Short Communication

Spectroscopic Characterization of a Photosystem II Preparation from the Blue-Green Alga (Cyanobacterium) *Anacystis nidulans**

Göran Lagenfelt,§ Örjan Hansson and Lars-Erik Andréasson

Department of Biochemistry and Biophysics, University of Gothenburg and Chalmers Institute of Technology, S-412 96 Göteborg, Sweden

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This communication describes a further development of the Schatz and Witt SB 12 preparation[1] and an adaptation to the species *Anacystis nidulans*. This work has yielded a Photosystem II (PS II) preparation almost free of phycobilisomes, but with retained high O_2-evolving activity.

The use of oxygen-evolving PS II particles has greatly contributed to the knowledge of Photosystem II. Although most work has been done with PS II-enriched membrane fractions from higher plant chloroplasts, Stewart and Bendall in 1979[2] isolated an oxygen-evolving PS II particle fraction from the blue-green alga *Phormidium laminosum* using the zwitterionic detergent LDAO (lauryldimethylamine-*N*-oxide). Recently, Schatz and Witt[1] used the rather closely related detergent SB 12 (*N*-dodecyl-*N,N*-dimethyl-3-ammonio-1-propanesulfonate) on *Synechococcus spec.* and obtained a preparation with very high O_2-evolving activity and yield. The allophycocyanin content of this preparation is, however, 33 per PS II, and the b_6f complex content has not been measured.

Aims with our further development of the Schatz-Witt preparation. A. nidulans is by far the most extensively studied species of blue-green alga, e.g. its phycobilisome antenna has been intensively studied and is rather well described. Our object was to adapt the Schatz-Witt method to this organism and to obtain well-defined and intact (i.e. with no essential polypeptides lost) PS II particles as a starting material for further studies of Photosystem II function. Desirable qualities included a high and stable O_2-evolving activity and the almost total absence of phycocyanin. The adaptation of preparations between different species is not a trivial task; most species of blue-green algae are not closely related. Direct use of the Schatz-Witt preparation on *A. nidulans* gave PS II particles with a considerable amount of residual phycocyanin and a O_2-evolving activity significantly lower than that reported in Ref. 1.

Experimental

Materials and methods. Culture. A. nidulans was grown in 5 l flasks in a medium based on "medium C"[3] supplied with air enriched with approx. 0.5 % CO_2. Illumination was provided with fluorescent lamps (warm-white and cool-white) and the temperature was 25–30 °C.

Preparation of thylakoid membranes. The method of Schatz and Witt[1] was followed with some exceptions: The culture was harvested at a density corresponding to 8–12 mg Chl l^{-1}, the ly-

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§To whom correspondence should be addressed.

sozyme treatment was performed for 2.5 h at 37 °C, and 2 mM $CaCl_2$ was added to all solutions after the osmotic disruption.

Extraction of PS II particles with the zwitterionic detergent SB 12. The thylakoid membrane/glycerol suspension (stored at −20 °C) was thawed and diluted with ice-cold MG-1 buffer (the MES–NaOH/20 % glycerol buffer "MMPM" of Ref. 1 with pH = 6.0, but with 2 mM $CaCl_2$ added) and centrifuged at 20 000 × *g*. This gave a very deeply coloured blue supernatant. The pellet was suspended in ice-cold MG-1, centrifuged again at the same *g*-value, and the resulting pellet was resuspended in MG-1. The detergent extraction followed that of Ref. 1 with some exceptions: The extraction medium contained 2 mM $CaCl_2$, the SB 12 concentration was 0.4 % (*w/v*) and the temperature was approx. 15 °C. No ultracentrifugation was used (cf. Ref. 1); instead, the suspension was centrifuged for 30 min at 48 000 × *g* (max.) and 0 °C. The supernatant was collected and rapidly mixed with an equal volume of polyethylene glycol (*Mw* = 1500) saturated at 6 °C in MG-1 (i.e. approx. 28 % *w/v*). The mixture was centrifuged for 30 min at 48 000 × *g* (max.) and 0 °C. The pellet was suspended in MG-1 with the addition of 0.05 % SB 12 at a chlorophyll concentration of 0.5 mg ml^{-1} and loaded on "Quickseal" tubes with sucrose gradients (15–40 %, with 0.05 % SB 12) in MG-2 (buffer similar to MG-1 but with 10 % glycerol and 10 mM $CaCl_2$). Ultracentrifugation was then performed for 16 h at 239 000 × *g* and 0 °C. This yielded a concentrated PS II band. Below the band a clear blue fraction was seen, and above the PS II band faintly coloured blue-green and green fractions appeared. The PS II fraction was collected, frozen as drops in liquid nitrogen and stored at −196 °C.

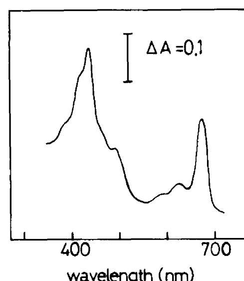

Fig. 1. Optical absorption spectrum of the PS II preparation. Chlorophyll concentration 2.90 μg ml^{-1} in MES buffer (pH 6.0) containing 20 % glycerol. Optical path 1 cm.

Table 1. Spectroscopically determined concentrations.

Component	Concentration/μM
Q_A (optical)	5.3
b-559, HP (optical)	3.5
b-559, LP (optical)	3.3
Signal II (EPR)	3.7
S_2 EPR signal	3.6
Signal I (EPR)	0.3
Rieske Fe-S (EPR)	0.3
Mn (EPR)	14
Chl (optical)	281

Analytical methods. O_2-evolving activity measurements were performed as in Ref. 1, but at pH 6.5 and 26 °C, and with 1 mM PPBQ and 2 mM $CaCl_2$. SDS-PAGE was performed as in Ref. 4 and EPR spectra were obtained as in Refs. 5 and 6. The primary acceptor Q_A was determined as in Ref. 7. High- and low-potential cytochrome *b*-559 were determined from oxidized (ferricyanide) minus reduced (hydroquinone and ascorbate, respectively) difference spectra.

Results

The optical absorption spectrum (Fig. 1) shows that the PS II particles are almost free from phycobilisomes. The absorption peak at 620 nm is mainly due to chlorophyll. The specific O_2-evolving activity was approx. 2000 μmol O_2 (mg Chl)$^{-1}$ h^{-1} at 26 °C. Table 1 summarizes the optical and EPR measurements. Fe-Q and *b*-559 were both detected by EPR but not quantified. The PS I/PS II and the b_6f/PS II quotients were both approx. 0.08, as judged from EPR. The two major light-harvesting chlorophyll-protein complexes of the PS I were not detectable by SDS-PAGE.

Conclusions

The application of a modified Schatz-Witt preparation method on *A. nidulans* has resulted in a PS II particle preparation with retained high O_2-evolving activity but with considerably reduced

phycobilisome content. In addition, the amount of the b_6f complex is very low. The *A. nidulans* PS II particles are suitable for: (i) *Optical studies.* The low pigment content in the PS II preparation and the negligible PS I and b_6f contents facilitate optical studies of PS II electron-transport components. (ii) *EPR studies.* The PS II preparation may be obtained at high concentrations. The low content of PS I components (for example iron-sulfur centres) and the b_6f-Rieske Fe-S complex facilitate investigations of PS II components. (iii) *Polypeptide composition studies.* The reduced number of bands in the SDS-PAGE gels, compared to that with thylakoid membranes, will aid in the identification of PS II functions with specific proteins.

Work on further characterization (optical and EPR properties, and polypeptide composition) is in progress in our laboratory.

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