Purification and properties of an oxygen-evolving reaction center complex from photosystem II membranes

A simple procedure utilizing a non-ionic detergent and elevated ionic strength

Demetrios F. Ghanotakis and Charles F. Yocum

Division of Biological Sciences and Department of Chemistry, The University of Michigan, Ann Arbor, MI 48109–1048, USA

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A method is reported for the isolation of a highly resolved oxygen-evolving photosystem II reaction center preparation. This preparation can be separated from the more complex photosystem II membranes isolated by the procedure of Berthold et al. [(1981) FEBS Lett. 134, 231–234] by use of octylglucopyranoside at elevated ionic strengths; the oxygen-evolving material can be collected by centrifugation at relatively low g values (40000 x g) in yields estimated to be more than 80%. This new preparation lacks the 17 and 23 kDa extrinsic polypeptides; addition of calcium and chloride produces activities approaching 1000 μmol O₂/h per mg chlorophyll. Although activity is maximal in the presence of 2,5-dichloro-p-benzoquinone, the response of activity to ferricyanide and 3-(3,4-dichlorophenyl)-1,1-dimethylurea indicates that the reducing side of photosystem II has been modified in this new oxygen-evolving reaction center preparation.

Oxygen evolution  Photosystem II  Polypeptide  Reaction center

1. INTRODUCTION

The isolation of PS II membranes by exposure of thylakoids to various detergents [1–4] has advanced knowledge of the polypeptide composition of PS II and has also provided a means to understand better the relationship between certain of these polypeptides and sites of catalytic activity. Preparation of a PS II ‘core’ complex [5–7] has made it possible to identify certain hydrophobic polypeptides which bind catalysts of PS II photochemistry. More recently, Tang and Satoh [8], Satoh et al. [9] and Ikeuchi et al. [10] isolated oxygen-evolving RC complexes from PS II membranes which were further solubilized by use of non-ionic detergents such as digitonin or OGP. Ikeuchi et al. [10] used a sucrose density gradient of OGP-solubilized PS II membranes, whereas Satoh et al. [9] used a combination of density gradient centrifugation followed by two-step column chromatographic separation. In all cases, the isolated RC complex was depleted of two water-soluble polypeptides (17 and 23 kDa) and thus required the presence of non-physiological concentrations of Ca²⁺ and Cl⁻ in order to retain oxygen-evolution activity [9,10].

Here, we report a new method for isolation of an oxygen-evolving PS II RC complex. This method does not require ultracentrifugation, sucrose density gradients or chromatographic separations and produces an oxygen-evolving RC complex in high yields.
2. MATERIALS AND METHODS

Subchloroplast membranes, free of PS I and having high rates of oxygen evolution, were prepared as described in [2]. PS II membranes were solubilized with 35 mM OGP, 0.4 M sucrose, 50 mM Mes, pH 6.0, 10 mM CaCl₂ and 0.5 or 1 M NaCl at a Chl concentration of 1.5 mg/ml. After incubation for 15 min in ice one part of the PS II membrane suspension was mixed with two parts of a solution containing 0.4 M sucrose, 50 mM Mes, pH 6.0, and 10 mM NaCl (fraction A). The supernatant was desalted by 90 min dialysis against a solution containing 0.4 M sucrose, 50 mM Mes, pH 6.0, and 10 mM CaCl₂, further diluted by 25% using the dialysis medium, and subsequently centrifuged for 90 min at 40000 × g. The pellet which resulted from this centrifugation step was resuspended in a medium containing 0.4 M sucrose, 50 mM Mes, pH 6.0, and 10 mM CaCl₂ (fraction B); the colorless supernatant was discarded. Ca²⁺- and Cl⁻-free samples were prepared by further dialysis of the above samples against a medium containing 0.4 M sucrose and 50 mM Mes, pH 6.0.

Gel electrophoresis was carried out as in [11] with the modification that a 15% acrylamide resolving gel was used and 6 M urea was present in the gel; O₂ evolution was measured with a Clark-type oxygen electrode. EPR spectroscopy was carried out on a Bruker ER-200D spectrometer operated at X-band and interfaced to a Nicolet 1180 computer.

