylcarbazide; DCPIP, 2,6-dichlorophenol-indophenol; DCMU, N,N'-3,4-dichlorophenyl-dimethylurea.

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available on the protein part of photosystem II, though many attempts have been made in this direction. Moreover, nearly all the existing information is on photosystem II of higher plants [3, 6–15], green algae [16–18] or *Euglena* [19]. Very little is known about the polypeptides characteristic for photosystem II in blue-green algae and how they compare to those of higher plants and other algae [20, 21]. In addition part of this information has only been gained by conclusion from analogy [21]. This is also true for some of the data for higher plants [11].

There are four principally different ways to approach the identification of the polypeptides involved in photosystem II activity:

1. One way is to isolate mutants which lack certain characteristics of photosystem II, and to investigate whether there are one or several protein components lacking in parallel to the defect. Very much work has been done in this field for higher plants [9, 14, 15] and also for green algae [16–18]. The disadvantage in this approach lies in the fact that it is difficult to find out whether the mutation has exclusively affected photosystem II or not.
2. Another way to identify the photosystem II polypeptides is to study developing chloroplasts and to relate the de novo synthesis of certain membrane protein components to the appearance of functions during the development [19].
3. A third way of identifying photosystem II polypeptides is the use of specific antisera against isolated membrane protein components for the localization of the antigens in the membrane and for their functional characterization [3, 5, 6, 8, 22–25]. There is no possibility, however, of deciding whether the inhibition of an electron transport reaction is due to the adsorption of an antibody to an electron carrier itself or to a neighboring molecule which is in close contact with the electron carrier.
4. The fourth and possibly best way to characterize photosystem II polypeptides is to isolate small active photosystem II particles from the thylakoid membrane and to analyze their polypeptide composition. This gives an immediate relation between function and structure [12, 13]. It is necessary, however, to remove or at least recognize contaminating protein components. Otherwise there is the danger of a faulty diagnosis [20].

Although the photosystem II activity of higher plants appears to be fairly stable, photosystem II is easily inactivated in most blue-green algae. Only recently it has been found that some thermophilic blue-green algae have a higher stability of photosystem II [26–28]. Among the non-thermophilic blue-greens the genus *Synechococcus* appears to have a more stable photosystem II [20].

The present paper gives a procedure for the isolation of active photosystem II particles from *Anacystis nidulans* (*Synechococcus* sp., strain 6301 [29]). Moreover, data are presented which allow for the identification of the polypeptides characteristic for photosystem II.

## Materials and Methods

*Conditions of cell culture:* *Anacystis nidulans* (*Synechococcus* sp., strain 6301 [29]) was grown in Allen's media [30]. The cultures were grown in constant light (5000 ergs/cm<sup>2</sup> · sec). They were shaken and gased with a mixture of air and CO<sub>2</sub> (about 5%). The growth temperature was 20–25 °C.

*Isolation of a thylakoid membrane fraction:* 6–7 day old cultures were harvested and used for the isolation of a thylakoid membrane preparation according to the method of Ono and Murata [31] which includes a lysozyme treatment and a subsequent short ultrasonication of the cells. The ultrasonication and all following operations were carried out at 2–5 °C in a darkened room. About 10% of the original amount of chlorophyll was found in this fraction.

The thylakoid membrane containing fraction was subsequently cleaned once or twice in a discontinuous sucrose gradient, the sucrose concentrations used being those of Chua and Bennoun [16], the buffer being 0.05 M Tricine-NaOH pH 7.5 with 10 mM MgCl<sub>2</sub>. Thylakoids corresponding to 0.5–0.8 mg chlorophyll were suspended in 10–12 ml of the above mentioned buffer containing 1.8 M sucrose. This suspension was overlaid with 4 ml of the MgCl<sub>2</sub>-containing Tricine buffer with 1.3 M sucrose. The top layer of the gradient consisted of about 7 ml 0.5 M sucrose in 0.05 M Tricine-NaOH buffer pH 7.5 which contained 10 mM MgCl<sub>2</sub>. The gradient was centrifuged for 90 min in an angle rotor (Ti60, Beckman) at 52000 rpm which corresponds to 270000 × *g*<sub>max</sub>. After the centrifugation the thylakoid membranes were found in the lower part of the 1.3 M sucrose layer while unbroken cells had pelleted. The pigment P 750 [32, 33] and phycocyanin were found in the 1.8 M sucrose containing bottom layer of the gradient. About 50% of the chlorophyll of the original thylakoid membrane preparation was found floating in the 1.3 M sucrose layer. When this cleaning step was repeated the yield was 40% of the original thylakoid membrane preparation.

