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Integrity and activity of photosystem 2 complexes isolated from the thermophilic cyanobacterium *Synechococcus elongatus* using various detergents

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Abstract

The efficiency in selective extraction of photosystem (PS) 2 oxygen evolving complexes was compared among seven detergents. These were applied to thylakoid membranes of the thermophilic cyanobacterium *Synechococcus elongatus*. Used were five non-ionic detergents with one ionic and one zwitterionic for comparison. To compare the suitability and efficiency of the detergents the following properties of the extracts were examined: maximum rate of oxygen evolution with various electron acceptors, the relative variable fluorescence (F_V/F_M), the contamination of the extract with photosystem (PS) 1, and the status of the electron acceptor side of PS2 reaction centre. None of the detergents yielded a highly selective extraction of the PS2 complexes (negligible contamination with PS1) which would simultaneously

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Abbreviations: BQ – *p*-benzoquinone; Chl – chlorophyll; DAB – 3,3′-diaminobenzidine; DCBQ – 2,6-dichlorobenzoquinone; DCMU – 3-(3,4-dichlorophenyl)-1,1′-dimethylurea; DGP – n-decyl-ß-glucopyranoside; DM – n-dodecyl-ß-maltoside; DMBQ – 2,6-dimethylbenzoquinone, ET – electron transport; FeCy - ferricyanide; HTG – n-heptyl-ß-thioglucoside; LDAO – lauryl-dimethylamine oxide; F_0, F_M, F_V - fluorescence constant, maximal, variable; MES – 2-(N-morpholino)-ethanesulfonic acid; MV – methylviologen; OG – β-octylglucoside; OTG – n-octyl-ß-thioglucoside; PHAR – photosynthetically active radiation; PS – photosystem; Q_A – primary quinone acceptor of PS2; Q_B – secondary quinone acceptor of PS2; RC – reaction centre; SB12 – N-dodecyl-N,N-dimethylammonio-3-propane sulfonate (sulfobetaine 12); SDS PAGE – sodium dodecylsulfate polyacrylamide gel electrophoresis; TL – thermoluminescence; TM – thylakoid membranes; Tris – tris-(hydroxymethyl)-amino methane. PS2 particles isolated upon extraction of the thylakoid membranes with a detergent are identified by the abbreviation of the detergent like this: DM-PS2, HTG-PS2, *etc.*

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display a high photochemical activity and high structural intactness. Heptylthioglucoside and dodecylmaltoside yielded the nearest approximation to the optimum result. Kinetic fluorometry was applied here for the first time to characterize the functional and structural properties of PS2 particles from cyanobacteria.

Additional key words: fluorescence induction; low temperature fluorescence spectra; oxygen evolution; PQ pool; Q_A^- reoxidation; Q_B pocket; thermoluminescence.

Introduction

Selective isolation of PS2 complexes from the thylakoids of cyanobacteria is, in general, more difficult than the corresponding procedure starting from chloroplasts of higher plants. In the latter, PS2 complexes are segregated in granal thylakoids. These may be mechanically separated from the single stromal membranes and used as starting material. Thus, the chance of selective PS2 extraction is substantially increased.

In cyanobacteria no such natural segregation exists and the selectivity of extraction entirely depends on the nature of the detergent. With one exception, concerning preferential extraction of PS1 particles with *Triton X-100* (Komenda *et al.* 1989), most other detergents applied to cyanobacterial thylakoids bring about a selective solubilization of the PS2 complexes, leaving the majority of PS1 complexes in the membrane. The selectivity is not absolute and its extent varies with the properties of the detergent and of the thylakoids from various species.

Oxygen evolving PS2 particles from the thermophilic species of cyanobacterium *Phormidium laminosum* were prepared first by Stewart and Bendall (1979). They used lauryldimethylaminooxide (LDAO) for the extraction and obtained particles with the maximum rate of Hill reaction 305 $mmol(O)_2\ mol^{-1}(Chl)\ s^{-1}$ (DMBQ + FeCy as electron acceptors). In spite of the substantial contamination of the extract with other proteins, the high O_2 evolution activity made them useful for functional studies. Later on, extraction with LDAO was used successfully with other cyanobacteria such as *Aphanocapsa* (England and Evans 1981), *Synechococcus lividus* (Smutzer and Wang 1984), and *Synechocystis* P803 (Noren *et al.* 1991) as well as with the red alga *Porphyridium cruentum* (Clement-Metral and Gantt 1984).

Schatz and Witt (1984) performed a systematic investigation of PS2 extractions from the thermophilic cyanobacterium *Synechococcus sp.* with sulfobetains differing in the lengths of the alkyl chain. Up to 95 % of all PS2 complexes present in the membrane could be extracted with sulfobetain 12 (SB12) without loss of water oxidizing activity. The ratio of PS2/PS1 electron transport activities in these extracts was about 20 : 1. The efficiency and selectivity of SB12 extraction was strongly dependent on the relative concentration of the detergent with respect to the membranes treated. These results stimulated the use of sulfobetains in many laboratories. The method was improved, first of all to maintain the intactness of the acceptor side of PS2 reaction centres (PS2RC).

Further progress was due to the introduction of nonionic glycosidic detergents. Ohno *et al.* (1986) purified PS2 complexes with β-octylglucoside (OG) and found about two plastoquinone molecules per Q_A, but the Q_B content varied from 0 to 50 % of the total Q_A reduced by flashes. In particles isolated with the same detergent, Tanaka-Kitatani *et al.* (1990) and Kashino *et al.* (1992) demonstrated noticeably different rates of the Hill reaction using various substituted benzoquinones as electron acceptors. Maximum rates of oxygen evolution [mmol(O_2) mol^{-1}(Chl) s^{-1}] decreased with decreasing hydrophobicity of the acceptor: 650 for 2,6-dichloro-*p*-benzoquinone (DCBQ), 130 for 2,6-dimethyl-*p*-benzoquinone (DMBQ), and 115 for *p*-benzoquinone. The difference was attributed to a different affinity of the individual reagents to the various reduced PQ species on the acceptor side of the PS2RC, including the vacant Q_B pocket (Satoh *et al.* 1995). The distinctly different rates of oxygen evolution with benzoquinone and its derivatives indicate the PS2 RCs which were not associated with the functional Q_B site.

