Isolation of oxygen-evolving phycobilisome–photosystem II particles from *Porphyridium cruentum*

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Photosystem II phycobilisome particles were isolated from the red alga *Porphyridium cruentum* in a sucrose–phosphate–citrate–magnesium medium. These particles showed high rates of oxygen evolution in the water-to-silicomolybdate reaction (2300 μmol O_2.mg $chl^{-1}.h^{-1}$). Photosystem I, according to fluorescence emission spectra (−196°C), was greatly reduced or absent. The results demonstrate a close physical relationship of photosystem II with the phycobilisomes which are the major light harvesting antennae in this organism.

Phycobilisome *Photosystem II* *Oxygen evolution* *Light-harvesting antenna*
(Porphyridium cruentum) *(Red alga)*

1. INTRODUCTION

In red, as well as in blue–green algae (cyanobacteria), the phycobilisomes are the major light harvesting antennae. The phycobilisomes exist on the external (stroma) surface of the thylakoid lamellae [1], grana stacks and chlorophyll *a/b* complexes do not exist in these organisms. With an apparently less complex thylakoid structure these organisms seemed more promising than green plants for establishing the direct structural relationship of photosystem II (PS II) (measured as O_2-evolution) with the antennae system. With the red alga *Porphyridium cruentum* a large fraction (95%) of the chlorophyll (chl) is associated with photosystem I (PS I) [2,3], and the energy absorbed by the phycobilisomes is transferred almost exclusively to a small amount of chlorophyll associated with PS II. Wang et al. [4] have made confirmatory observations, although according to their interpretation the phycobiliproteins make a relatively greater contribution to PS I than assumed in [3]. The fluorescence results, together with structural data of phycobilisomes and thylakoid particles [1,5], supported the idea of a direct structural relationship between the phycobilisomes and PS II.

PS II preparations which had high rates of O_2-evolution were isolated from the thermophilic blue-green algae *Phormidium laminosum* in [6]. Such preparations have now been further characterized [7]. Photochemically active PS II particles had been obtained from another blue-green alga [8]. However, in all PS II blue-green algal preparations [6–9] the isolation medium used caused a loss of the phycobilisomes. Phycobilisomes, along with normal O_2 activity could be retained on thylakoids of red and blue-

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Abbreviations: SPCM, sucrose–phosphate–citrate–magnesium chloride medium; chl, chlorophyll; PS I, PS II, photosystem I and II, respectively; LDAO, lauryldimethylamine oxide; DMSO, dimethylsulfoxide; DMBQ, 2,6-dimethyl-*p*-benzoquinone; SiMo, 12-silicomolybdate ($Na_4Mo_{12}O_{40}$); DCMU, 3-(3,4-dichlorophenyl)-1,1 dimethylurea; NH_2OH, hydroxylamine

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green algae [10,11] with a medium containing sucrose, phosphate, citrate and magnesium. Using this medium on *P. cruentum*, we present the first isolation procedure for highly purified O_2-evolving PS II preparations which are functionally joined to phycobilisomes.

2. MATERIALS AND METHODS

2.1. *Isolation of phycobilisome–PS II particles*

Porphyridium cruentum was grown according to [11] and harvested after 7 days growth in the late exponential phase. The isolation was carried out at 5°C. The isolation medium consisted of 0.5 M sucrose, 0.5 M potassium phosphate, 0.26 M sodium citrate, and 10 mM magnesium chloride (SPCM) with a final pH of 7.0. Cells of *P. cruentum* were washed quickly with distilled H_2O and 1 g (wet wt) was directly suspended into 4 ml isolation medium. The cell suspension was passed through an Aminco-French pressure cell at 18000 (128 MPa) $lb.in^{-2}$. These membrane fragments (~0.30 mg chl/ml) were treated with 0.12% (v/v) lauryl–dimethyl–amine oxide (LDAO) giving a detergent : chl ratio of 4 : 1 (w/w). The mixture was incubated for 30 min in the dark at 5°C with gentle stirring, then centrifuged 30 min at 27000 × *g* to remove cell debris and starch.