The preparation of washed thylakoids was collected and diluted with two volumes of 0.05 M Tricine buffer pH 7.5 which was 10 mM in MgCl<sub>2</sub>. The thylakoids were collected from this suspension by a 70 min spin at 80000 × *g*<sub>max</sub>.

*Separation of photosystem I and II:* Thylakoids corresponding to 0.5–0.8 mg chlorophyll were sus-

pended in 2–3 ml of a solution containing 0.24% of the zwitterionic detergent Miranol S2M-SF [34, 35], 5% sucrose and 10 mM  $\text{MgCl}_2$  in 0.01 M Tricine-NaOH buffer pH 7.5 and then were loaded on a linear 10–18% sucrose gradient. The buffer in the gradient was 0.01 M Tricine-NaOH pH 7.5 with 10 mM  $\text{MgCl}_2$  and 0.24% Miranol S2M-SF. Thus, 6–15 mg detergent were used per mg chlorophyll. The gradients were prepared by means of an ISCO Dialagrad Model 382. The loaded gradients were centrifuged for 12–16 h in a swinging bucket rotor (SW27, Beckman) at  $131\,000 \times g_{\text{max}}$ . After the run, the gradients were fractioned into equal fractions of about 1.5 ml; and then the fractions were analyzed.

*Removal of coupling factor and carboxylase from the membranes* prior to the separation of photosystem I and photosystem II particles was carried out according to Strotmann *et al.* [36].

*Inhibition of proteases* during the cleaning procedure of the thylakoids and during the separation of photosystem I and II was done with phenylmethyl sulfonylfluoride, with 1,10-phenanthroline and with iodoacetate. The concentrations of the inhibitors used were those published by Weber and Osborn [37].

*Isolation of the coupling factor of photophosphorylation  $CF_1$*  from the thylakoids of *Anacystis nidulans* was performed according to the method of Binder and Bachofen [38].

*Sodium dodecylsulfate polyacrylamide gel electrophoresis of the membrane polypeptides:* The membrane proteins were dissolved in 0.03 M sodium borate/HCl solution [52] with 2% sodium dodecylsulfate (SDS) and 2%  $\beta$ -mercaptoethanol. The pH of the mixture was 7.4. The samples were allowed to sit for 12 to 16 h at room temperature prior to the electrophoresis. Heating of the samples was generally avoided as this leads to a selective aggregation of some polypeptides. If it was done for comparison reasons, the samples were heated for 2 min at 100 °C.

For the separation of the polypeptides a discontinuous electrophoresis system (System IV) developed by Machold *et al.* was used [39]. Gels were stained with Coomassie brilliant blue as published by Chua and Bennoun [16]. The apparent molecular weight of the various polypeptide components was calculated using the following protein standards: Bovine serum albumin (68 kD), ovalbumin (45 kD),

creatine phosphokinase (40 kD), carbonic anhydrase (30 kD), chymotrypsinogen A (25 kD) and lysozyme (14.3 kD).

*Measurement of electron transport reactions:* Photosystem I activity was measured as anthraquinone-2-sulfonate Mehler reaction as described by Koenig *et al.* [40] with an oxygen electrode from Yellow Springs Instruments (YSI Model 53). Photosystem II activity was measured as photoreduction of dichlorophenol-indophenol (DCPIP) with either water or diphenylcarbazide (DPC) as the electron donor as described by Vernon and Shaw [41]. The buffer in the assay mixture was the one used by Fredricks and Jagendorf [42]: 0.01 M sodium phosphate buffer pH 5.8, which contained 23  $\mu\text{M}$  NaCl, 7  $\mu\text{M}$   $\text{CaCl}_2$  and 5% polyethylene glycol 4000 (Carbowax PEG 4000, Union Carbide Corporation).

*Chlorophyll concentrations* were determined using the procedure published by Klein and Vernon [4].

*Absorption spectra* were recorded with a Cary Model 118.

*The low temperature fluorescence emission spectrum* was recorded as described by Butler and Strasser [43] in Dr. Butler's laboratory at the University of California, San Diego.

*The cytochrome determination* was performed as done by Newman and Sherman [20]. Chemically reduced minus oxidized difference spectra were recorded with a Cary Model 118. A millimolar extinction coefficient of 20 was used for cytochrome  $b_{559}$  [44].

