

Photosynthetic Oxygen Evolution Is Stabilized by Cytochrome c_{550} against Heat Inactivation in *Synechococcus* sp. PCC 7002¹

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We investigated the factors responsible for the heat stability of photosynthetic oxygen evolution by examining thylakoid membranes from the cyanobacterium *Synechococcus* sp. PCC 7002. We found that treatment of the thylakoid membranes with 0.1% Triton X-100 resulted in a remarkable decrease in the heat stability of oxygen evolution, and that the heat stability could be restored by reconstituting the membranes with the components that had been extracted by Triton X-100. The protein responsible for the restoration of heat stability was purified from the Triton X-100 extract by two successive steps of chromatography. The purified protein had a molecular mass of 16 kD and exhibited the spectrophotometric properties of a c-type Cyt with a low redox potential. The dithionite-minus-ascorbate difference spectrum revealed an α band maximum at 551 nm. We were able to clone and sequence the gene encoding this Cyt from *Synechococcus* sp. PCC 7002, based on the partial amino-terminal amino acid sequence. The deduced amino acid sequence revealed a gene product consisting of a 34-residue transit peptide and a mature protein of 136 residues. The mature protein is homologous to Cyt c_{550} , a Cyt with a low redox potential. Thus, our results indicate that Cyt c_{550} greatly affects the heat stability of oxygen evolution.

When plants are exposed to temperatures above the normal physiological range, photosynthesis is permanently inactivated. The inactivation of photosynthesis occurs at relatively low temperatures; additional severe damage and the thermal breakdown of cellular integrity occur at higher temperatures (Berry and Björkman, 1980). Thus, it is likely that the heat stability of photosynthetic activity determines the cellular heat tolerance.

The PSII complex is the most susceptible to heat among various components of the photosynthetic apparatus (Berry and Björkman, 1980; Mamedov et al., 1993). Among the partial reactions taking place in PSII, the oxygen-evolving process is particularly heat sensitive (Kato and San Pietro, 1967; Yamashita and Butler, 1968; Santarius, 1975; Mamedov et al., 1993). Therefore, the heat stability of oxygen evolution should determine the overall heat tolerance of the photosynthetic process. Nash et al. (1985) demonstrated that the heat

inactivation of oxygen evolution is caused by the release of functional manganese ions from the PSII complex. To understand the mechanisms contributing to the heat stability of photosynthesis, we must examine the biochemical factors responsible for the heat stability of oxygen evolution.

The glycerolipids of thylakoid membranes were initially thought to play a key role in the heat stability of photosynthesis (Quinn, 1988). Several studies suggested that levels of saturated fatty acids in thylakoid glycerolipids correlated with the heat stability of PSII activity (Pearcy, 1978; Raison et al., 1982; Thomas et al., 1986). Recently, however, direct experimental evidence has been obtained, using mutants and transformants defective in desaturases (Gombos et al., 1991; Mamedov et al., 1993; Wada et al., 1994), that contradicts the previously suggested importance of saturated lipids in maintaining heat stability. Therefore, factors other than membrane lipids must be involved in determining the heat stability of photosynthetic oxygen evolution.

In the present study, we examined thylakoid membranes from the cyanobacterium *Synechococcus* sp. PCC 7002 to find the molecular basis for the heat stability of oxygen evolution. We found that destruction of the vesicular structure in the thylakoid membranes resulted in a radical decrease in the heat stability of oxygen evolution and that the heat stability could be restored by adding the components that had been extracted with Triton X-100. Using a reconstitution assay, we determined that the protein component responsible for the heat stability of oxygen evolution is Cyt c_{550} , a Cyt with a low redox potential.

MATERIALS AND METHODS

Preparation of Thylakoid Membranes

Synechococcus sp. PCC 7002 was obtained from the Pasteur Culture Collection. The cells were grown photoautotrophically at 40°C for 3 d, and thylakoid membranes were isolated from the cells as previously described (Nishiyama et al., 1993). The isolated thylakoid membranes were then suspended in medium A (50 mM Hepes-NaOH, pH 7.5, 800 mM sorbitol, 30 mM CaCl_2 , 1.0 M glycinebetaine, 1 mM 6-amino-*n*-caproic acid) and stored at 0 to 4°C for a maximum of 1 d.