Similar differences in the rates of the Hill reaction with substituted quinones as electron acceptors were measured for cyanobacterial PS2 particles prepared with other non-ionic detergents. Thus, Ichimura *et al.* (1992) applied sucrose monolaurate (SML) to the thermophilic cyanobacterium *Synechococcus elongatus,* Satoh *et al.* (1995) used *n*-heptyl-β-thioglucoside (HTG), and Egashira *et al.* (1995) a mixture of HTG and OG with the thermophilic *Synechococcus vulcanus*.

N-dodecyl-β-maltoside (DM) was first used by Bowes *et al.* (1983) for the stabilization of PS2 particles extracted from *Phormidium laminosum* with LDAO. Later on, DM alone was used in several laboratories for selective extraction of highly active PS2 particles from cyanobacteria which were used in the structural and functional studies (Rögner *et al.* 1987, Dekker *et al.* 1988, Gounaris *et al.* 1989, Shen *et al.* 1992). Burnap *et al.* (1989) using extraction with DM or a mixture of OG+DM obtained PS2 particles from *Synechocystis sp.* PCC6803 with a high rate of oxygen evolution [typically 300-650 mmol(O_2) mol^{-1}(Chl) s^{-1}], which also displayed a very good stability (Tang and Dinner 1994).

The papers quoted above represent an extensive body of experience with detergent supported selective extractions of PS2 particles from the thylakoids of cyanobacteria. With the exception of the paper by Schatz and Witt (1984), however, any systematic comparison of the efficiency and selectivity of PS2 extraction with different detergents is missing.

In this paper we try to fill this gap by comparing a set of detergents which were reported in the literature and which yielded encouraging results. The efficiency and selectivity of the extraction is compared using one starting material and the particles obtained are characterized with respect to their purity, activity, and structural integrity.

Materials and methods

Growth of the cyanobacteria: *Synechococcus elongatus* strain KOVROV 1972/8 was grown at 57 °C in a chemostat, in the medium of Kratz and Myers (1955) modified

by adding 10 mM $NaHCO_3$. Cells were grown in glass cylinders with an inner diameter of 30 mm, irradiated from one side by 500 W incandescent lamps, and aerated by a mixture of air with 2 % of CO_2. Irradiance of the cultures was adjusted by wire-net filters from 50 to 200 W m^{-2} of PHAR according to a time schedule designed to maximize the ratio of PS2 to PS1 as described in Ritter *et al.* (1992).

Isolation of the thylakoid membranes: Cells from the culture were settled by centrifugation (3000×*g*, 5 min, 4 °C) and washed with buffer A (30 mM K_2HPO_4, 5 mM $MgCl_2$, pH 7.5). Cells were resuspended in buffer B (20 mM MES, 500 mM mannitol, 10 mM $MgCl_2$, 10 mM $CaCl_2$, and 1 mM ε-aminocaproic acid, pH 6.5) containing 0.3 % lysozyme, and incubated at 37 °C for 1 h in the dark with shaking. The spheroplasts resulting from this treatment were pelleted (3000×*g*, 15 min, 4 °C) and resuspended in the hypotonic buffer C (buffer A with added ε-aminocaproic acid, 1 mM). The osmotic shock ruptured the spheroplasts and most phycobilisomes were released from thylakoid membranes. The rest of biliproteins was further reduced by two successive washings of thylakoids with buffer C, followed by a centrifugation at 6000×*g* for 15 min at 4 °C. Finally, the thylakoid membranes were pelleted (24 000× *g*, 30 min, 4 °C), resuspended in buffer B containing 10 % (v/v) of glycerol, and stored in a deep-freeze at –74 °C.

Isolation of PS2 particles: The solubilization procedure was similar with all detergents tested. Thylakoid membranes resuspended in buffer B at a concentration corresponding to 1 kg(Chl) m^{-3} were stirred in the dark with the detergent under conditions given in Table 1. The non-solubilized remnants of membranes were settled by centrifugation and the supernatant, containing preferentially solubilized PS2 particles, was either used immediately for measurements or stored at –74 °C upon addition of 20 % of glycerol.

Table 1. Conditions of incubation of thylakoid membranes and of subsequent centrifugation in the course of the extraction of photosystem 2 complexes with various detergents. TM concentrations were 1 kg(Chl) m^{-3}, centrifugation temperature 2 °C.

Detergent name	concentration [kg m^{-3}]	Incubation time [min]	Incubation temp. [°C]	Centrifugation gravity [×*g*]	Centrifugation time [h]
SML, DGP	5.0	30	20	160 000	2
SB12	3.5	30	20	160 000	2
DM	10.0	60	4	300 000	1
OTG, HTG	9.0	10	20	300 000	1

Analytical methods: For Chl *a* determination, 50 mm^3 of the samples were extracted with 5 cm^3 of methanol at room temperature. Absorbance of filtered extracts was measured at 666 nm and Chl *a* concentration calculated using $\varepsilon_M = 65.8$.

Protein composition of various fractions was analyzed by means of polyacrylamide gel electrophoresis of samples solubilized with 2 % of SDS (SDS-PAGE). Slabs of 5 % stacking gel and 15 % resolving gel containing 8 M urea were

used. The buffer system was basically that of Laemmli (1970) with slight modifications. The gels were stained with *Coomassie Brilliant Blue G 250.*

Photochemical activities: The rates of electron transport were assessed by measuring the rates of oxygen exchange with a Clark electrode in a measuring chamber described by Bartoš *et al.* (1975). 10 cm^3 of buffer B contained particles equivalent to 100 μg Chl *a*. For measuring the rate of PS2 electron transport the quinone electron acceptor was added to the final concentration of 4 mM and oxygen concentration was reduced by nitrogen aeration to a pO_2 of approximately 1 kPa. After 2 min of dark, the irradiation with 1000 μmol m^{-2} s^{-1} of red radiation from a halogen lamp transmitted by the *RG5 Schott* glass filter ($\lambda > 650$ nm) was started. PS1 activity was measured as the consumption of oxygen with the same irradiation in the presence of 2 mM DAB, 4 mM MV, and 1 μM DCMU.

