Subunit Structure of Chloroplast Photosystem I Reaction Center*

CARMELA BENGIS AND NATHAN NELSON
From the Department of Biology, Technion-Israel Institute of Technology, Haifa, Israel

Purified Photosystem I reaction center preparation catalyzed a plastocyanin-dependent cytochrome f photooxidation. Euglena cytochrome 552 replaced plastocyanin in all photochemical reactions catalyzed by the purified reaction center and was also photooxidized.

A reaction center preparation lacking Subunit III was obtained by means of Triton X-100 treatment and DEAE-cellulose chromatography or sucrose gradient centrifugation. This preparation was incapable of cytochrome f photoreduction in the presence of plastocyanin. However, the reaction center lacking Subunit III photoreduced NADP when plastocyanin was replaced by N-methylphenazonium 3-sulfonate, serving as direct electron acceptor, in the reducing site of Photosystem I.

We previously purified the reaction center of Photosystem I from chloroplasts (6, 7). The purified reaction center consisted of six polypeptides that were designated as Subunits I, II, III, IV, V, and VI in the order of decreasing molecular weights of 70,000, 25,000, 20,000, 18,000, 16,000, and about 8,000, respectively (6, 7). We have also shown in the previous papers that P700 is associated with the 70,000-dalton polypeptide (Subunit I), which was purified by treating the reaction center with SDS and by subsequent sucrose gradient centrifugation. The latter preparation, called the P700 reaction center, was active in the light-induced reversible bleaching of P700. However, this preparation was incapable of NADP photoreduction or of cytochrome f photooxidation.

At this stage of investigation, the functions of the low molecular weight polypeptides of the purified reaction center in NADP photoreduction were not understood.

McIntosh et al. (8) and Evans et al. (9) have recently suggested that two membrane-bound iron-sulfur centers of ferredoxin type serve in chloroplasts as intermediates in the photosynthetic electron transport from the primary electron acceptor of Photosystem I to soluble ferredoxin. However, this is disputed by Bearden and Malkin (10) who suggested that bound ferredoxin is the primary electron acceptor of Photosystem I. These authors were first to discover bound ferredoxin in chloroplasts by low temperature EPR spectroscopy (11).

We have reported previously (12) that bound ferredoxin was detected in the purified Photosystem I reaction center by EPR spectroscopy at 18 K. The P700 reaction center, associated with the single 70,000-dalton polypeptide, did not contain any EPR-detectable bound ferredoxin.

The present communication deals with the results of further studies on the subunit structure, functions and arrangement of the Photosystem I reaction center in the thylakoid membrane.

EXPERIMENTAL PROCEDURES

Materials — DEAE-cellulose (DE11) was obtained from Whatman Biochemicals Ltd. and was washed and equilibrated as previously described (13). Digitonin, Triton X-100, NADP, Tricine, Tris, PMS, and Mes were obtained from Sigma. Sodium dodecyl sulfate, acrylamide, and methylenebisacrylamide were obtained from Bio-Rad Laboratories. Sodium dithionite was obtained from BDH Chemicals. PMS-S was a generous gift from Dr. Gunter Hauska, Universitat Regensburg, Fachbereich Biologie und Vorklinische Medizin, Regensburg, Germany.

1 The abbreviations used are: SDS, sodium dodecyl sulfate; Tricine, N-tris(hydroxymethyl)methylglycine; Mes, 2-(N-morpholino)ethanesulfonic acid, PMS, N-methylphenazonium methosulfate; PMS-S, N-methylphenazonium-3-sulfonate.
Preparations—Chloroplasts and Photosystem I dguotonin particles from Swiss chard leaves were prepared as previously described (6, 7, 14). The Photosystem I reaction center was prepared as previously described (6, 7), with slight modification in Step III in order to obtain large scale preparations. The active fractions after DEAE-cellulose column chromatography were layered (4 ml on each tube) onto gradients of 5 to 25% sucrose, containing 50 mm Tris/Cl (pH 8) and 0.2% Triton X-100 and centrifuged for 20 h in the SW 27 Spinco Rotor at 25,000 rpm. The lower green band formed upon centrifugation contained the Photosystem I reaction center. Plastocyanin, ferredoxin, ferredoxin NADP reductase, cytochrome f and Euglena cytochrome 552 were prepared by published procedures (15–17).

Analytical Methods—Gel electrophoresis in the presence of sodium dodecyl sulfate was performed as described by Weber and Osborn (18). The gels were fixed, stained, and destained as previously described (15). Cytochrome f photooxidation (14), Euglena cytochrome 552 photooxidation (14), and NADP photoreduction (6, 7) were assayed by the published procedures. All of the above photochemical reactions as well as the light-induced Pn0 signal at 430 nm, were assayed spectrophotometrically by the illumination of the samples in the Cary 118C spectrophotometer cuvette with actinic beam and by recording the light-induced absorbance changes at the appropriate wavelength. The actinic beam was provided by a 150 W slide projector and passed through a red filter (Corning 2403). The photoelectrode was protected from the actinic beam by a blue filter (Corning 9782). The light intensity was 5 × 10^{-6} ergs/cm^2/s at the level of the cuvette. The specific activity of NADP photoreduction was calculated using a millimolar extinction coefficient of 5.85 at 330 nm. Cytochrome f photooxidation was recorded at 553.5 nm, and a millimolar extinction coefficient of 20.6 was used for calculation of the amount of photooxidized cytochrome f.