Treatment of Thylakoid Membranes

To disrupt the closed vesicular structure of the thylakoid membranes, the membranes were incubated in the dark at

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4°C for 5 min in medium A containing 0.1% (w/v) Triton X-100 at a Chl concentration of 0.5 mg mL⁻¹, at which the ratio of Triton X-100 to Chl was 2:1 (w/w). After the components were dissociated with Triton X-100, they were separated into soluble and membrane fractions by centrifugation at 200,000g for 2 h. The Triton X-100 was removed from the soluble fraction using column chromatography with a 1- × 10-cm column of Bio-beads (Bio-Rad, Richmond, VA) equilibrated with medium A. The fractions eluted with medium A (nonadsorbed fractions) were collected and concentrated approximately 20-fold using a Centricon-10 centrifugal micro-concentrator (Amicon, Beverly, MA).

Reconstitution of Thylakoid Membranes

To reconstitute the thylakoid membranes, 10 μL of the thylakoid membrane suspension previously treated with 0.1% Triton X-100 was mixed with 90 μL of the concentrated extract or Cyt *c*₅₅₀. The mixture was incubated in the dark at 25°C for 10 min. The suspension was then mixed with 900 μL of medium B (50 mM Tricine-NaOH, pH 7.5, 600 mM Suc, 30 mM CaCl₂, 1.0 M glycinebetaine) and subjected to heat treatments at designated temperatures for 20 min in the dark. Following each heat treatment, the suspension was cooled to 25°C, and the oxygen-evolving activity was measured.

A similar procedure was performed to assay the column chromatography fractions for heat-stabilization activity. However, in this case the 20-min heat treatment was always performed at 38°C. The extent that each fraction prevented heat inactivation was measured, and the measurement was considered as the heat-stabilization activity.

Protein Purification

Thylakoid membranes, with concentrations of 20 mg of Chl, were treated with 0.1% Triton X-100 under the same conditions as described above, except that medium A was replaced with medium C (20 mM Hepes-NaOH, pH 7.5, 800 mM sorbitol, 1.0 M glycinebetaine). The membrane suspension was then separated into soluble and membrane fractions by centrifugation at 200,000g for 2 h. The soluble fraction was applied to a 2.6- × 8-cm column of DEAE-Toyopearl 650C (Tosoh, Tokyo, Japan), equilibrated with medium D (20 mM Hepes-NaOH, pH 7.5, 0.2% *n*-octyl-β-D-glucopyranoside). The column was washed with 100 mL of medium D and was then developed with a linear gradient ranging from 0 to 0.3 M NaCl in 150 mL of medium D. Active fractions were collected and dialyzed in medium E (25 mM *N*-methylpiperazine-HCl, pH 5.5), and the dialysate was applied to a Mono-P HR 5/5 column (Pharmacia LKB Biotechnology, Uppsala, Sweden) equilibrated with medium E. The column was developed with 20 mL of medium F (10% [v/v] Polybuffer 74 [Pharmacia]-HCl, pH 4.0). The active fractions were recovered, and the medium was replaced with medium G (20 mM Hepes-NaOH, pH 7.5, 10 mM NaCl) by centrifugal concentration in a Centricon-10.

Measurement of Photosynthetic Activity

Photosynthetic oxygen evolution was measured by monitoring the concentration of oxygen with a Clark-type oxygen

electrode. Measurements were carried out at 25°C in the presence of 100 μM phenyl-1,4-benzoquinone to be used as an electron acceptor. Red actinic light, at an intensity of 430 W m⁻², was provided by an incandescent lamp used in conjunction with a heat-absorbing optical filter (filter HA50; Hoya, Tokyo, Japan) and a red optical filter (filter R-60; Toshiba, Tokyo, Japan).

Analysis of the Amino Acid Composition and Amino-Terminal Amino Acid Sequence

Purified protein was hydrolyzed in 5 N HCl at 110°C for 24 or 72 h, and the amino acid composition was determined using an automatic amino acid analyzer (model 835; Hitachi, Tokyo, Japan). The amino-terminal sequence was determined by automatic Edman degradation using a protein sequence analyzer (model 477A; Applied Biosystems, Foster City, CA).