Chlorophyll fluorescence and thermoluminescence measurements: F_V/F_M was measured with the *PAM 101-103* fluorometer (*Waltz*, Germany) equipped with the temperature controlled measuring chamber *ED-101US*. To avoid the contribution of free phycobilin, fluorescence was excited at 450 nm using the emitter *US-L450* covered with the short-pass filter *SP 580*. The detector was protected with a long-pass filter *RG 645*. Samples were diluted with the measuring buffer (15 mM MES, pH 6.5, 100 mM NaCl, 5 mM $MgCl_2$, 500 mM mannitol) to the final concentration 2 g(Chl) m^{-3}.

The Q_A reoxidation was followed and induction curves for assessment of the PQ pool were recorded with the Dual-Modulation Kinetic Fluorometer (*Photon Systems Instruments*, Brno, Czech Republic, for description see Nedbal *et al.* 1999). The 1 cm^3 cuvette was filled with samples adjusted to a concentration corresponding to 2 g (Chl) m^{-3}. Q_A was reduced by a single turnover saturating flash (actinic flash - AF) and fluorescence was recorded at three points per time decade from 100 μs (after the AF) to 10 s. Measuring flash (MF) duration was 5 μs, intensity 100 %, blue LEDs ($\lambda_{max} = 450$ nm), actinic flash (AF) was 25 μs long, applied from both flashing units (blue $\lambda_{max} = 450$ nm and red $\lambda_{max} = 650$ nm) simultaneously at 100 % intensity. F_0 was assessed by three MFs at 750, 500 and 250 μs before the AF; F_V was measured by MFs starting at 100 μs (after the AF) and following at times giving an equal distribution of three points per time decade (factors 1.00; 2.15; 4.64; 10.00, *etc.*). The deconvolution of the decay curves was performed with the Marquardt-Lavenberg algorithm available in the *SigmaPlot* curve fitting software. For DMF record, AF was applied in 1 ms intervals up to 12 s (120 000 flashes), MF was applied 200 μs after the AF. Numerical integration of the area above the fluorescence curve was performed using *SigmaPlot* version *4.01*.

Fluorescence emission spectra were recorded with the *Fluorolog* spectrofluorometer (*SPEX*, USA) with excitation at $\lambda = 435$ nm, and resolution set at 0.5 nm. The concentration of the samples corresponded to 5 g(Chl) m^{-3}.

Thermoluminescence was measured with a computerized laboratory-built apparatus (see Prášil *et al.* 1996). Samples were gently spread on the support filter (*Millipore HA*, pore size 4 μm, 25 mm diameter) and placed on the sample holder. The sample was kept in the dark at 30 °C for 1 min and then cooled (10 °C s^{-1}) to the

temperature of irradiation (5 °C). Sample was irradiated by single turnover flash from *Xe* flashlamp (*EG&G*). TL was recorded during linear heating (0.5 °C s^{-1}).

Results

The following properties of the PS2 oxygen evolving particles were measured: (*1*) Activity of electron transport through PS2. (*2*) Selectivity of the extraction, *i.e.*, contamination of the preparation by PS1. (*3*) Processes on the electron acceptor side of the PS2 reaction centre.

Photosystem 2 activities estimated as the rate of Hill reaction with BQ were compared with the values of relative variable fluorescence, F_V/F_M (Fig. 1). The values of F_V/F_M paralleled the electron transport activities even in the isolated particles, in which many factors can disturb the relations which tie the two parameters in intact thylakoids.

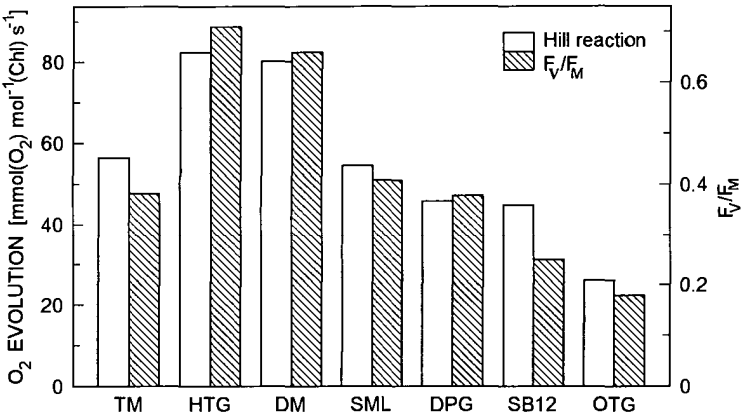

Fig. 1. Irradiance saturated rates of oxygen evolution with BQ as electron acceptor (*white columns*) and values of the F_V/F_M ratio (*hatched columns*) of thylakoid membranes (TM) and of particles extracted by various detergents. For names of the detergents see the list of abbreviations.

Particle purity in the extracts, *i.e.*, the stoichiometric ratio of PS1 to PS2, was evaluated according to three criteria: (*1*) Oxygen exchange measurements provided the ratio of radiant-energy-saturated electron transport rates through PS2 and PS1; (*2*) PAG electrophoresis revealed the relative amounts of PS2 and PS1 polypeptides; (*3*) the 77 K fluorescence emission spectra offered the ratio of areas under the emission peaks attributed to PS2 and PS1.

In a semiquantitative comparison of the results (Table 2) obtained by the three methods the only values independent of any assumption were the ratios of the PS1/PS2 activities, *i.e.*, of rates of radiant-energy saturated electron transport through the photosystems. In all following quantifications we assumed that the

Table 2. Contamination of PS2 particle extracts with PS1 complexes as indicated by the ratio of PS1 and PS2 photochemical activities, constituent proteins detected on electrophoresis gels, and the areas of low temperature chlorophyll *a* fluorescence emission peaks.

Detergent	HTG	DM	SML	DGP	SB12	OTG
PS1/PS2 ET activity	0.02	0.05	0.05	0.05	0.11	0.07
PS1/PS2 proteins	0.04	0.03	0.04	0.05	0.10	0.05
PS1/PS2 fluorescence	0.03	0.07	0.06	0.06	0.12	0.10

stoichiometrical ratio of PS1/PS2 in the thylakoid membranes was the same as the ratio of their activities, *i.e.*, 1.9 : 1.0, even if we have no proof that this is really so.

The quantity of individual proteins in the electrophoretograms (Fig. 2) was approximately estimated by integrating the area under the peaks in the records of absorbance of the stained gels. The bands of the D1 and D2 proteins and their dimer on one side and of the CP43 and CP47 proteins on the other were taken as representative of PS2. The band of PS1 trimer at 210 kDa and of its monomer at 75 kDa represented the PS1. The absorbances of these bands were assessed also for the thylakoid preparation. The latter values were then used to normalize the values obtained for the particles with respect to the assumed stoichiometry of the photosystems in thylakoids.