3. RESULTS

As shown in fig.1, the pellet (fraction A) isolated from the first centrifugation of the procedure described in section 2 consists mainly of the LHC (lanes 3,5), whereas the final pellet (fraction B) contains a complex which has been depleted of the LHC and several other polypeptides (lanes 2,6). Even though the presence of 1 M NaCl during exposure to the detergent results in the quantitative isolation of a PS II RC complex, it also releases the water-soluble 33 kDa polypeptide and thus affects oxygen-evolution activity. On the other hand, the presence of 0.5 M NaCl during solubilization results in a preparation which retains the 33 kDa protein and shows high oxygen-evolution activity (table 1). In contrast to the preparation reported by Ikeuchi et al. [10], this PS II RC complex is very active in the presence of DCBQ as an electron acceptor (table 1); Fe(CN)₆³⁻ appears to be a more effective acceptor in this new preparation when compared to control PS II membranes but is not as effective as DCBQ (table 1). As shown in table 1, oxygen-evolution activity of the PS II RC complex is sensitive to DCMU addition. The observation that Fe(CN)₆³⁻ is a relatively effective acceptor in this complex as well as the fact that DCMU is not as inhibitory as in control PS II membranes suggest that some changes have taken place at the
Table 1

<table>
<thead>
<tr>
<th>Additions</th>
<th>Activity (μmol O₂/mg Chl per h)</th>
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<tbody>
<tr>
<td>PS II membranes</td>
<td>PS II RC complex</td>
</tr>
<tr>
<td>DCBQ (500 μM)</td>
<td>640</td>
</tr>
<tr>
<td>Fe(CN)₆⁻ (2 mM)</td>
<td>140</td>
</tr>
<tr>
<td>DCBQ (500 μM) + Fe(CN)₆⁻ (2 mM)</td>
<td>600</td>
</tr>
<tr>
<td>DCBQ (500 μM) + DCMU (5 μM)</td>
<td>0</td>
</tr>
<tr>
<td>Fe(CN)₆⁻ (2 mM) + DCMU (5 μM)</td>
<td>20</td>
</tr>
</tbody>
</table>

* Assay medium: 50 mM Mes, pH 6.0, and 10 mM CaCl₂

Reducing side of PS II. Since the main difference, in terms of polypeptide content, between our preparation and that of Ikeuchi et al. [10] is a polypeptide of approx. 20 kDa (fig.1, lane 6), it is possible that this species is involved in some function on the reducing side of PS II. An examination of the manganese content of the PS II RC complex is shown in fig.2. Acidification of PS II membranes and the PS II RC complex reveals that the latter shows a 3.7-fold Mn enrichment on a Chl basis. A quantitation of Z⁺, the primary donor to P680, in Tris-treated preparations of the PS II RC complex also revealed a 3.7-fold enrichment, on a Chl basis, when compared to Tris-treated PS II membranes (not shown). As shown in the gel of fig.1, the PS II RC complex has been depleted of the water-soluble 17 and 23 kDa polypeptides; it is known that under these conditions, non-physiological concentrations of Cl⁻ and Ca²⁺ are required for oxygen-evolution activity [12–16].

Fig.2 shows the Cl⁻ requirement of the PS II RC complex, while fig.3 shows a Ca²⁺ titration in the presence of sufficient Cl⁻ (15 mM NaCl). The titrations of figs 3 and 4 clearly demonstrate the in-

![EPR signal of hexa-aquo Mn](image)

Fig.2. EPR signal of hexa-aquo Mn in (A). PS II membranes and (B) the PS II RC complex, both acidified with HClO₄. Instrumental conditions: microwave power, 64 mW; modulation amplitude, 32 Gpp; gain, 4 × 10⁵; time constant, 200 ms.

![Rates of oxygen evolution](image)

Fig.3. Rates of oxygen evolution of the PS II RC complex as a function of added NaCl. Assay medium: 50 mM Mes, pH 6.0, 500 μM DCBO and the indicated concentration of NaCl (7 μg Chl/ml). Each point is the average of 2 measurements. Variation was less than 5%.
Fig. 4. Rates of oxygen evolution of the PS II RC complex as a function of added CaCl₂. Assay medium: 50 mM Mes, pH 6.0, 15 mM NaCl, 500 μM DCBQ and the indicated concentration of CaCl₂ (7 μg Chl/ml). Other conditions as in fig. 3.