Nucleotide Sequence Analysis

The DNA fragment corresponding to 25 of the amino acid residues in the amino terminus of the purified protein was amplified using PCR. First, genomic DNA was isolated from *Synechococcus* sp. PCC 7002 cells, according to the method described by Williams (1988), and was used as template DNA for the PCR. Mixed oligonucleotides, encoding for the amino acids at positions 1 to 5 and 21 to 25, were synthesized using a DNA synthesizer (model 391; Applied Biosystems Japan, Tokyo, Japan) and used as forward and reverse primers, respectively. The PCR amplification product of approximately 70 bp was then cloned into a plasmid vector using the TA cloning system (Invitrogen, San Diego, CA). The nucleotide sequence was determined to ensure that the cloned gene coded for a portion of the purified protein.

We also created a genomic library from *Synechococcus* sp. PCC 7002 DNA. The genomic DNA was partially digested with *Sau*3AI, and the resulting DNA fragments were ligated into the *Bam*HI site of the phage vector, λDASHII (Stratagene, La Jolla, CA). The genomic library was then screened by plaque hybridization as described by Ausubel et al. (1987). Approximately 4000 plaques were transferred onto nylon membranes (GeneScreen Plus; NEN Research Products, Boston, MA) and hybridized at 50°C for 20 h in a medium containing 10% dextran sulfate, 5× Denhardt's solution, 6× SSC, 0.1% SDS, and 100 μg mL⁻¹ salmon sperm DNA. The library was screened using the 70-bp PCR product described above, labeled with [α -³²P]dCTP (Random Primer Labeling Kit; Takara, Tokyo, Japan). Following hybridization with the probe, membranes were washed at 50°C for 2 h in a solution containing 1× SSC and 0.5% SDS and were then exposed to x-ray film (WIF50; Konica, Tokyo, Japan).

A total of 24 positive clones were obtained, and DNA fragments from the clones were analyzed using restriction digestion and Southern blotting. The Southern blots were probed with the previously described 70-bp probe under the same hybridization and wash conditions used to screen the genomic library. A 5.6-kb *Eco*RI fragment, contained in the 12-kb insert of one positive clone, hybridized strongly to the probe and was subcloned into a plasmid vector (pBluescript II KS+; Stratagene). The recombinant plasmid was designated pCC55.

We then determined the nucleotide sequence of a 1.3-kb *Bam*HI-*Pst*I fragment within the insert of pCC55 using a DNA sequencer (model 373A; Applied Biosystems Japan) to carry out dideoxy sequencing (Sanger et al., 1977). The sequencing reactions were performed using either the Dye Dedoxy Terminator Cycle Sequencing Kit or the Dye Primer Cycle Sequencing Kit (Applied Biosystems Japan) as described by the manufacturer.

Other Analytical Methods

The Chl concentration was determined using the method of Arnon et al. (1974). The absorption spectrum of the purified protein was measured with a slit-beam spectrophotometer (model UV300; Shimadzu, Kyoto, Japan) in the presence or absence of sodium dithionite and sodium ascorbate. SDS-PAGE was performed according to the method of Laemmli (1970) using a 15% polyacrylamide gel. After electrophoresis, the gel was stained with 0.1% (w/v) Coomassie brilliant blue R-250 (Sigma). Protein concentrations were determined using a protein assay solution (Bio-Rad) with BSA (Sigma) as a standard (Bradford, 1976).

RESULTS

Dissociation of Factors Contributing to Heat Stability from the Thylakoid Membranes

When thylakoid membranes were treated with 0.1% (w/v) Triton X-100, the heat stability of oxygen evolution decreased dramatically (Fig. 1). Following treatment, the temperature resulting in 50% inactivation of oxygen evolution decreased from 43 to 38°C. This suggests that the treatment resulted in the dissociation of components from the thylakoid membranes that are essential for maintaining the heat stability of oxygen evolution.

The components dissociated with Triton X-100 were separated from Triton X-100, concentrated 20-fold, and then added back to the thylakoid membranes that had been treated with Triton X-100. The concentrated components were able to restore the heat stability of oxygen evolution; the temperature for 50% inactivation increased from 38 to 42°C (Fig. 1). These observations indicate that at least one of the components extracted with Triton X-100 is essential for the heat stability of oxygen evolution. Furthermore, when the extracted components were filtered through a Centricon-10 filter, they lost the activity to restore heat stability (data not shown). Exposure of the extracted components to a temperature of 100°C for 10 min also resulted in the loss of activity (data not shown). Thus, the functional component appears to be a protein.