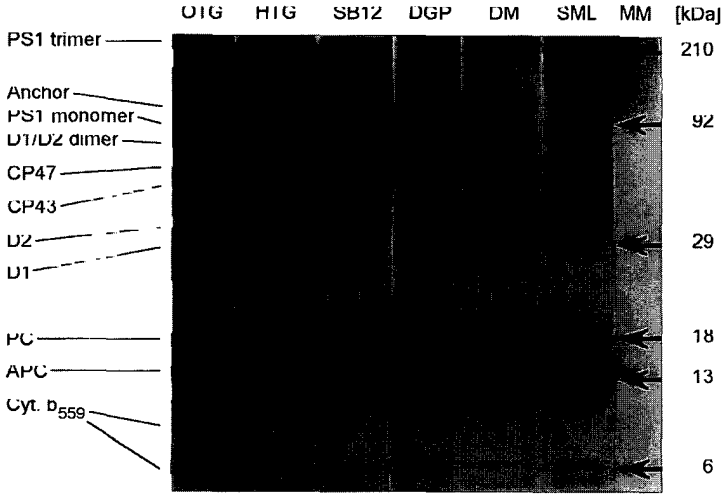

Fig. 2. SDS PAGE of photosystem (PS) 2 particles extracted by the detergents tested. PS1 is mainly present as a trimer with M_r = 210 kDa and partly as monomer with M_r = 67 kDa. *Anchor* – the band of the large linker polypeptide, PC – phycocyanine, APC – allophycocyanine. *Arrows on the right* indicate the position of protein molecular standards (the band not shown) with M_r = 6, 13, 18, 29, 92, and 210 kDa.

A similar approach was applied to the semiquantitative estimation of the photosystem stoichiometry according to the low temperature fluorescence emission

bands (Fig. 3). We started from the assumption that the fluorescence at $\lambda_{max} = 685$ and 695 nm is emitted from PS2 units and the broad band between 720 and 760 nm from PS1 units. We also assumed that PS2 has a low band in the far-red and we identified its shape with the band seen in HTG particles (Fig. 3). The latter appear to be inappreciably contaminated with PS1 according to oxygen exchange rate measurements. The ratio of the emission peak areas (upon deconvolution) found in the thylakoids was taken as characteristic of the 1.9 ratio of the photosystems. This value was used to normalize the ratio of peak areas in the particle preparations for the estimation of the PS1/PS2 ratios.

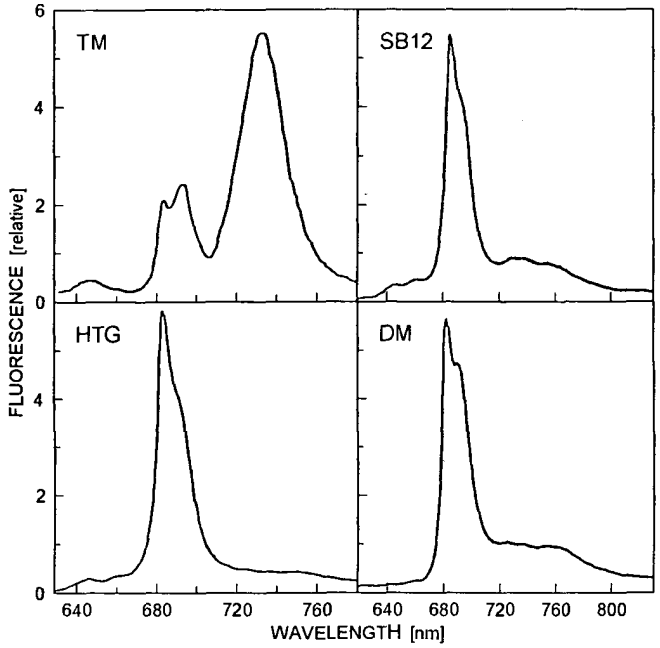

Fig. 3. 77 K emission spectra of thylakoid membranes (TM) and PS2 particles extracted by the detergents indicated by their abbreviations. Emission spectra of SML-PS2 and DGP-PS2 preparations were similar to HTG–PS2 spectra while OTG-PS2 spectra resembled those of SB12-PS2.

For a semiquantitative estimation of the PS2 effective antenna size we have used the Dual-Modulation Fluorimeter and two approaches. One used the protocol for measuring induction with relative flash irradiance of 30 % which gave a curve similar to that obtained under weak continuous irradiation (cf. Fig. 7). The curves over the induction curves recorded in the presence of DCMU were compared. In the other approach we read from the induction curves recorded with a sequence of subsaturating flashes the fraction of F_V represented by the first flash in the sequence. Both values are given in Table 4 as qualitative indications of the light-harvesting capacity in various preparations.

The pattern of the acceptor side of PS2RC: The following approaches were used to define the state of the acceptor side of the particles:

(*a*) Measurement of the rates of oxygen evolution in the Hill reaction with *p*-benzoquinone (BQ), 2,6-dichlorobenzoquinone (DCBQ), and 2,6-dimethylbenzoquinone (DMBQ) which differ in their affinities to various sites on the acceptor side of PS2 (see Discussion).

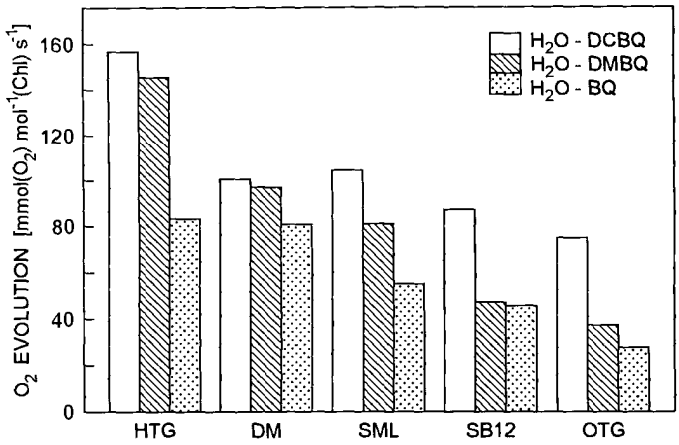

Fig. 4. Irradiance saturated rates of oxygen evolution by particles extracted with various detergents. *White columns* show the rates with DCBQ as electron acceptor, *hatched* with DMBQ, and *stippled* with BQ as electron acceptor.