Euglena cytochrome 552 photooxidation was recorded at 552 nm and amounts of photooxidized cytochrome were calculated using a differential millimolar extinction coefficient of 19.6. The reduced-minus-oxidized difference spectrum of Pn0 was recorded with the Cary 118C spectrophotometer as previously described (7). The reaction mixture for the light-induced Pn0 signal contained, in a final volume of 1 ml, 20 μmol of Tricine/Mes (pH 7), 2 μmol of sodium ascorbate, 1 nmol of PMS, and reaction center preparations, equivalent to 0.2 to 0.4 nmol of Pn0. An extinction coefficient of 45/mM/cm was used for calculation of the amount of Pn0 (19).

RESULTS

Further Purification of Pn0 Reaction Center on Chlorophyll Basis—In our previous communications (6, 7), a purification procedure was described which yielded the Pn0 reaction center (Subunit I) containing 40 to 50 chlorophyll a molecules/Pn0. Subunit I with about 30 chlorophyll a molecules/Pn0 can be obtained by increasing the Triton concentration in the sucrose gradient to 1% and prolonging the centrifugation time to about 40 h. However, recovery of the light-induced Pn0 signal was rather low under these conditions (20 to 40%). A "blue shift" of the chlorophyll a absorption band to 670 to 672 nm probably reflected partial solubilization of antennae chlorophyll a from the reaction center complex as a result of Triton X-100 treatment.

The experiment that is depicted in Fig. 1 is in line with this assumption. The extent of Pn0 photooxidation in control Pn0 reaction center was similar in the presence of ascorbate or ascorbate plus PMS. After the Triton treatment, the reaction center was depleted of 50% of its chlorophyll a and maintained comparable extent of Pn0 photooxidation in the presence of ascorbate. However, the rate of Pn0 photooxidation was slower and the extent of Pn0 photooxidation in the presence of PMS was markedly decreased. This was due to fast reduction of Pn0 by reduced PMS and the slower rate of Pn0 photooxidation. The rate of Pn0 reduction by reduced PMS was not altered by the Triton treatment. This means that some of the chlorophyll a molecules in this preparation failed to transfer the light energy to the Pn0 pigment.

Further depletion of chlorophyll from Pn0 reaction center can be obtained by the use of DEAE-cellulose column. Pn0 reaction center equivalent to about 2 mg of chlorophyll was applied to a DEAE-cellulose column (1 × 10 cm), which was equilibrated with a buffer solution, containing 50 mm Tris/Cl (pH 8) and 0.5% Triton X-100. The column was washed with 200 to 300 ml of the same buffer for several hours in the dark at 4°C. The Pn0 reaction center was eluted with 50 ml of 0.2 M NaCl in the same buffer. The preparation contained one Pn0/10 to 20 chlorophyll a molecules, and its absorption spectrum showed a maximum at 676 nm. In order to achieve the chlorophyll a to Pn0 ratio of 10, the column had to be washed overnight but the recovery of Pn0 was lower than 10%. Table I summarizes the purification of Pn0 reaction center on the chlorophyll basis and recovery of Pn0 signal through the purification procedure. The described Pn0 reaction center, highly enriched in Pn0, appeared to be quite similar in other properties to the Pn0 reaction center preparation described previously (6, 7). It migrated as a single band (Mr = 70,000) on the SDS gels and was completely free of carotenoids.

Partial Resolution of Photosystem I Reaction Center—As described previously (6, 7), the purified Photosystem I reaction center was active in NADP photoreduction. Fig. 2 shows that the purified preparation is also active in cytochrome f photooxidation, which is completely dependent upon plastocyanin. Another assay for the oxidizing side of the Photosystem I reaction center is photooxidation of Euglena cytochrome 552. This cytochrome replaces plastocyanin in plant chloroplasts (20). In accord with this, we have observed that cytochrome 552 replaces plastocyanin in NADP photoreduction by the Photosystem I reaction center equivalent to about 2 mg of chlorophyll.

TABLE I

<table>
<thead>
<tr>
<th>Chlorophyll</th>
<th>Chlorophyll a/Pn0</th>
<th>Total Pn0</th>
<th>Recovery of</th>
<th>Recovery of</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg</td>
<td>a/Pn0</td>
<td>signal</td>
<td>Pn0 signal</td>
<td>Pn0 signal</td>
</tr>
<tr>
<td>DEAE-active fractions</td>
<td>10.3</td>
<td>124</td>
<td>5.3</td>
<td>100</td>
</tr>
<tr>
<td>Pn0 reaction center</td>
<td>1.6</td>
<td>32</td>
<td>3.2</td>
<td>60</td>
</tr>
<tr>
<td>after sucrose gradient in 1% Triton</td>
<td>0.5</td>
<td>18</td>
<td>1.8</td>
<td>34</td>
</tr>
<tr>
<td>DEAE-cellulose column</td>
<td>0.5</td>
<td>18</td>
<td>1.8</td>
<td>34</td>
</tr>
</tbody>
</table>
purified reaction center. The specific activity was quite similar to that obtained with plastocyanin.

