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CHARACTERIZATION OF A PHOTOSYSTEM II REACTION CENTER COMPLEX ISOLATED BY EXPOSURE OF PSII MEMBRANES TO A NON-IONIC DETERGENT AND HIGH CONCENTRATIONS OF NaCl[1]

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ABSTRACT

A highly resolved PSII reaction center complex has been prepared by exposure of PSII membranes to the detergent octylglucopyranoside at elevated ionic strengths; oxygen evolution activity is about 1,000 μmoles O_2/hr/mg Chl in the presence of $CaCl_2$. A Mn quantitation and a kinetic study of Z, the donor to P_{680}, reveals that on a Chl basis this new preparation shows an almost four-fold enrichment in Mn and the electron transport components of PSII.

1. INTRODUCTION

Research in several laboratories (3, 9, 10, 13) has produced highly refined preparations of a PSII "core" complex which, although photochemically active, does not possess the capacity to evolve oxygen. More recent work (6, 11, 12) has produced isolated oxygen evolving reaction center complexes from PSII membranes by dissociating these membranes with non-ionic detergents such as digitonin or octylglucopyranoside. The product of these procedures is a purified PSII reaction center complex depleted of the water soluble 17 and 23 kDa polypeptides, and these preparations therefore require the presence of non-physiological concentrations of Ca^{2+} and Cl^- for optimum oxygen evolution activity (5, 6, 11, 12). In this communication we report results from a new method (5) for isolation of a PSII reaction center complex. A manganese quantitation and a spectroscopic study of Z, the primary donor to P_{680}, reveals a four-fold enrichment, on a Chl basis, in the Mn-content as well as the electron transport components of PSII.

[1] Dedicated to the memory of Warren Butler, whose research provided new insights and ideas about the structure and function of PSII.

Abbreviations: BZ, benzidine; Chl, chlorophyll; DCBQ, 2,5-dichloro-p-benzoquinone; EPR, electron paramagnetic resonance; LHC, light harvesting complex; OGP, 1-O-n-octyl-b-D-glucopyranoside; PSII, Photosystem II; R.C.C, reaction center complex; Tris, 2-amino-2-(hydroxymethyl)-1-3-propanediol.

2. MATERIALS AND METHODS

PSII membranes, prepared as in (4), were treated as in (5) to produce the highly active oxygen evolving PSII reaction center complex. Tris treated systems were prepared by exposure of PSII preparations to 0.8M Tris plus 1mM EDTA. Oxygen evolution activity was measured with a Clark-type oxygen electrode. Gel electrophoresis was carried out as in (2) with the modifications described in the figure captions. EPR spectroscopy was carried out on a Bruker ER-200D spectrometer operated at X-band and interfaced to a Nicolet 1180 computer. Instrument modifications, as well as the flash lamp circuitry and the protocol for signal averaged, flashing-light kinetic experiments, are described in ref. 15.

3. RESULTS

Fig. 1, lane 2 shows that the new PSII Reaction Center Complex (R.C.C.) consists of the hydrophobic polypeptides observed in "core" complex preparations from PSII, along with two other hydrophobic polypeptides (with molecular weights which we estimate to be about 20 and 28 kDa), and the hydrophilic 33 kDa species; the complex has been depleted of the water-soluble 17 and 23 kDa polypeptides and thus the presence of non-physiological concentrations of both Ca^{2+} and Cl^- are required for oxygen evolution activity (see ref. 5). In contrast to the preparation described in (6), the PSII reaction center complex isolated by our procedure is very active when DCBQ is present as an electron acceptor; $Fe(CN)_6^{3-}$ appears to be a more effective acceptor in this new preparation when compared to control PSII membranes, but is not as effective as DCBQ (data not shown).

As shown in Table I, an analysis of the manganese content of this new preparation reveals an almost four-fold enrichment of manganese on a Chl basis. Since a series of experiments carried out by Matsuda and Butler (8), has clearly demonstrated that high potential Cyt b_{559} reveals the structural integrity of the photosynthetic membrane and that disruption of that integrity causes Cyt_{559} to be modified to lower potential forms, we studied the state of Cyt_{559} in this new preparation by use of EPR spectroscopy. We have found that a significant amount of high potential Cyt_{559} has shifted to lower potential form(s) in the Complex; even though addition of $CaCl_2$ reconstituted oxygen evolution activity, it did not restore Cyt_{559} to its high potential form(s) (data not shown).