The ratio of Hill reaction rates with the three quinones as acceptors was different in the different preparations (Fig. 4). The highest rate was obtained invariably with DCBQ as electron acceptor. As for the other two quinones, all possible variants are present. There was a stepwise decrease of the rate in the sequence DCBQ→DMBQ→

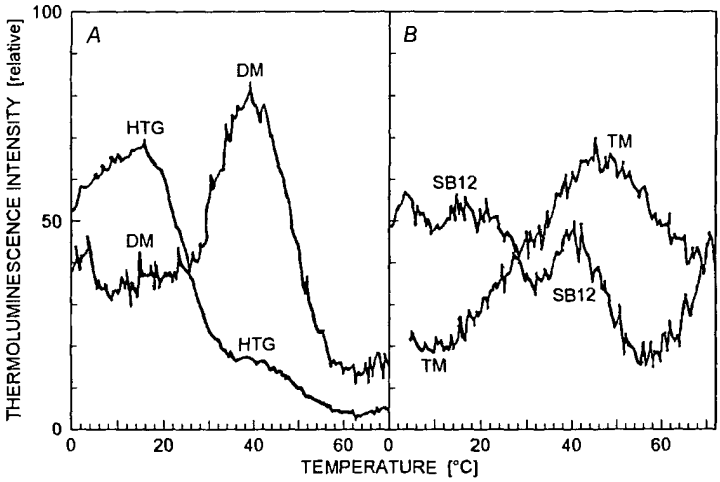

Fig. 5. Thermoluminiscence glow curves for thylakoid membranes and selected particles extracted with HTG, DM, and SB12. For detergent labelling see the list of abbreviations. TM show the isolated B band. With DM particles the B band predominates, with HTG the Q band. SB12 show both bands of approximately the same height.

BQ with small (DM–PS2) or noticeable (SML–PS2) steps. With HTG–PS2 the substituted quinones supported similar high rates, while the rate with BQ was nearly half of this value. Finally, in the HTG–PS2 and SB12–PS2, the DMBQ- and BQ-supported rates were approximately the same and about half the DCBQ supported rate.

(*b*) We have also tested the situation on the acceptor side of PS2 by thermoluminescence measurement. Large differences were found in the shape of the glow curves between the various particles (Fig. 5). The records with TM-PS2 display the classical shape of the B-band which arises from the charge recombination between Q_B^- and the S2 state of the OEC. It indicates that electrons from all Q_A^- reduced by the flash could pass without obstruction to the Q_B. The other extreme is represented by the HTG-PS2 in which the Q-band is outstanding and the B-band appears only as a very feeble shoulder. This indicates a very inefficient electron transfer on the reducing side of PS2RC. In the SB12-PS2 an intermediary situation was encountered with the Q-band only slightly higher than the B-band. This might imply a heterogeneity of the preparation with a fraction of PS2RCs in which the electron transfer from Q_A to Q_B is blocked.

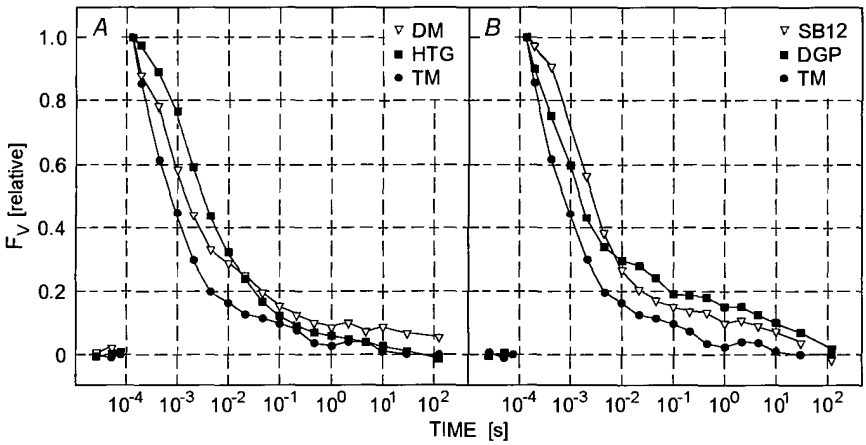

Fig. 6. Q_A^- reoxidation followed as decline of variable fluorescence in TM and in the PS2 particles extracted by various detergents. For labelling of the detergents see the list of abbreviations. The curves are displayed in a logarithmic time scale. The first measurement was taken 150 μs after the 30 μs flash to avoid artefacts arising from the hysteresis of the detector. Curves starting with a convex slope are those characterized by a slow first phase.

(*c*) Kinetics of Q_A^- reoxidation following its reduction by a single turnover saturation flash provides also information on the situation on the PS2 acceptor side. Typical recordings from which the kinetics of reoxidation was evaluated are shown (Fig. 6) on the logarithmic time scale.

In the deconvolution analysis three exponential components were assumed (Table 3). The parameters of the fast phase (f-phase) are labelled by the subscript 1, those of the medium one (m-phase) by the subscript 2, and those of the slow one (s-phase) by 3. The τ values of all three components for TM were similar to the rates

Table 3. Amplitudes (A_i), rate constants (k_i), and characteristic times (τ_i) of the three components of fluorescence decline accompanying the reoxidation of Q_A^-. A_i is expressed in percent of total amplitude.

	TM	HTG	DM	SML	DGP	SB12
A_1 [%]	57.28	61.30	68.72	61.51	67.41	70.09
k_1 [s^{-1}]	5401	509.7	999.6	954.7	1102	467.5
τ_1 [ms]	0.18	1.97	1.00	1.04	0.90	2.14
A_2 [%]	33.02	30.47	23.29	25.81	16.91	17.62
k_2 [s^{-1}]	516.6	29.27	17.35	47.03	21.26	32.69
τ_2 [ms]	1.93	34.2	57.6	21.3	47.0	30.6
A_3 [%]	9.69	8.21	7.98	12.66	15.67	12.27
k_3 [s^{-1}]	3.06	0.20	0.08	0.051	0.039	0.054
τ_3 [ms]	326	4926	11627	19607	25640	18520

reported for chloroplasts. With all particles the characteristic times were distinctly longer, *i.e.*, τ_1 and τ_2 approximately by one order of magnitude and τ_3 even more.