The supernatant was layered on a sucrose step gradient (0.25–2 M sucrose in 0.5 M potassium phosphate, 0.26 M sodium citrate, 10 mM magnesium chloride (pH 7.0)) similar to the gradient used in [12]. The particle fraction was recovered from the 1–2 M sucrose interface after 11 h centrifugation at 130000 × *g* in an angle head rotor. It was then diluted with 2 parts SPCM medium and centrifuged 30 min at 27000 × *g* to pellet and discard contaminating thylakoid vesicles. Finally the PS II–phycobilisomes were concentrated by centrifugation for 3 h at 269000 × *g*.

2.2. *Absorption spectra and fluorescence emission spectra*

Absorption spectra were recorded at room temperature using a Cary 17 spectrophotometer. Fluorescence spectra were recorded at −196°C on an Aminco-Bowman spectrofluorometer in the standard mode. Fluorescence emission spectra were made by suspending 1 part of the sample into 2.5 parts potassium glycerophosphate (ICN Pharmaceuticals, Plainview NY) with a final $A_{545\ nm}$ = 0.05.

The [chl] was estimated from DMSO extracts, at room temperature, using MacKinney's extinction coefficient [13]. Comparisons of the values obtained with DMSO, and with cold 80% acetone followed by cold methanol showed very good agreement.

2.3. *Photosynthetic O_2 evolution*

A Clark-type O_2 electrode was used to measure photosynthetic O_2 evolution. Temperature was kept constant at 20°C. Light from a slide projector lamp was filtered through 10 cm water and a Schott KG 1 heat absorbing filter.

Table 1

Rates of oxygen evolution of various isolation fractions

Fraction	$H_2O \longrightarrow$ SiMo	$H_2O \longrightarrow$ DMBQ + FeCy
	(μmol O_2.mg $chl^{-1}.h^{-1}$)	
Thylakoid–phycobilisome fraction	272	270
LDAO–27000 × *g* supernatant	300	300
PS II–phycobilisome particles	2330 ± 940 (± DCMU)	2140[a] ± 560

[a] Mean of 3 preparations; the other numbers represent the mean of 8 preparations

Activity was usually measured ~18 h after harvesting the cells, with a Clark-type electrode, at 20°C, in SPCM, either with DMBQ (2,6-dimethyl *p*-benzoquinone) 1 mM + ferricyanide 2 mM, or silicomolybdate 100–200 μM ± DCMU 5 μM [14]. White light was filtered through 10 cm of water + Schott KG 1 heat absorbing filter (250 $nE.cm^{-2}.s^{-1}$) (0.1–3.6 μg chl)

3. RESULTS AND DISCUSSION

The PS II–phycobilisome particles prepared by LDAO treatment in SPCM medium had about the same density and size as isolated phycobilisomes. This was determined by their recovery from the 1–2 M sucrose interface of the same type of gradient as used for isolating phycobilisomes [12]. Under these sedimentation conditions thylakoid fragments with attached phycobilisomes sedimented higher on the gradient than the PS II–phycobilisomes. The thylakoid–phycobilisome fraction, derived from broken cells, and the LDAO-treated fraction (27000 × *g* supernatant) had O_2-evolution rates that were essentially the same (table 1) and were close to those obtained for whole cells [11]. The PS II–phycobilisome particles had substantially higher rates. With 2,6-dimethyl *p*-benzoquinone (DMBQ) plus ferricyanide as acceptor they showed an 8-fold enhancement, and with silicomolybdate (SiMo) plus DCMU an 8.6-fold enhancement over the original thylakoids.

O_2-evolution was light dependent, and when assayed with DMBQ + ferricyanide it was sensitive (100%) to 10 mM hydroxylamine, indicating that it is a true PS II reaction. Furthermore, the reaction with SiMo was insensitive to DCMU, which would be expected since the acceptor site of SiMo is close to the PS II reaction center [14].

The O_2-evolution rates observed here are some of the highest reported thus far [6,7,9]. With the cyanobacterium *Phormidium laminosum* ~1340 μmol O_2 $mg^{-1}.chl^{-1}.h^{-1}$ was obtained with DMBQ + ferricyanide [6]. The even higher rates obtained here with the red alga *P. cruentum* may reflect a species difference, but could also be due to the presence of the phycobilisomes, or to the SPCM medium. It is possible that the phycobilisome being functionally coupled to PS II could at the same time be functionally protective of an O_2-evolving complex.

It should be noted that with 0.5–2.0% LDAO, and with 1% Triton X-100, O_2-activity was lost, as was the chlorophyll, but the phycobilisomes were otherwise unchanged. These results suggest that exhaustive detergent treatments result in the removal of the O_2 components along with the chlorophyll.