Identification of Cyt c_{550} as a Component Involved in Heat Stability

We purified the component responsible for the restoration of the heat stability of oxygen evolution from thylakoid membranes by extraction with Triton X-100, followed by two successive steps of column chromatography, first with DEAE-Toyopearl 650C and then with Mono-P HR 5/5. The fractions obtained from the DEAE-Toyopearl column that were able

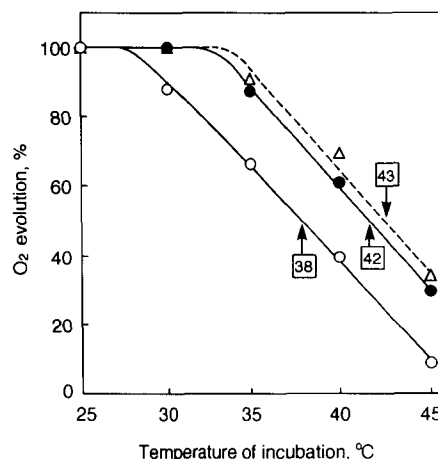


Figure 1. Temperature profiles of the heat inactivation of photosynthetic oxygen evolution in thylakoid membranes from *Synechococcus* sp. PCC 7002. The thylakoid membranes were treated with 0.1% Triton X-100 and were then reconstituted with the concentrated Triton X-100 membrane extract. The membranes were incubated at designated temperatures in the dark for 20 min, and oxygen evolution was measured at 25°C in the presence of phenyl-1,4-benzoquinone as an electron acceptor. Δ , Thylakoid membranes with no treatment; \circ , thylakoid membranes after treatment with Triton X-100; \bullet , thylakoid membranes reconstituted with the extract. The oxygen-evolving activities taken as 100% were 238, 202, and 204 $\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl h}^{-1}$, respectively. The numbers within the squares signify temperatures for 50% inactivation.

to restore heat stability were recovered at NaCl concentrations of 0.2 to 0.3 M NaCl in medium D. No activity was recovered in effluent that contained 0.3 to 1.0 M NaCl.

The activity was recovered at pH 4.0 as a single peak on the Mono-P column, whereas proteins with no activity were eluted within a pH range of 4.0 to 5.5 (Fig. 2). This result indicates that the functional protein has an isoelectric point of 4.0. Figure 3 shows the profiles obtained by SDS-gel electrophoresis of the active fractions from each purification step. The active fraction obtained from Mono-P chromatography contained a single protein of 16 kD, without any sign of contamination.

The 16-kD protein exhibited a unique absorption spectrum with a maximum at 409 nm (Fig. 4). Upon the addition of sodium dithionite, the absorption maxima appeared at 551, 523, and 417 nm (Fig. 4). An absorption change of this nature, caused by a reducing reagent such as sodium dithionite, is typical of *c*-type Cyt. A similar absorption change did not result from the addition of sodium ascorbate. Thus, the 16-kD protein appears to be one of the low redox potential Cyts c_{550} , which are present in a number of cyanobacterial and algal species (Alam et al., 1984). The amino-terminal amino acid sequence of the 16-kD protein is TAL-REVDRTVNLNETETVVLSDQQV. Since this sequence is similar to the amino-terminal sequences of Cyt c_{550} from other cyanobacterial species (Cohn et al., 1989; Shen et al., 1992), we have identified the 16-kD protein as a low redox potential Cyt c_{550} of *Synechococcus* sp. PCC 7002.

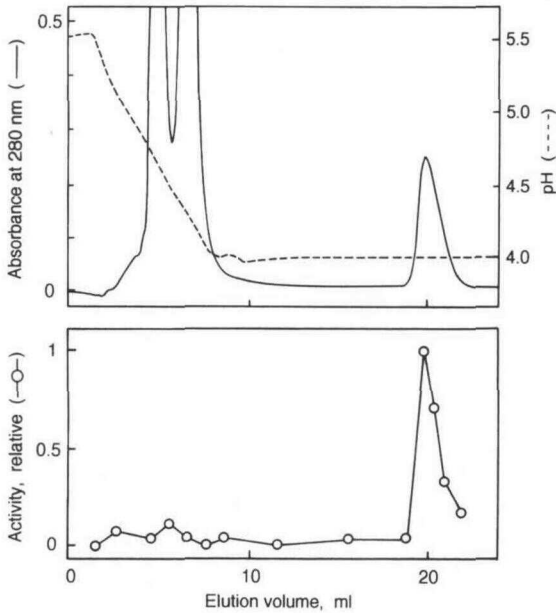


Figure 2. Chromatofocusing using Mono-P HR 5/5 column chromatography of the active fractions obtained from DEAE-Toyopearl 650C column chromatography. Fraction activity was assayed as described in "Materials and Methods."