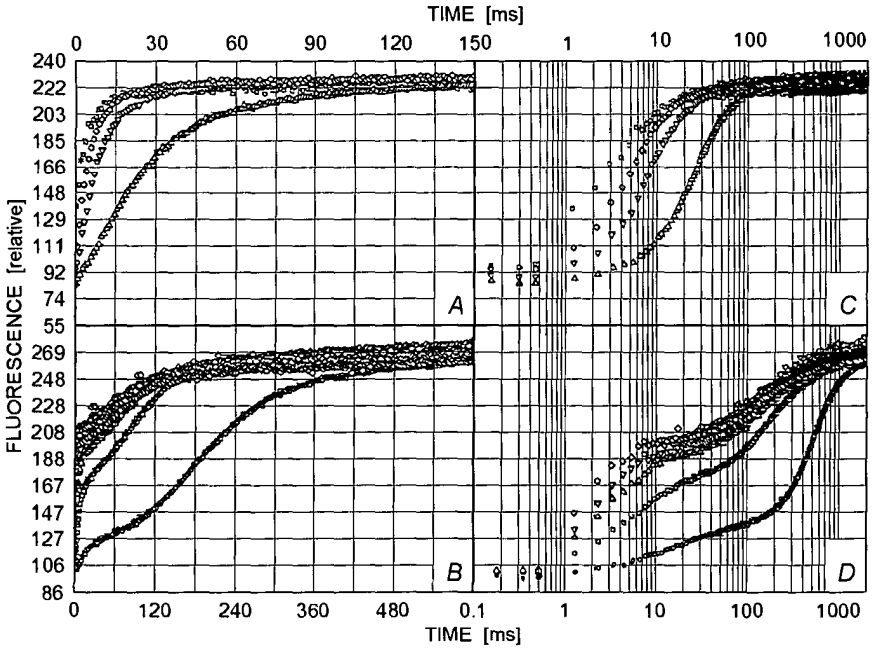

Fig. 7. Fluorescence induction curves of DM-PS2 particles elicited by sequences of subsaturating radiation pulses. *A* and *C* (in presence of 5 mmol DCMU), and *B* and *D* (without DCMU) are the same records displayed on a linear (*A*, *B*) and logarithmic (*C*, *D*) time scale. The relative power of actinic flashes was for the curves from bottom to top 10, 20, 30, and 50 % in *A* and *C*, and in *B* and *D* there is still one curve with flashes of 100 % power.

Table 4. Semiquantitative assessment of the PQ pool size and the effective antenna size in the photosystem 2 particles extracted by various detergents. PQ pool – number of PQ molecules referred to one Q_A obtained as ratio of the areas over induction curves without and with DCMU (see Fig. 8); 1/area – the reciprocal value of the area above the induction curve recorded in presence of DCMU. The value for thylakoid membranes was put equal to 1. 1st flash – the height of the first flash divided by the value of F_V.

	TM	HTG	DM	SML	DGP	SB12	OTG
PQ pool	10.0	1.7	6.0	1.7	1.5	3.5	1.5
1/area	1.0	0.75	0.51	0.24	0.28	0.37	0.25
1st flash	1.0	0.81	0.62	0.42	0.36	0.48	0.38

Our results show distinct and reproducible, even if not dramatic, differences among the parameters of the decay curves measured with different particles. Least variation displayed the amplitude and the rate constant of the fast component. 60 to 70 % of the Q_A^- became reoxidized within 1 to 2 ms, presumably by fast electron transfer to Q_B. Both the amplitude (16 to 30 %) and the τ (20 to 60 ms) of the middle phase varied more conspicuously. The third phase with τ in tens of seconds was much slower than usual and, at the same time, it displayed the most pronounced variation in τ (5 to 50 s).

(*d*) We recorded fluorescence induction curves in presence and absence of DCMU to estimate the size of the PQ pool accessible to one RC. This was more intricate than expected. The comparison gives the desired estimate only if the irradiance applied is strong enough to produce, in the absence of DCMU, a transient complete closure of the PS2 reducing side in a reasonably short time. The induction curves of our TM and PS2 particles displayed the predominantly two-step character described by Strasser *et al.* (1996) with cyanobacterial cells. With higher irradiances the fluorescence displayed a steep rise at the very beginning of the irradiation which completely distorted the customary shape of the induction curve (Fig. 7*A,B*).

This behaviour made it sometimes very difficult to find the proper irradiance to reach the peak P without DCMU and to produce an exactly measurable area above the curve so that comparison with the curve in presence of DCMU makes sense. In spite of these difficulties we succeeded in recording curves that allowed a reasonable comparison of the areas. According to these records we estimated the numbers of PQ molecules per one Q_A in TM and in the various PS2 particles (Table 4).

Discussion

The most detailed investigations of the functional properties of cyanobacterial PS2 particles obtained by selective extraction with detergent are those of Schatz and Witt (1984), Takahashi and Katoh (1986), Tanaka-Kitatani *et al.* (1990), Kashino *et al.* (1992), Satoh *et al.* (1995), *etc.*

The maximum rates of oxygen production referred to unit Chl were conspicuously lower with our particles than the rates given by other authors. Our most active

Table 5. Maximum rates of oxygen evolution [mmol(O_2) mol^{-1}(Chl) s^{-1}] from PS2 particles isolated by various authors from solubilized cyanobacterial thylakoids. Abbreviations of detergent names are explained in the list of abbreviations. PBQ - phenyl-*p*-benzoquinone.