When a higher ionic strength (1M NaCl) was used during exposure of PSII membranes to OGP, the PSII reaction center complex which resulted was also depleted of the water soluble 33 kDa polypeptide (Fig. 2) as well as most of the manganese (Table I). This preparation shows very little oxygen evolution activity (data not shown). This modified isolation procedure (35 mM OGP + 1M NaCl) was also applied in Tris-treated PSII membranes; the polypeptide content of the reaction center complex which results from such a treatment

is shown in Fig. 2. The kinetic behavior of Z^+ in this Tris-PSII R.C.C. was studied by EPR and was compared to the kinetic behavior of Z^+ observed in Tris-PSII membranes. As shown

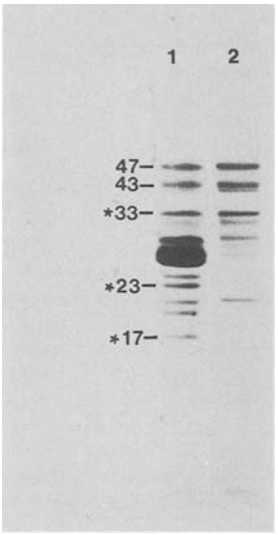


Fig. 1. Gel electrophoresis patterns of (1) untreated PSII membranes; (2) O_2 evolving reaction center complex prepared by exposure of PSII membranes to 35 mM OGP + 0.5 M NaCl. A 15% acrylamide resolving gel was used and 6M urea was present in the gel. (*) denotes water soluble extrinsic polypeptides.

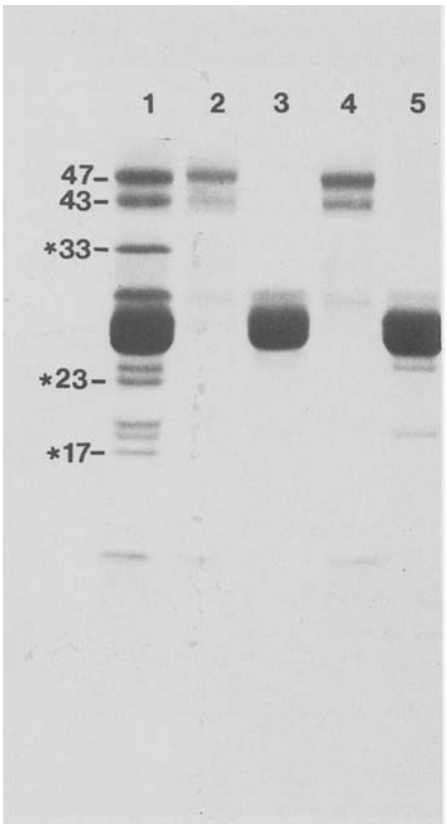
Table II: Mn Content of Various PSII Preparations

Preparation	Mn-Content (atoms/250 Chl)
PSII Membranes	4[a]
PSII Reaction Center Complex (35:1.0)	6.0
Tris-PS II Membranes	0.6
Tris-PSII Reaction Center Complex (35:1.0)	0
PS II Reaction Center Complex (35:0.5)[b]	14.8

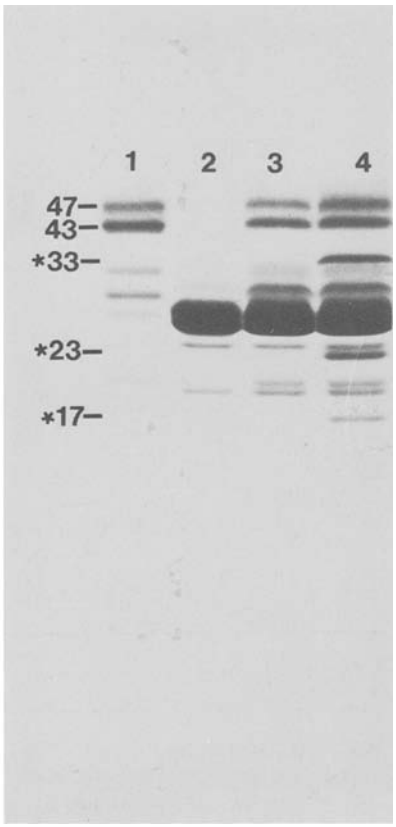
a. See refs. 1 and 4
b. (x:y); x = concentration of OGP (mM), y = concentration of NaCl (M) used for the isolation of the R.C.C.