The reduction of the chl content in the PS

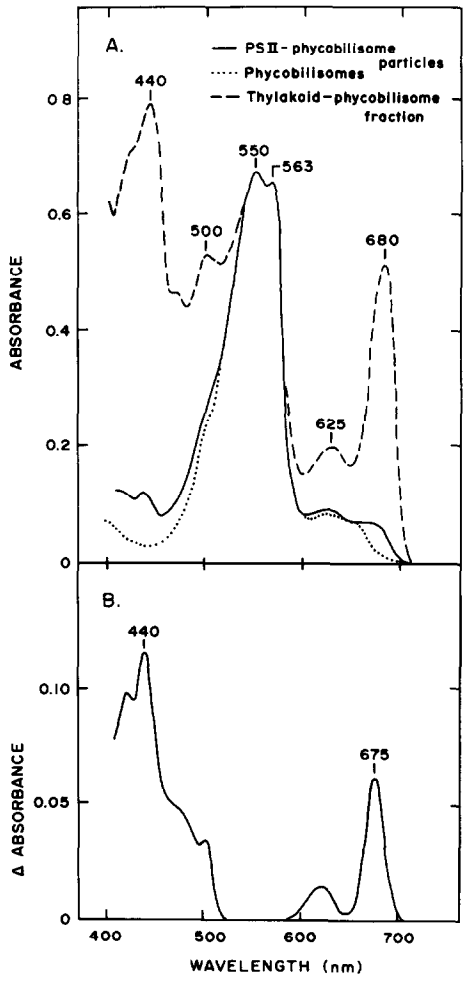

Fig.1. (A) Absorption spectra of PS II–phycobilisome particles (——), phycobilisomes (···) isolated with Triton X-100 [12], and thylakoid (– – –) in SPCM medium at room temperature normalized at 550 nm. (B) Difference spectrum showing chlorophyll content in PS II–phycobilisome particles. PS II–phycobilisome particles absorption spectrum (——) minus phycobilisome absorption spectrum (···).

II–phycobilisome particles is apparent (fig.1A) when this fraction is compared with the normal thylakoid–phycobilisome fraction. The chl component, although visible only as small shoulders at ~440 nm and ~680 nm (fig.1A) was clearly resolved in the difference spectrum in fig.1B (of PS II–phycobilisome particles minus phycobilisomes isolated in Triton X-100). In fact, the long wavelength maximum was at 675 nm which cor-

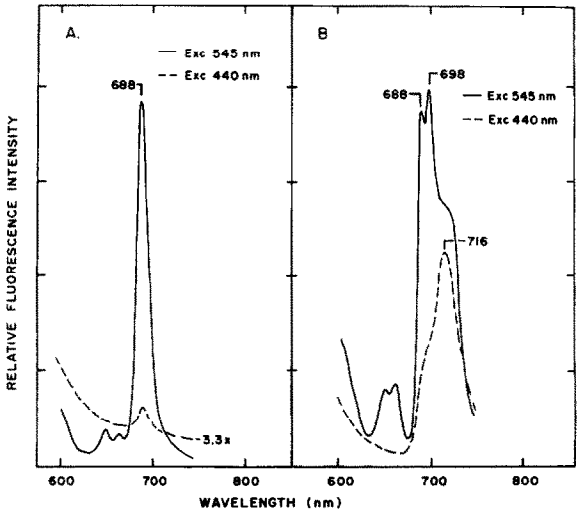

Fig.2. Fluorescence emission spectra with excitation of phycoerythrin (545 nm) and chlorophyll (440 nm): (A) PS II–phycobilisome particles, 0.07 μg chl/ml; (B) unfractionated phycobilisome–thylakoid preparations, 0.94 μg chl/ml, obtained as in [11].

responds exactly to the absorption peak of isolated PS II–chl-complexes from the same alga [15]. Furthermore, the 688 nm fluorescence emission of the PS II particles (fig.2A) also suggests a major PS II component. Of considerable importance also is the reduction of the PS I emission peak in the 715–730 nm region. By comparison, the thylakoid–phycobilisome fraction (fig.2B) has major components at ~695 nm, and at ~720 nm (PS I) [3]. Collectively these data indicate that PS I is greatly reduced and PS II is clearly present with these phycobilisome particles. This provides one of the most promising PS II preparations thus far isolated.

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