Reference	Organism	Detergent	O_2 evolution	Electron acceptor
England and Evans (1981)	*Anacystis nidulans*	LDAO	76	1 mM FeCy
Schatz and Witt (1984)	*Synechococcus* sp.	SB12	300	1 mM FeCy
Schmutzer and Wang (1984)	*Synechococcus lividus*	LDAO	440	2 mM FeCy +1 mM DMBQ
Burnap *et al.* (1989)	*Synechocystis* PCC6803	OG/DM	308	0.6 mM DCBQ
Tanaka-Kitatani *et al.* (1990)	*Synechococcus elongatus*	OG	750	0.4 mM PBQ
Satoh *et al.* (1995)	*Synechococcus vulcanus*	HTG	1000	0.5 mM DCBQ

particles (DM-PS2, HTG-PS2) yielded 83 mmol(O_2) mol^{-1}(Chl) s^{-1} with BQ as electron acceptor and 160 mmol(O_2) mol^{-1}(Chl) s^{-1} with DCBQ. The rate of oxygen production by the TM with BQ was about 55 mmol(O_2) mol^{-1}(Chl) s^{-1}. As shown in Table 5, the majority of good PS2 particle preparations described in the literature display rates higher than 250 and the top value is 1000 mmol(O_2) mol^{-1}(Chl) s^{-1}. We do not have any reasonable explanation for this discrepancy. Since our particles were isolated from a thermophilic organism and the measurement of oxygen evolution was done at 30 °C, the low oxygen evolution rates could be caused by the low temperature. But the extremely high rates reported by Tanaka-Kitatani *et al.* (1990) and Satoh *et al.* (1995) were also measured with particles from a thermophilic strain at 30 °C.

The reduction of the oxygen evolution rate with both BQ and DCBQ as electron acceptors down to one half or one third if particles with maximum efficiency are compared with the least efficient ones (Figs. 1 and 4) is most probably due to the damage of the oxygen-evolving complex. This can be also supported by the measurements of F_V/F_M values.

The three methods used to determine the degree of contamination of the extracts with PS1 complexes agree in most cases well. This is an auspicious finding in view of the fact that neither the photometry of Coomassie blue stained polypeptides nor the areas under 77 K fluorescence bands are reliably quantitative methods. The fluorescence measurements are no doubt the least time expensive to perform and, as long as the necessary equipment is available, this is the method of choice. The values of PS1/PS2 ratio found with all the preparations fall into the range found for good preparations in the literature, *i.e.*, from 1.5 to 10 %. Comparison of the values presented in Table 2 and Fig. 1 may suggest that in some cases higher selectivity of extraction is paid for by low (OTG-PS2) or at least reduced (SML-PS2, DGP-PS2) activity. HTG-PS2 particles are, however, the purest and most active in oxygen evolution, and SB12 are the most contaminated and display only medium activity.

The idea was launched by Tanaka-Kitatani *et al.* (1990) and Satoh *et al.* (1995) and also carefully examined by Srivastava *et al.* (1995) that BQ and the various BQ derivatives differ in their ability to accept electrons either directly from Q_A^-, Q_B^{2-},

and QBH_2 or in entering the vacant Q_B pocket. The differences which we found in O_2 evolution rates with BQ, DMBQ, and DCBQ as electron acceptors fall in line, in most cases, with the conclusions of the above authors. Using the model proposed by Satoh *et al.* (1995) we can deduce from our results (see Fig. 4) that DM-PS2 can efficiently transport electrons through the PQ/PQH_2 shuttle since the rates are nearly equal with all three quinone derivatives. All the other particles display distinctly faster rates with DCBQ than with BQ which indicates that the transfer of e^- through the Q_B-pocket is barred in some way. An alternative explanation (not entirely corresponding with the proposals of Satoh and coworkers) would be that the higher rate with DCBQ is a general property of the particles. This may be possible since DCBQ can accept electrons through an alternative pathway that bypasses Q_B. In DM-PS2 it would then not be open. Whether the higher rate supported by DMBQ in the cases of HTG-PS2 and SML-PS2 could indicate that DMBQ takes electrons from Q_B^-, Fe, or some other site inaccessible to BQ cannot be decided on the basis of our results. They are, however, not at variance with such explanation since this occurs in particles containing about one PQ per RC. The difference in electron transport rates between the DCBQ and BQ is similar to that found by the mentioned authors on the basis of their measurements of Q_A reduction by one turnover saturation flashes.

We have characterized several features of the PS2 particles by fluorescence measurement. In cyanobacterial cells low values of the F_V/F_M ratio are usually encountered. This is mainly do to the relatively high value of F_0 which has two causes: (*a*) Contribution of fluorescence from phycobilins and PS1 complexes, the latter being higher in cyanobacteria than in chloroplasts of higher plants. (*b*) Exciton transfer from PS2 to PS1 complexes (spillover) which may further reduce the F_V from PS2. Since PS2 particles are strongly impoverished in both phycobilins and PS1, they may display F_V/F_M values higher than the cyanobacterial cells. This was actually confirmed by all our measurements. In addition, since absence of PS1 complexes and phycobilisomes are desirable properties of isolated PS2 particles, high F_V/F_M values can mark their good quality (Fig. 1).

We fitted the decay curves of Q_A^- reoxidation successfully by assuming three exponential components (Table 3). The values of τ for all three components were more than one order of magnitude higher for various particles as compared to the thylakoid membranes (Table 3).

The values for τ_1 and τ_2 in the particles are similar to what Tanaka-Kitatani *et al.* (1990) measured with the β-octylglucoside particles isolated from *S. elongatus* and Satoh *et al.* (1995) with the heptyl-β-D-thioglucoside particles from *S. vulcanus*, respectively. In both papers the τ-values were obtained by spectrophotometric measurements of Q_A and Q_B reduction states. A more important difference with respect to our measurements was, however, that the mentioned authors measured the kinetics of Q_A^- reoxidation in presence of artificial electron acceptors in the measuring medium. Consequently, Tanaka-Kitatani *et al.* (1990) ascribe the f-phase (in our results characterized by τ_1) to electron transfer from Q_A^- to Q_B, and the m-phase to oxidation of Q_A^- by the exogenous quinone present in the reaction mixture. The latter explanation cannot be applied to our results and this holds also for the interpretation of the s-phase. Tanaka-Kitatani *et al.* (1990) ascribe their s-phase in

absorbance changes to the reoxidation of Q_B^- by an external electron acceptor. Our s-phase of fluorescence decay can hardly have any similar cause and it is also by up to two orders of magnitude longer than found by Tanaka-Kitatani *et al.* (1990) and than the s-phase of whole thylakoids. We are unable to propose any hypothesis for the interpretation of this process. Most probably it is not due to the recombination of the electron with a hole on the oxidizing side of the RC, since this takes place in several seconds at most.