Molecular Cloning of Cyt c_{550}

A 5.6-kb *EcoRI* fragment in one of the positive clones hybridized to the probe prepared from the 70-bp PCR product that corresponded to a portion of the gene for Cyt c_{550} . An open reading frame of 510 bases was located in a 1.3-kb *BamHI-PstI* fragment within the 5.6-kb region that coded for

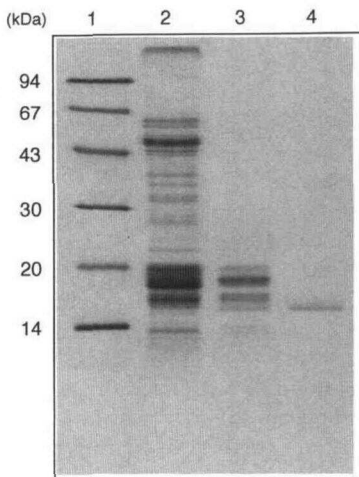


Figure 3. Electrophoretic analysis of the active fractions from each purification step. Proteins were analyzed by SDS-PAGE on a 15% polyacrylamide gel. Lane 1 shows the molecular mass markers. Lanes 2, 3, and 4 show the active fractions obtained after extraction with Triton X-100, DEAE column chromatography, and chromatofocusing, respectively.

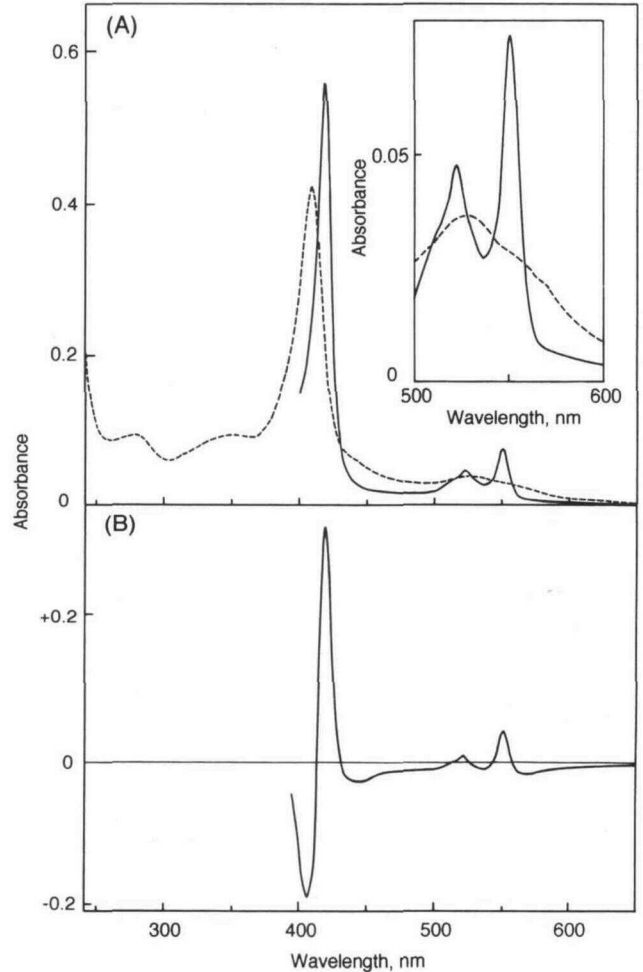


Figure 4. A, Absorption spectra of the protein purified from thylakoid membranes of *Synechococcus* sp. PCC 7002. The spectra were measured in the presence (solid line) and absence (dashed line) of sodium dithionite. B, The dithionite-minus-ascorbate difference spectrum.

Cyt c_{550} (Fig. 5). The initiation codon was located 102 bases upstream of the amino terminus of Cyt c_{550} . A typical ribosomal binding site, GAGG, was found 10 bases upstream of the initiation codon. The deduced amino acid sequence indicates that the product of this open reading frame consists of a transit peptide of 34 residues and a mature protein of 136 residues. The deduced amino acid composition of the mature protein is identical with that of purified Cyt c_{550} (data not shown); thus, the identity of the gene is confirmed. The presence of the transit peptide suggests that Cyt c_{550} is located on the luminal side of the thylakoid membranes. In addition, a conserved heme-binding site, Cys-X-X-Cys-His, was found at residues 70 to 74.