Involvement of both Ca²⁺ and Cl⁻ in oxygen-evolution activity. Since Ikeuchi et al. [10] reported that addition of digitonin in the assay medium enhanced oxygen-evolution activity by a factor of 1.5, we added digitonin to our assay system, but observed no stimulation of activity (not shown).

4. DISCUSSION

In this communication we have reported a very simple procedure for the isolation of an active PS II RC complex. Our procedure does not involve ultracentrifugation, sucrose density gradients or chromatographic separation; a simple exposure to the detergent OGP in the presence of high ionic strength followed by selective manipulation of the detergent and salt concentrations results in separation of the PS II membranes into two complexes. One of these consists mainly of the LHC whereas the other contains the hydrophobic polypeptides of the core complex as well as the water-soluble 33 kDa polypeptide, a 10 kDa polypeptide [17] and a species with a molecular mass estimated to be 20 kDa in our gel electrophoresis system. The presence of high concentrations of NaCl in the isolation procedure reported here has two effects. First, it promotes aggregation of the LHC [18,19], and second, it facilitates the dissociation of the PS II RC complex from the rest of the photosynthetic membrane.

It is instructive to compare this new oxygen-evolving PS II RC complex with the preparation described by Ikeuchi et al. [10]. As the data of table 2 demonstrate, both preparations effect an enrichment in manganese of about 3.7-fold on a Chl basis; for the present preparation, this enrichment is also observed for Z⁺, suggesting that no loss of manganese has occurred during the enrichment process. We also note that for our preparation, the manganese is EPR-silent at room temperature. Inspection of table 2 also shows that both preparations are capable of oxygen-evolution activity with rates which exceed 900 μmol O₂/h per mg Chl. Differences in the conditions necessary for this activity are shown in table 2. The preparation of Ikeuchi et al. [10] operates most efficiently with ferricyanide as the PS II acceptor in a DCMU-

<table>
<thead>
<tr>
<th>PS II RC complex prepared according to:</th>
<th>Component enrichment on a Chl basis</th>
<th>Activity (μmol O₂/mg Chl per h)</th>
<th>DCMU (5 μM) sensitivity</th>
<th>Detergent required for optimal activity</th>
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<tbody>
<tr>
<td></td>
<td>Mn</td>
<td>Z</td>
<td>+ Fe(CN)₆³⁻</td>
<td>+ DCBQ</td>
</tr>
<tr>
<td>This paper</td>
<td>3.7</td>
<td>3.7</td>
<td>530</td>
<td>940</td>
</tr>
<tr>
<td>Ikeuchi et al. [10]</td>
<td>3.7</td>
<td>-</td>
<td>846</td>
<td>461</td>
</tr>
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</table>

a The PS II RC complex was compared to PS II membranes prepared by exposure of thylakoids to Triton X-100
b Assay medium contained CaCl₂
insensitive reaction. The material produced by our procedure is optimally active in the presence of DCBQ and this activity is sensitive to DCMU (see also table 1). This new oxygen-evolving PS II RC complex has the advantage that its reducing side is relatively intact and moreover does not require the presence of a detergent for maximum activity. It is possible that the observation of Ikeuchi et al. [10] that their PS II preparation does not require DCBQ may derive from the requirement, in their preparation, for digitonin to produce enhanced rates of oxygen-evolution activity. A common property of both preparations is that in spite of substantial enhancements of components (manganese, Z) associated with the oxidizing side of PS II and the oxygen-evolving complex, oxygen-evolution activity is not comparably enriched. The explanation for this observation is unclear, but may relate to subtle changes in the reducing side of PS II, loss of LHC or both of these factors and other considerations which are not obvious at present. The enrichment in manganese content of O2-evolving RC preparations provides a very attractive system for spectroscopic characterization of the oxidizing side of PS II. These studies, an immunological characterization of the various polypeptides of this oxygen-evolving RC PS II complex, and further experiments to improve oxygen-evolution activity are now in progress.

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