Preparation of Active Fractions Enriched in Photosystem I and Photosystem II from the Thermophilic Blue-Green Alga

Phormidium laminosum

ALISON C. STEWART and DEREK S. BENDALL

Department of Biochemistry, University of Cambridge, Cambridge CB2 1QW, U.K.

Attempts to separate Photosystem I and Photosystem II in blue-green algae with retention of oxygen evolution activity, have hitherto proved unsuccessful. Ogawa et al. (1969) reported a sucrose-density-gradient separation of Triton-treated membranes from Anabaena variabilis into 'light' and 'heavy' fractions which by spectral and fluorescence criteria were identified with Photosystem II and Photosystem I respectively. However, neither fraction retained any electron-transport activity. Arnon et al. (1974) found that, in Nostoc muscorum, O₂ evolution was very resistant to digitonin, but digitonin did not effect a separation of the photosystems. More recently, Pullin & Evans (1977) have reported separation of the photosystems by detergent treatment of membrane preparations from Chlorogloeae fritschii, but little activity was retained.

We have previously reported (Stewart & Bendall, 1977) the preparation of cell-free membrane fragments from the thermophilic blue-green alga Phormidium laminosum which are stable and active in O₂ evolution. O₂ evolution in the fragments is unusually stable to treatment with non-ionic detergents such as Nonidet P40. The present report concerns the fractionation of P. laminosum membranes into two fractions that are enriched in O₂-evolution activity (Photosystem II) and in Photosystem I respectively.

Cultures of P. laminosum strain OH-1-p Cl 1 (Castenholz, 1970) were obtained from Dr. R. W. Castenholz, University of Oregon, and were grown in an orbital incubator at 45°C in continuous light in an atmosphere enriched with 5% CO₂. The culture medium was Medium D of Castenholz (1970), supplemented with NaHCO₃ (0.4g/l). Late-exponential-phase cultures were harvested by centrifuging, and spheroplasts were prepared by the method of Binder et al. (1976), with the modification that 2mM-EDTA was included in the medium during lysozyme treatment. Spheroplasts were ruptured by hypo-osmotic shock, and the resulting membrane fragments were separated from the solubilized phycobilin by centrifuging. The pellet of membrane fragments was resuspended in 25% glycerol containing 10mM-MgCl₂ and 10mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid]/NaOH, pH 7.5.

Membrane fragments at a concentration of 1 mg of chlorophyll a/ml were treated for 20min with 0.5% Nonidet P40 (detergent/chlorophyll = 5:1). After centrifuging at 100000g for 1h, the supernatant and resuspended pellet had the characteristics shown in Table 1.

The supernatant, which contained 15–20% of the total chlorophyll a, had 5–6 times the rate of O₂ evolution (expressed per mg of chlorophyll a) compared with the pellet. O₂ evolution was light-dependent and sensitive to 3-(3,4-dichlorophenyl)-1,1-dimethylurea, an indication of a true Photosystem II reaction. In contrast, the ratio chlorophyll a/pigment P700 in the supernatant was 2–3-fold that in the pellet.

The absorption spectra for the two fractions showed a predominance of shorter-wavelength forms of chlorophyll a in the supernatant, as well as larger amounts of carotenoids (absorbing in the 400–500 nm region of the spectrum) and phycobilin (absorbing at 615nm). Reduced-minus-oxidized difference spectra showed that almost all the membrane-bound cytochromes (f, b-563 and b-559) were also to be found in the supernatant.

Negative staining with 2% phosphotungstic acid and examination under the electron microscope showed that the supernatant fraction consisted predominantly of vesicles 100–200 nm in diameter, bounded by a single membrane.

The precise conditions during the detergent treatment were essential for optimal fractionation of the membranes and stability of activity in the fractions. Higher detergent/chlorophyll ratios led to greater solubilization of chlorophyll, but yielded supernatants that contained more pigment P700 and other long-wavelength-absorbing
Table 1. Properties of fractions obtained by detergent treatment of P. laminosum membrane fragments

Rates of O₂ evolution were measured at 25°C in a Hansatech oxygen electrode. The reaction medium contained (in a final volume of 1 ml) 0.5m-sorbitol, 10mM-MgCl₂, 10mM-Hepes/NaOH, pH 7.5, 5mM-phosphate buffer, pH 7.5 (NaH₂PO₄/K₂HPO₄), 2mM-K₃Fe(CN)₆, 1mM-2,6-dimethyl-p-benzoquinone, and particles containing 10μg of chlorophyll a. Pigment P700 was assayed chemically by recording difference spectra [2mM-ascorbate minus 1mM-K₃Fe(CN)₆] of particles at a concentration of 20μg of chlorophyll a/ml. A value of Δε₇₃₀ = 64 litre:mmol⁻¹·cm⁻¹ was used (Hiyama & Ke, 1972). Spectra were recorded on a sensitive split-beam spectrophotometer.

<table>
<thead>
<tr>
<th></th>
<th>Supernatant</th>
<th>Pellet</th>
<th>Original membrane fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll (%)</td>
<td>15–20</td>
<td>80–85</td>
<td>100</td>
</tr>
<tr>
<td>Rate of O₂ evolution (μmol of O₂/h per mg of chlorophyll)</td>
<td>550–600</td>
<td>90–110</td>
<td>200–250</td>
</tr>
<tr>
<td>Chlorophyll/P700 (molar ratio)</td>
<td>250–300</td>
<td>110–130</td>
<td>130–150</td>
</tr>
</tbody>
</table>

forms of chlorophyll a, and were less enriched in O₂-evolution activity. The presence of 25% (v/v) glycerol in the incubation medium stabilized O₂ evolution in the supernatant. Fractionation could be achieved in the absence of glycerol, but O₂-evolution activity in the supernatant gradually decayed over 24h at 4°C.

In some respects our preparation is similar to that of Ogawa et al. (1969), in that they also found that, in contrast with higher plants, Photosystem II in Anabaena was localized in a lighter particle that was more easily removed from the membranes by low concentrations of a non-ionic detergent. However, we have extended their work by obtaining fractions from P. laminosum that retain photochemical activity, particularly O₂ evolution, as well as the spectral characteristics associating them with Photosystem I and Photosystem II.

We are grateful to Dr. R. W. Castenholz for the culture of Phormidium laminosum and to the Shell Company of Australia for a scholarship to A. C. S.

Alteration in Concentration of Carotenoids in Rhodopseudomonas capsulata Transferred from Dark to Light Growth

JACQUELINE MANWARING, CAROLE A. PULLIN and E. HILARY EVANS

Biology Division, Preston Polytechnic, Corporation Street, Preston, Lancs., U.K. and GEORGE BRITTON

Department of Biochemistry, University of Liverpool, P.O. Box 143, Liverpool L69 3BX, U.K.

It has been known for many years that the carotenoid complement of the Athiorhodaceae is dependent on the oxygen tension of the growth medium and the