The constants for the three decay components that we calculated from the values obtained with whole thylakoid membranes are not much different from those for other organisms. Our measurements, similarly to those of Tanaka-Kitatani *et al.* (1990), were performed at room temperature, while the growth temperature of the cells was by 30 °C higher. Tanaka-Kitatani *et al.* (1990) assess an important temperature dependence of the Q_A^- reoxidation kinetics, which points to an activation energy for the f-phase as high as 42 kJ mol^{-1}. If this applies also to our thylakoid membranes, the rate of the reoxidation reactions at 55 °C would be surprisingly high. Further measurements of all processes at the growth temperature of *Synechococcus* are required to clarify this point and to formulate any serious proposal on the processes that may take place in the middle and slow phase.

The differences in the parameters of individual phases of fluorescence decay between various particles indicate that various detergents affect differently the structure of the complexes. In the thermoluminescence measurements we observed that the temperature at which the maximum of a given glow peak occurs differed in extracts obtained with various detergents; this suggests that the stability of the separated charges changes. Without a more detailed and systematic investigation the TL records do not allow any strictly quantitative conclusions. They are very useful, however, as a qualitative indication which appropriately supports other measurements. Thus the HTG-PS2 glow curves confirm that high rates of the Hill reaction with DCBQ as electron acceptor can be obtained even when the electron transfer $Q_A \rightarrow Q_B$ occurs with reduced efficiency while DM-PS2 records show that in these particles the $Q_A \rightarrow Q_B$ electron transfer is very efficient.

The fluorescence induction curves of the cyanobacterial thylakoids and particles would deserve a separate systematic investigation. The main difference in shape between these induction curves and the conventional O-J-I-P transient in green plants is that the J step becomes rather pronounced already at medium irradiances and at high irradiances there is only one early rising step before P which is responsible for the substantial part of the whole O-P rise. It is often difficult to decide whether under irradiation which brings about a sharp rise of J the latter merges with I or whether I merges with P. From comparison of several series of measurements in which irradiance was gradually increased (Fig. 8) we prefer the first alternative. But this aspect of the cyanobacterial induction curves certainly needs closer examination.

The experience from the comparison of particles obtained with various detergents can be summarized as follows: None of the detergents yields ideal particles in the sense specified in the introduction. Highest oxygen evolution yields substantiated by the intactness of the donor side are displayed by the HTG-PS2 and DM-PS2. The simultaneous use of several methods gives a possibility to get a closer idea on the

properties of the particles. Thus the HTG-PS2 show the high rates of O_2 evolution, and a high value of the F_V/F_M ratio although their acceptor side is in a poor shape as manifested by: a 90 % increase in O_2 evolution rate with DCBQ as compared to BQ (Fig. 4), the presence of 1.7 PQ molecules per Q_A (Table 4), a pronounced Q band

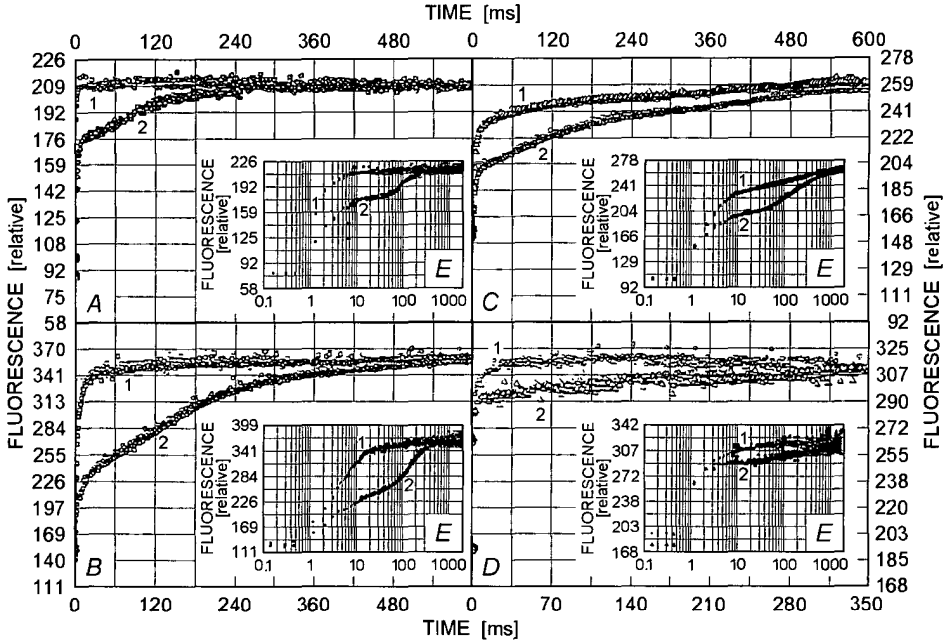

Fig. 8. Examples of fluorescence induction curves, which were used for estimation of the number of PQ molecules relative to one Q_A. All curves were elicited by flashes with the relative power 100 %. *Curve 1* is recorded in presence of 5 μM of DCMU, *curve 2* without DCMU. The *inserts* (*E*) show the same curves in the logarithmic time scale. *A* – TM, *B* – DM-PS2, *C* – SB12-PS2, *D* – HTG-PS2.

in the TL glow curve with the B band as a small shoulder only (Fig. 5*A*) and a slow first phase of the Q_A^- reoxidation (Fig. 6*A*, Table 3). On the other hand, the DM-PS2 match the HTG-PS2 in the O_2 evolution rate with BQ as acceptor, but they are not stimulated to higher rates by DCBQ since the access to the e^- acceptance site is paradoxically barred by the good shape of the acceptor side of the particles. This good condition is further manifested by: the presence of 6 PQ molecules per Q_A (Table 4), an outstanding B band with a very small touch of the Q band (Fig. 5*A*), and a fast first phase of Q_A^- reoxidation (Fig. 6*A*, Table 3). Finally, the SB12-PS2 can be described as a rather intricate mixture of contradictory properties. With respect to the reduced O_2 evolving capacity the OEC may be non-functional in half of the reaction centres present. Electron transfer $Q_A \rightarrow Q_B$ is hampered as manifested by the 90 % stimulation of O_2 production with DCBQ as e^- acceptor (Fig. 4), by the slow first phase of Q_A^- reoxidation (Fig. 6*B*), and by both Q and B bands visible on the TL glow curve, with slight predominance of the Q band. Similar detailed analyses which point to an agreement between the indications provided by the various methods, may be elaborated also for the other particles.

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