*P 700* was determined from the ascorbate minus ferricyanide difference spectrum using an extinction coefficient of 64  $\text{mM}^{-1} \cdot \text{cm}^{-1}$  [45].

*The photoreduction of C 550 and the accompanying photooxidation of cytochrome  $b_{559}$*  was measured by Dr. Butler of the University of California, San Diego, as described by Erixon and Butler [46].

*Manganese* was determined by flameless atomic absorption spectrophotometry with a Perkin Elmer Model 603.

*Chemicals* were used analytical reagent grade where possible. Sodium dodecylsulfate purchased from Schwarz/Mann was routinely worked with, but sometimes SDS from Pierce was used for comparison. Acrylamide and N,N'-methylene-bis-acrylamide were purchased from Sigma.

## Results

A thylakoid membrane containing fraction was isolated from the blue-green alga *Anacystis nidulans* according to the procedure published by Ono and Murata [31]. This fraction contained, besides thylakoids, unbound phycocyanin and the pigment P 750 [32, 33]. The contaminants were removed by floating the thylakoids in a discontinuous sucrose gradient. When this cleaning step was repeated the resulting thylakoid preparation was nearly completely free of the pigment P 750 (Fig. 1). About 50% of the chlorophyll of the original thylakoid membrane preparation was recovered after the first discontinuous sucrose gradient and 40% after the second cleaning step.

The cleaned thylakoid membranes showed a photosystem II activity of about 130  $\mu\text{mol}$  DCPIP reduced per mg chlorophyll and hour with water as the electron donor and a value of about 170, sometimes 200, when DPC was used as the electron donor. Both reactions were almost completely inhibited by DCMU. The reason for the generally low activity is not known. The use of donors and

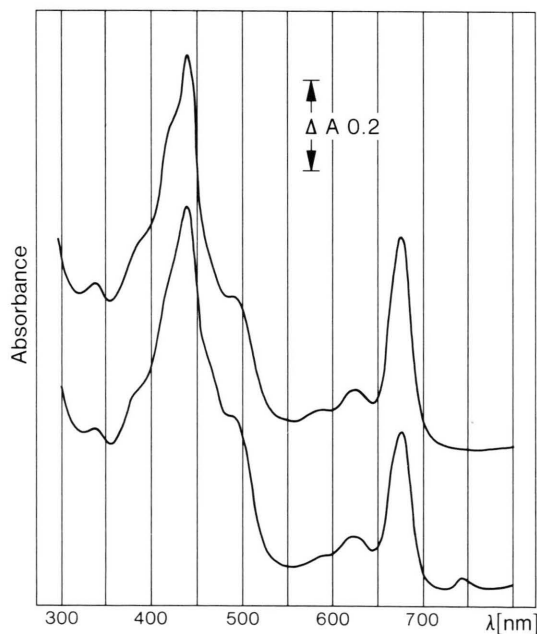


Fig. 1. Absorption spectrum of the thylakoid membrane preparation before (lower curve) and after (upper curve) floating the thylakoids in a discontinuous sucrose density gradient. The spectra were recorded at room temperature. After floating the thylakoid membrane preparation is free of the pigment P 750.

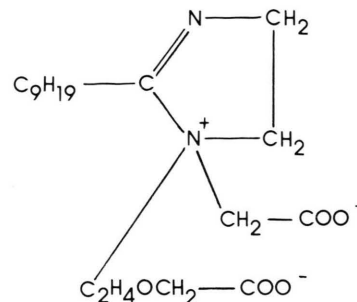


Fig. 2. The zwitterionic detergent Miranol S2M-SF.

acceptors other than DPC and DCPIP did not give better results.

The solubilization of the thylakoid membrane proteins was done with the zwitterionic detergent Miranol S2M-SF (Fig. 2) [34, 35]. In contrast to many other detergents Miranol S2M-SF does not completely destroy the photosystem II activity in *Anacystis*, but only reduces it. When this detergent was used in a concentration of 0.24% in the buffer at a ratio of detergent to chlorophyll of 6–15 mg/mg the photoreduction of DCPIP with DPC as the electron donor went down to 30–60  $\mu\text{mol}$  DCPIP reduced per mg chlorophyll and hour within the first five hours. When the Tricine buffer in which the thylakoids were exposed to the Miranol contained 10 mM  $\text{MgCl}_2$  and 5% sucrose, a higher activity was maintained than when it did not contain sucrose and  $\text{MgCl}_2$ . The  $\text{MgCl}_2$  precipitated some of the Miranol; but enough detergent stayed in the solution to dissolve the membranes.