The Thermoprotective Role of Cyt c_{550}

Figure 6A shows the effect of the addition of Cyt c_{550} on the temperature profile of heat inactivation of oxygen evolution in thylakoid membranes treated with Triton X-100.

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GGATCCTAAAATAAAAGTAAACAGCAGCAGAAATATGCTATTTTTACGGATTATAAG 60
AAAAATTCACCCCAATGGCATGATATGATGTAGTTGGCTGGCTGACTGGTGGCGT 120
GGCAACACAAATTTAAATTAACAAAGGATGACACATTTACTTACAGCCCTGTAATAATCT 180
TGCCCTAGGCTGCTCCTTCCAGCTTAAATAAGTGGAGATAGATTATTTACGTTATTTGCT 240
GTATTCGGAAAACATGAATAAATTCCTGGGATCGCCACCTCAAAAATTTATTTTC 300
      SD  M N K I L G I D P L K K P I F 15'
GGGATTAGCGCATTCGTTTACTATTTTGGCAACTCAATGTGGGGCTGCCAATGCCACC 360
G I S A F V L L F W Q L N V G A A N A T 35'
GCCCTCGGGAGTGAACCGCAGCGTCACTCAATGAACCGAAACGGTCTCTCTCAGT 420
A L L R E V D R T V N L N P E T E T V V L S 55'
GACCAGCGGTTGCCAAAGGAGGACGGGATCTTTATCAACCTGTTCACCTGCCACAC 480
D Q Q V A K G E R I F I N T C S T C H N 75'
AGCGCGGACAAAGACCAACCCCAAGCTAACCCCTTCTAGTGCATTCGAAGGGGCA 540
S G R T K S N P N V T L S L V D L R G A 95'
GAACCCCGCGGATAATATCTCTGGCATGTGGACTATCTCAAAAATCCACCTCTAC 600
E P R R D N I L A M V D Y L K N P T S Y 115'
GATGTGAATAGACCTATCTCACTTACACCCATACCGTCCGCGCAGCATCTGGAGT 660
D G E L D L S Q L H P N T V R A D I W S 135'
AGCATGGTAACCTCAACGAAGGATCTGCAAAATGTTCCGGTATGTCTCTGTTCAA 720
S M R N L N E E D L Q N V S G Y V L V Q 155'
GCCAGGTGCTGGTGTCTTGGGGTGGTGTATTAACCGTTACTAAACACGATGAT 780
A Q V R G V A W G G G K T V H * 170'
CGACTTTATTAATCTCTTTTATCTAAATTCGCAAAATGATTCGGAAGACAC 840
ATAATGAGGTGTCTTTTAAATGTCTTAAAGCGATCGCTCGGTTTCGACACAAA 900
    
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Figure 5. The nucleotide sequence of the gene for Cyt *c*₅₅₀ from *Synechococcus* sp. PCC 7002. The deduced amino acid sequence is shown in one-letter code under the nucleotide sequence. The amino-terminal sequence that was determined directly from the protein is underlined. The putative ribosomal binding site (SD) and heme-binding site (HBS) are double-underlined. The arrow indicates the cleavage site for removal of the transit peptide.

Cyt *c*₅₅₀ was added to the thylakoid membrane suspension, with a Chl concentration of 5 μg mL⁻¹, at a final concentration of 2 μg mL⁻¹. The addition of Cyt *c*₅₅₀ clearly resulted in an increase in the heat-inactivation profile of the membranes; the temperature for 50% inactivation shifted from 38 to 42°C. Thus, the addition of Cyt *c*₅₅₀ restored the heat stability of