in Fig. 3B, Z^+ decays relatively slowly in Tris-treated PSII R.C.C., but its decay is dramatically accelerated upon addition of an exogenous donor such as benzidine. A calculation of the second order rate constant for benzidine from the data of Fig. 3B gives $k = 6.0 \times 10^6\ M^{-1}.s^{-1}$; this rate constant is higher compared to that observed in Tris PSII membranes ($k = 1.0 \times 10^6\ M^{-1}.s^{-1}$). A comparison of the amplitude of the kinetic traces of Z^+ in the Tris-PSII reaction center complex (Fig. 3B) with that observed in Tris-PSII membranes (Fig. 3A) reveals that, on a Chl basis, an enrichment of Z^+ approaching four-fold has occurred in the Tris-PSII R.C.C.

2A.

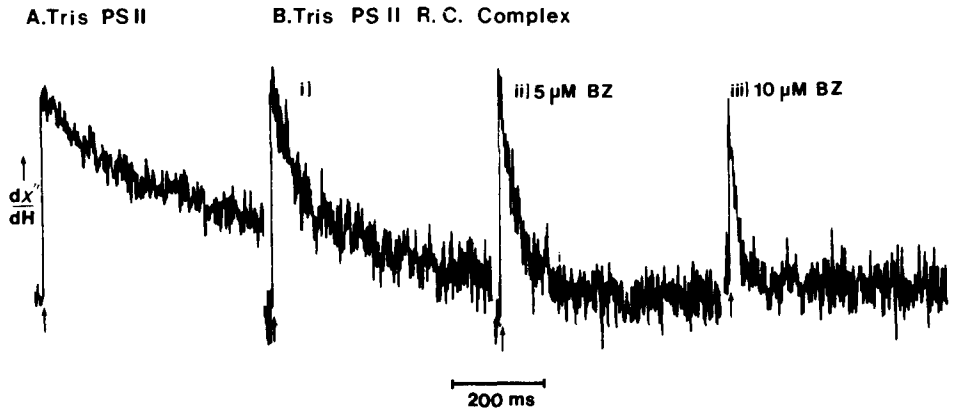


2B.



2A. Gel electrophoresis patterns of (1) untreated PSII membranes; (2) the reaction center complex prepared by exposure of PSII membranes to 35mM OGP plus 1M NaCl (Fraction B); (3) Fraction A obtained from treatment in 2; (4) the reaction center complex prepared by exposure of Tris-treated membranes to 35mM OGP plus 1M NaCl (Fraction A); (5) Fraction A obtained from treatment in 4. A combination of a 12% (upper part) and 18% (lower part) acrylamide resolving gel was used and 6M urea was present in the gel.

2B. Gel electrophoresis patterns of (1) the reaction center complex prepared by exposure of Tris-PSII (2) Fraction A obtained from treatment in 1; (3) Tris-treated PSII membranes; (4) untreated PSII membranes. Conditions as in Fig. 1.



3. Kinetic transients of Z^+ at room temperature in A) Tris-treated PSII membranes (4.16 mg Chl/ml) and B) the PSII reaction center complex prepared from Tris treated PSII membranes (0.56 mg Chl/ml); i) 0 μM Bz, ii) 5 μM Bz and iii) 10 μM BZ. Instrument conditions: time constant 1 ms, flash rate 0.25 Hz, modulation 4 Gpp, gain 10 x 10^5 and number of scans averaged 100 (for A) and 200 (for B). A mixture of 3 mM $Fe(CN)_6^{3-}$ and 3 mM $Fe(CN)_6^{4-}$ served as an artificial acceptor system.

4. DISCUSSION

Although the isolation of Photosystem II membranes (1, 7, 14) has advanced knowledge of the polypeptide composition of PSII, further resolution of the PSII system is necessary for effective spectroscopic studies of both the primary reactions and the water cleavage process. Recently, a series of techniques have been reported for isolation of oxygen evolving reaction center complexes (5, 6, 11, 12). As shown in Table I, the active PSII reaction center complex is enriched in Mn by a factor of four, and it is therefore a very attractive system for a spectroscopic characterization of the Mn-complex. Low temperature ESR and EXAFS experiments, which require very concentrated samples, will benefit by use of this new system. The kinetic study of Z^+ shown in Fig. 3 also demonstrates a 3.7-fold enrichment in a component of the PSII electron transport system. In addition to its use in spectroscopic studies, the reduced number of polypeptides present in the PSII reaction center complex will facilitate further elucidation of the structural role of the various polypeptides as well as their relationship to sites of catalytic activity.

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