For the separation of photosystem I and II a linear sucrose density gradient was used which also contained Miranol. While there was no separation into photosystem I and II on a 5–25% gradient, the routinely used 10–18% gradient showed a clear separation into three distinct bands, two of which were green and one was orange (Fig. 3). The dark green band located at the bottom of the gradient contained photosystem I particles. Photosystem I activity was measured as anthraquinone-2-sulfonate Mehler reaction and was determined to be 250–300  $\mu\text{mol}$   $\text{O}_2$  taken up per mg chlorophyll and hour. There was no detectable photosystem II activity in this band (Fig. 4). The particles contained cytochrome f and one molecule P 700 per 70–100 molecules chlorophyll a. The red absorption maximum of the particles in this band was at 675 nm.



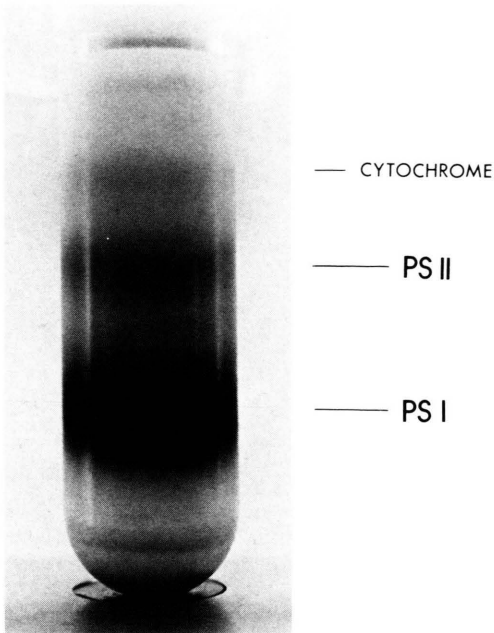


Fig. 3. 10–18% linear sucrose density gradient loaded with thylakoids of *Anacystis nidulans*. The two green bands correspond to photosystem I (PS I, lower band) and photosystem II (PS II, upper band), respectively.

The top band of the gradient was orange and contained mainly cytochrome  $b_6$  and some cytochrome  $b_{559}$ .

The middle band was always much lighter green than the lower band. It contained only about 20% of the chlorophyll of the photosystem I band (Fig. 4). The particles in this band showed photosystem II

activity measured as photoreduction of DCPIP with DPC as the electron donor. There was no water splitting activity detectable. The highest values measured were 100–110  $\mu\text{mol}$  DCPIP reduced per mg chlorophyll and hour. In the majority of the experiments only values of about 40–60 were found (Fig. 4). Photosystem II activity was only found in the lighter green middle band when the buffer in the gradient was Tricine. With Tris buffer of the same concentration no photosystem II activity could be detected. About 70% of the observed photosystem II activity was found to be sensitive to DCMU. Pure preparations of the photosystem II particles did not contain any detectable P700. The red absorption maximum of the particles at room temperature was at 672 nm (Fig. 5). The maximum of the low temperature fluorescence emission spectrum was found at 685 nm (Fig. 6) upon excitation with blue light. The particles were enriched in cytochrome  $b_{559}$ . They contained this cytochrome at an average ratio of one molecule per 40–50 molecules chlorophyll a. Cytochrome  $b_{559}$  was the only cytochrome found in the particles (Fig. 7). For unknown reasons, however, the photosystem II particles were not enriched in C 550. Photoreduction of C 550 accompanied by photooxidation of cytochrome  $b_{559}$  was observed in the particles, although in smaller amounts than the cytochrome  $b_{559}$  content of the particles would have suggested (Fig. 8). Manganese was not enriched in the photosystem II band of the gradient.