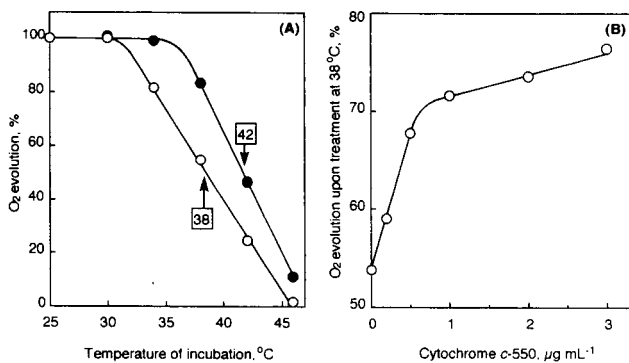


Figure 6. Effect of Cyt *c*₅₅₀ on the heat stability of oxygen evolution in the thylakoid membranes of *Synechococcus* sp. PCC 7002. A, Temperature profile of the heat inactivation of oxygen evolution. ●, Cyt *c*₅₅₀ at a final concentration of 2 μg mL⁻¹ was added to the thylakoid membrane suspension that had been treated with Triton X-100; ○, control (no Cyt *c*₅₅₀). The oxygen-evolving activities taken as 100% were 195 and 191 μmol O₂ mg⁻¹ Chl h⁻¹, respectively. The numbers within the squares signify temperatures for 50% inactivation. B, The relationship of Cyt *c*₅₅₀ concentration to the restoration of heat stability. Various amounts of Cyt *c*₅₅₀ were added to the thylakoid membrane suspension that had been treated with Triton X-100. The heat stability is shown as the proportion of oxygen evolution upon treatment at 38°C compared to that resulting from treatment at 25°C.

thylakoid membranes to initial levels (Fig. 1).

The restoration of heat stability in the thylakoid membranes was examined using various concentrations of Cyt *c*₅₅₀ (Fig. 6B). Heat stability increased linearly with increases in the amount of added Cyt *c*₅₅₀ up to a concentration of 0.6 μg mL⁻¹, at which point the molecular ratio of added Cyt *c*₅₅₀ to the PSII reaction center was approximately 1.4. The addition of greater amounts of Cyt *c*₅₅₀ resulted in a much slower increase in heat stability (Fig. 6B).

DISCUSSION

In the present study, we investigated a factor involved in sustaining the heat stability of oxygen evolution in *Synechococcus* sp. PCC 7002. We assumed that the factor would be located near the oxygen-evolving site of the PSII complex, i.e. on the luminal side of the closed vesicle in the thylakoid membrane. After disrupting the vesicular structure of the thylakoid membranes with a low concentration of Triton X-100, we observed a dramatic decrease in the heat stability of oxygen evolution. We were able to restore heat stability by reconstitution of the membranes with the components extracted by Triton X-100 (Fig. 1). We demonstrated that the component responsible for the restoration of the heat stability of oxygen evolution in *Synechococcus* sp. PCC 7002 is a Cyt *c*₅₅₀. In addition, the nucleotide sequence obtained from our cloned Cyt *c*₅₅₀ gene revealed the presence of a transit peptide, indicating that Cyt *c*₅₅₀ is located on the luminal side of the thylakoid membranes.

We compared the amino acid sequence of the mature Cyt *c*₅₅₀ from *Synechococcus* sp. PCC 7002 with Cyt *c*₅₅₀ sequences from other cyanobacteria (Fig. 7). The sequence of Cyt *c*₅₅₀ from *Synechococcus* sp. PCC 7002 showed 48% identity with the Cyt sequence from *Microcystis aeruginosa* (Cohn et al., 1989) and 47% identity with a partial sequence of 119 residues from *Aphanizomenon flos-aquae* (Cohn et al., 1989).

Cyt *c*₅₅₀ is a c-type, monoheme Cyt, with a molecular mass of 15 kD and an unusually low redox potential (-260 mV) (Holton and Myers, 1967a, 1967b). Holton and Myers (1963) first discovered Cyt *c*₅₅₀ in *Anacystis nidulans*, and it has since

S. PCC7002	TALREVDRTVNLNET-ETVVLSDQQVAKGERIFINT	CSTCH	NSGRTKSNPN	50
M. aeruginosa	LE*D*KTL*IT**DAG*S*T*TSE*ATE*QKL*VAN	*TK**	LQ*K**T*N*	51
A. flos-aquae	LE*D*TI**P**DKGG*****LE**-*EGKL*NYA	*AQ**	AG*V**T*Q*	50
S. PCC7002	VTLSLVDLEGAPRRDNLAMVDYLKNPTSYDGLDLSQLHPNTRADIWS			101
M. aeruginosa	*S*G*G*AK**P***L**LI**EH*****D***E****VS*P**FP			101
A. flos-aquae	*G*EPEA*A*L*N*????????M*****E**EI?*SIKS*N*F-			90
S. PCC7002	SMRNLNEEDLQNVSGYLVQAQVRGVAMGGKTVN			136
M. aeruginosa	EL***T*D*VY**AA*M**APRLDER-***TIYF			135
A. flos-aquae	--**TD***KAIABHI*LEPL*V*TK***-			119