The photosystem I and II particles were dissolved under strongly denaturing conditions with 2% SDS and 2%  $\beta$ -mercaptoethanol. The samples were ex-

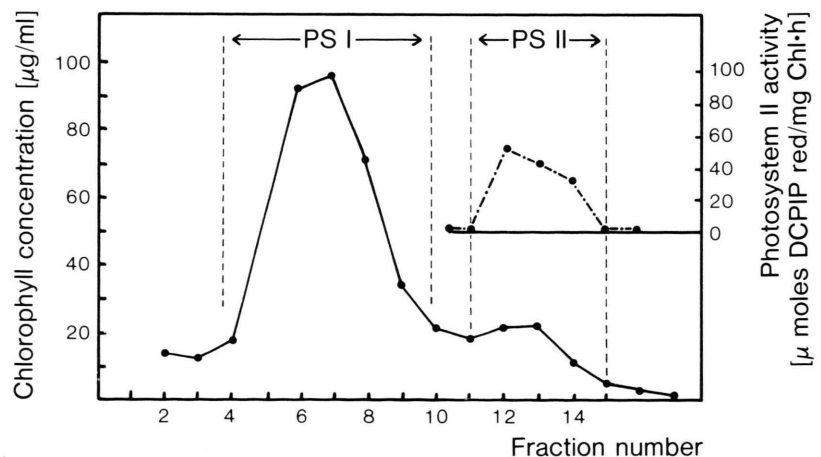


Fig. 4. Distribution of chlorophyll and photosystem II activity in the sucrose density gradient. The photoreduction of DCPIP with DPC as the electron donor was followed. The activity was found to be DCMU-sensitive.

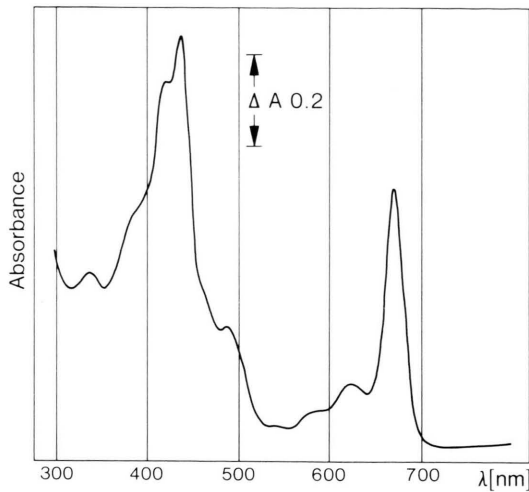


Fig. 5. Absorption spectrum of the photosystem II particles recorded at room temperature. The maximum of the red band was found at 672 nm.

posed to the denaturing agents for 12 to 16 h at room temperature prior to the electrophoresis. Consequently, no green bands were observed on the electropherograms. As heating of the samples before the electrophoresis leads to a selective aggregation of some polypeptides this was generally avoided, except for comparison reasons where indicated.

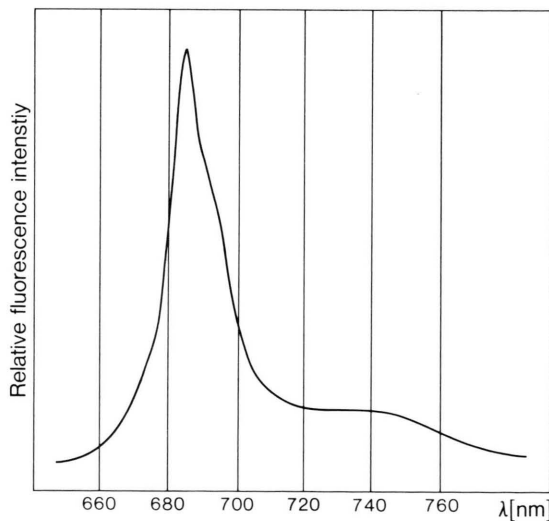


Fig. 6. Fluorescence emission spectrum of the photosystem II particles at  $-196^\circ\text{C}$ . The maximum of the emission band was found at 685 nm. Excitation was done with blue light.

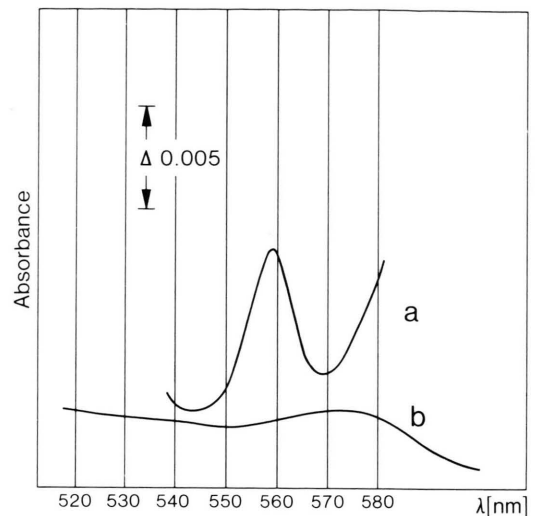


Fig. 7. Ferricyanide-oxidized minus dithionite-reduced spectrum of the photosystem II particles recorded at room temperature. a: difference spectrum, b: baseline.

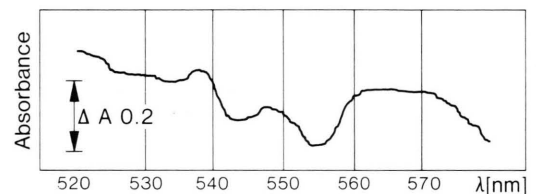


Fig. 8. Light-induced C550 reduction and accompanying cytochrome  $b_{559}$  photooxidation observed in the photosystem II particles at  $-196^\circ\text{C}$ . (Light minus dark difference spectrum.)

Despite the long incubation time of the samples in the absence of protease inhibitors it could be proven that none of the polypeptides described below are due to proteolytic activity: Addition of inhibitors of three different protease types (phenylmethyl sulfonylfluoride, *o*-phenanthroline and sodium iodoacetate) during the isolation of the photosystem II particles and also during the incubation of the particles with SDS and mercaptoethanol prior to the electrophoresis did not affect the obtained polypeptide pattern.

Slightly different polypeptide patterns were obtained for the photosystem II particles depending on the treatment of the thylakoid membranes before the separation into photosystem I and II in the presence of Miranol. Routinely, the freshly isolated thylakoids were stored frozen over night at  $-15^\circ\text{C}$  before being cleaned in the discontinuous sucrose

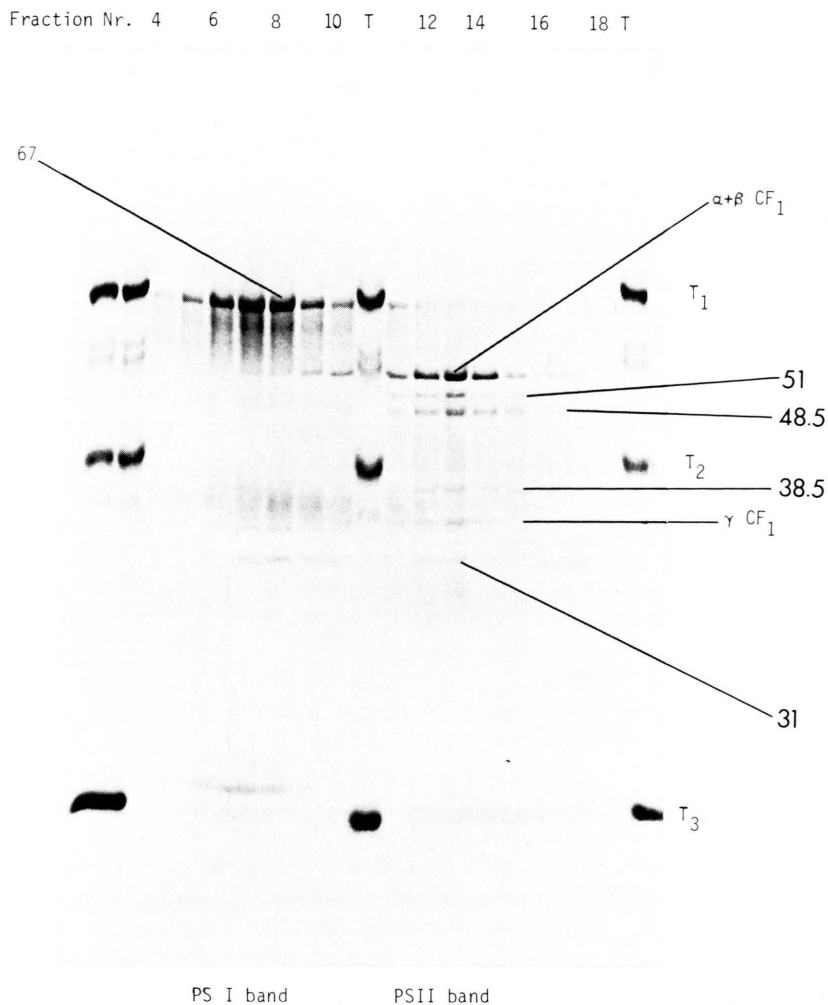


Fig. 9. Sodium dodecylsulfate polyacrylamide gel electropherogram of the fractions of the 10–18% sucrose density gradient starting from bottom (left) to top (right). T: Test,  $T_1$ : Bovine serum albumin 68,  $T_2$ : Ovalbumin 45,  $T_3$ : Lysozyme 14.3,  $\alpha + \beta$   $CF_1$  53.5,  $\gamma$   $CF_1$  35. ( $CF_1$ : Coupling factor of photophosphorylation.) The apparent molecular weights are given in kilodalton. The numbers in extra bold print mark the polypeptides characteristic for photosystem II.