Figure 7. Aligned amino acid sequences of cyanobacterial Cyt *c*₅₅₀. * indicates amino acids in the Cyt *c*₅₅₀ of *Synechococcus* sp. PCC 7002 identical with those in *M. aeruginosa* or *A. flos-aquae* Cyt *c*₅₅₀. Gaps introduced to optimize alignment are indicated by "-". The heme-binding sites are boxed.

been found in a variety of cyanobacteria (Ho et al., 1979; Alam et al., 1984) and in some eukaryotic algae (Yamanaka et al., 1967; Kamimura et al., 1977; Evans and Krogmann, 1983). The physiological function and cellular localization of Cyt c_{550} have not yet been established, although there are several hypotheses. The cellular content of Cyt c_{550} varies greatly among different species of cyanobacteria and may depend on growth conditions (Ho et al., 1979). Krogmann and Smith (1990) observed that, in some cyanobacterial species, the abundance of Cyt c_{550} increases with cell density when the availability of light and oxygen becomes limited. They suggested that Cyt c_{550} may eliminate excess electrons in a fermentative pathway to maintain cell viability in a dark, anaerobic environment.

In addition to the water-soluble Cyt c_{550} described above, a membrane-bound Cyt c_{550} has been observed in some cyanobacteria. The membrane-bound Cyt c_{550} resembles the water-soluble Cyt c_{550} in size, spectrum, and redox properties. The bound Cyt can be separated from the thylakoid membranes using Tween 20 and acetone (Ho et al., 1979), Triton X-100 (Krisner et al., 1982), or 1.0 M NaCl (Hoganson et al., 1990). It is unclear whether the membrane-bound Cyt c_{550} is the same as the water-soluble one. Hoganson et al. (1990) observed that the two Cyts differ slightly in electron paramagnetic resonance spectra. Additional data, such as amino acid sequences, are needed to address the question (Krogmann, 1991). It should be noted here that the Cyt that is located on the luminal side of the thylakoid membranes (as in the present study) can also be released from the membranes during cell breakage by treatment with a French pressure cell, shaking with glass beads, or freeze-thawing and still be recovered in the aqueous phase.

Recently, Shen et al. (1992) found that a PSII core complex, prepared from the thermophilic cyanobacterium *Synechococcus vulcanus* using lauryldimethylamine *N*-oxide and dodecyl β -D-maltoside, contained a stoichiometric amount of Cyt c_{550} as an extrinsic component of the PSII complex. They demonstrated that Cyt c_{550} enhanced oxygen evolution and facilitated the binding of another extrinsic protein, of 12 kD, to the PSII complex (Shen and Inoue, 1993a). In addition, they noted that the Cyt was exclusively located on the luminal side of the PSII complex (Shen and Inoue, 1993b). It is unclear whether the Cyt from *S. vulcanus* is the same as the Cyt in the present study. The partial amino-terminal sequence of the Cyt c_{550} from *S. vulcanus* (Shen et al., 1992) is similar to that from *Synechococcus* sp. PCC 7002 with 12 of 35 identical residues (data not shown). However, the two Cyts differ in their association with the thylakoid membranes. The Cyt c_{550} from *S. vulcanus* required the presence of 1.0 M CaCl_2 or 1.0 M Tris (pH 8.5) during sonic oscillation to release it from the thylakoid membranes (Shen et al., 1992; Shen and Inoue, 1993b). A Cyt c_{550} identical with that from *S. vulcanus* was also found in PSII particles prepared from the thermophilic cyanobacterium *Phormidium laminosum* (Bowes et al., 1983). In contrast, the Cyt c_{550} from *Synechococcus* sp. PCC 7002 could be dissociated from the thylakoid membranes at neutral pH and under low salt conditions. This suggests that the Cyt in *Synechococcus* sp. PCC 7002 is loosely bound to the PSII complex. Omata and Murata (1984) observed that Cyt c_{550} from *A. nidulans* was also released from the thylakoid mem-

branes by sonic oscillation under low salt conditions. Thus, it appears that there may be two distinct types of Cyt c_{550} .

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The GenBank accession number for the sequence reported in this article is D29788.

LITERATURE CITED

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