gradient. In this case the following polypeptide pattern was obtained for the photosystem II particles isolated from the thylakoids by means of Miranol (Fig. 9):

1. A predominant pair of bands corresponding to apparent molecular weights (app. mol. wts.) of about 50 000 and 48 000. The latter band ran sometimes a little bit faster, then representing a 46 000 polypeptide instead. These two bands do only occur in the photosystem II band of the gradient. They are unique for photosystem II.
2. A relatively weak band corresponding to an app. mol. wt. of about 38 000.
3. An equally weak band corresponding to an app. mol. wt. of about 31 000. Both, the polypeptide of app. mol. wt. 38 000 and that of app. mol. wt. 31 000 appear to be centered in both, the photosystem I and II bands of the gradient, the 31 000 polypeptide always and the 38 000 polypeptide sometimes. There is no evidence, however, whether the polypeptides in both bands are different and have only identical molecular weight or whether they are equal.
4. Sometimes – under unknown conditions – a very weak band corresponding to an app. mol. wt. of 27 000 to 28 000 was detected in the photosystem II band of the gradient.
5. Frequently a very strong band corresponding to an app. mol. wt. of about 14 000 was observed all over the gradient; it was not centered in the photosystem II band. Therefore, it cannot be concluded

whether this component is really an element of the photosystem II particles or not. But as DCMU-sensitive photosystem II activity can also be observed in the absence of this component, it is obvious that this component is not necessary for photosystem II activity.

Additional polypeptides which can be found in the photosystem II band or overlapping with it could be identified as subunits of the coupling factor of photophosphorylation  $CF_1$  (Fig. 9). This was proven by coelectrophoresis of coupling factor isolated from *Anacystis nidulans* according to Binder and Bachofen [38]. The  $\alpha$  and  $\beta$  subunits were found to run together as one band when SDS from Schwarz/Mann was used. They then showed an app. mol. wt. of 53 000 to 54 000. The well-known double band for the  $\alpha$  and  $\beta$  subunits of coupling factor was observed when SDS from Pierce was used instead. The  $\gamma$  subunit showed an app. mol. wt. of about 35 000.

Another, more indirect way of identifying the subunits of coupling factor in the polypeptide pattern was washing the thylakoids before the separation into photosystem I and II particles according to the method of Strotmann *et al.* [36]. This cleaning step, originally developed to remove coupling factor and carboxylase from the thylakoid membrane, weakened very much the polypeptide bands in question. Often these bands completely disappeared upon the Strotmann treatment. However, there was no longer any detectable photosystem II activity left after this washing step. Therefore, this way was not further pursued.

If the thylakoids were not stored frozen over night at  $-15^\circ\text{C}$  as in the routinely used procedure but instead were stored at  $2-4^\circ\text{C}$ , the polypeptide 48 000 was found many times only in reduced amounts, sometimes also with slightly increased mobility, then representing a polypeptide of app. mol. wt. 46 000. Sometimes, this band was not visible at all. In these cases also the polypeptide 27 000 could not be detected. The reason for this variability is not known.

Two minute boiling of the sample in the presence of SDS and mercaptoethanol prior to the electrophoresis changed the polypeptide pattern of the photosystem II particles only in one point: The polypeptide of the app. mol. wt. 38 000 aggregated and was refound as a 50 000 dalton polypeptide.

## Discussion

From the here presented data about the activity, the absorption and low temperature fluorescence emission maxima and the cytochrome content of the photosystem II particles isolated from *Anacystis nidulans* it can be concluded that they are very similar to the photosystem II particles isolated by Newman and Sherman [20] from *Synechococcus cedrorum* and by Vernon *et al.* [47] and by Satoh and Butler [12] from *Spinacia*. It is not clear, however, why there was not also an enrichment of C 550 in parallel to the enrichment of cytochrome  $b_{559}$  in the particles as found by other authors [12], especially as the cytochrome  $b_{559}$  in the here described fractions was still ascorbate-reducible.

The interpretation of the position of the maximum of peak and shoulder in the low temperature fluorescence emission spectrum is still obscure [20, 48]. It is clear, however, that Katoh and Gantt's interpretation [49] is not valid in the case of the here described photosystem II particles from *Anacystis*. These authors conclude from their experiments with vesicles from the blue-green alga *Anabaena variabilis* that the 685 nm emission peak of the low temperature fluorescence emission spectrum is to be attributed to phycocyanin and not to the chlorophyll of photosystem II. This is not compatible with the here presented data: The absorption spectrum of the photosystem II particles shows that phycocyanin is, if at all present, only there in minimal concentrations (Fig. 5). In contrast to this absence of phycocyanin the maximum of the low temperature fluorescence emission spectrum of the here described photosystem II particles is still found at 685 nm (Fig. 6).

By careful observation of the behaviour of the polypeptides and by means of control experiments it was found that the number of polypeptides characteristic for photosystem II in blue-green algae is much smaller than the results published by Newman and Sherman [20] would suggest. The photosystem II preparation of these authors contains in any case at least the coupling factor of photophosphorylation  $CF_1$  as an impurity, something, they do not realize.

According to the here presented investigation the following polypeptide components are in close relation to photosystem II activity: A predominant pair of polypeptides with app. mol. wts. of 50 000 and 48 000 (46 000), polypeptides with app. mol. wts. of 38 000 and 31 000 in much lower amounts and some-

times in trace amounts a polypeptide with the app. mol. wt. of 27 000. As the photosystem II activity of the particles was found to be DCMU-sensitive, it can be concluded that one of the polypeptides described is the DCMU-binding one. There is no clue from the experiments presented here as to which this could be. By comparison with the literature, however, it can be concluded that the polypeptide with the app. mol. wt. of 31 000 may be the candidate for this component [50].

Despite the fact that cytochrome  $b_{559}$  is one of the major components of the here described photosystem II particles it could not yet be unequivocally identified in their polypeptide pattern. There is preliminary evidence, however, that the complex molecule of cytochrome  $b_{559}$  [51] does not completely dissociate into the single polypeptide chains under the electrophoretic conditions used and, instead, is found as one of the major polypeptides of higher molecular weight in the photosystem II particles: By means of anion exchange chromatography in the presence of a neutral detergent all the small molecular weight components like the 14 000 dalton polypeptide can be removed from the preparation, but not the cytochrome  $b_{559}$ . The characteristic difference spectrum (Fig. 7) is still obtained after this purification step. The observation of the behaviour of isolated cytochrome  $b_{559}$  under different dissolution and electrophoresis conditions will help to further clarify this question.

In the course of these investigations we did not only get the answer to the question: "Which are the polypeptides characteristic for photosystem II in *Anacystis nidulans*?" We got much more information. The result of the present investigation is that the membrane of the blue-green algae thylakoids is by no means so completely different from the thylakoid membrane of higher plants and green algae concerning the structure of photosystem II as the very controverse literature published in the past would suggest. Going different routes we have found that higher plants [9, 14] green algae [16–18] and the blue-green algae are at least very similar con-

cerning the structural elements of photosystem II. Even the endosymbiotic cyanelle, *Cyanocyta korschikoffiana*, from *Cyanophora paradoxa*, an organism of unique systematic position [53] has obviously a similar polypeptide composition of photosystem II. Particles isolated from the thylakoids of the cyanelle by means of the method developed for the isolation of photosystem II particles from *Anacystis*, were, unfortunately, inactive in photosystem II; they showed, however, a polypeptide pattern qualitatively similar to that of the active particles from *Anacystis*: Two polypeptides with app. mol. wts. 49 000 and about 28 000 were predominant.

There are still different opinions as to which are really the polypeptides characteristic for photosystem II; it is not clear at all for instance why Satoh's results obtained for spinach [13] are so different from those of most other investigators [9, 14, 16–18]. On the other hand Satoh's polypeptide 27 000 could correspond to the polypeptide 26 000 found characteristic for photosystem II by Schmid *et al.* [6] and to the 27 000 polypeptide observed sometimes in *Anacystis*. The authors would like to agree with Wessels and Borchert [10] that most of the observed differences in the polypeptides found characteristic for photosystem II are surely not due to species specificities, but that the diverging results are due to differences in the treatment of the thylakoids before the isolation of the photosystem II particles and to differences in the treatment of the photosystem II particles during and prior to the electrophoresis.

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