In looking for treatment that will modify specifically the donor site of Photosystem I, it was found that this is sensitive to Triton treatment. Partial inhibition of NADP photoreduction and cytochrome 552 photooxidation can be obtained by increasing the Triton concentration during the sucrose gradient centrifugation to 1%. Complete inhibition of these reactions was obtained by the adsorption of the Photosystem I reaction center to a DEAE-cellulose column (1 x 10 cm), which was equilibrated with 50 mM Tris/Cl (pH 8), containing 0.5% Triton X-100. After the column was washed with the same buffer for several hours, the reaction center was eluted with 50 mM Tris/Cl (pH 8), 0.2% Triton, and 0.2 M NaCl. The SDS-gel electrophoresis pattern and relative amounts of the subunits of the eluted preparation are shown in Fig. 3 and in Table II, respectively, in comparison to those of the control reaction center. It is seen that the preparation eluted from the DEAE-cellulose column was completely free of Subunit III. Relative amounts of the remaining reaction center subunits were unaltered. The preparation contained about 35 chlorophyll a molecules./P700 and was almost free of β-carotene.

This reaction center preparation completely lost both NADP photoreduction and cytochrome f photooxidation activities. Fig. 4 shows that the cytochrome 552 photooxidation was markedly decreased in the reaction center lacking Subunit III. If Subunit III participated in NADP photoreduction in the reducing side of Photosystem I, one would expect cytochrome f photooxidation to be uninhibited in preparations depleted in this subunit. Therefore, we have assumed that Subunit III probably mediates the electron transfer from plastocyanin to P700. To test this assumption, we needed a suitable electron donor which could bypass plastocyanin and reduce P700 directly at a rate sufficiently high to accomplish NADP photoreduction, by the Photosystem I reaction center. Under these con-  

Subunit III photoreduced NADP when PMS-S replaced plastocyanin.

The data presented in Table III confirmed the suggestion that Subunit III might be located between plastocyanin and P700, mediating the electron transfer from the former to the latter.

Preparation and Properties of Reaction Center Partially Depleted in Low Molecular Weight Subunits—Active frac-
Subunit Structure of Chloroplast Photosystem I Reaction Center

Fig. 4. Photooxidation of cytochrome 552 by Photosystem I reaction center and the reaction center depleted of Subunit III. The reaction mixture contained, in a final volume of 1 ml, 20 μmol of Tricine/Mes (pH 7), 1 μmol of sodium ascorbate, 0.6 nmol of Euglena cytochrome 552, and Photosystem I reaction center or reaction center depleted of Subunit III containing 0.7 nmol of P700. L, light; D, dark.

Table III

<table>
<thead>
<tr>
<th>Reaction center</th>
<th>NADP photoreduction</th>
<th>Cytochrome 552 oxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control reaction center</td>
<td>214</td>
<td>0.6</td>
</tr>
<tr>
<td>Reaction center lacking Subunit III</td>
<td>0</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Table IV

<table>
<thead>
<tr>
<th>Subunits</th>
<th>NADP photoreduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plastocyanin</td>
<td>PMS-S</td>
</tr>
<tr>
<td>Photosystem I reaction center</td>
<td>100</td>
</tr>
<tr>
<td>Reaction center depleted of Subunit III</td>
<td>80</td>
</tr>
</tbody>
</table>

Fig. 5. Photochemical activities of preparations that were partially depleted in the low molecular weight subunits by means of Triton X-100 and SDS treatments. The reaction mixture for NADP photoreduction contained in a final volume of 1 ml, 20 μmol of Tricine/Mes (pH 7), 2 μmol of sodium ascorbate, 0.1% Triton X-100, 0.5 μmol of NADP, 3 nmol of ferredoxin, 0.5 nmol of ferredoxin NADP reductase, 2.5 nmol of plastocyanin, and reaction center preparations containing 0.7 nmol of P700. The activities and the subunit amounts are expressed as percentage of control reaction center that was obtained from the sucrose gradient containing 0.2% Triton X-100. The DEAE-fractions were treated with the specified SDS concentrations and then were centrifuged in sucrose gradients containing 1% Triton X-100 (right). The control rate of NADP photoreduction was 190 μmol NADP/mg of chlorophyll/h.

Table IV

<table>
<thead>
<tr>
<th>Subunits</th>
<th>NADP photoreduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photosystem I reaction center</td>
<td>100</td>
</tr>
<tr>
<td>Reaction center depleted of Subunit III</td>
<td>80</td>
</tr>
</tbody>
</table>

Electrophoresis in the presence of SDS was performed as described under "Experimental Procedures" and in the legend of Fig. 3. The control rate of NADP photoreduction with plastocyanin was 210 μmol of NADP/mg of chlorophyll/h and with PMS-S 30 μmol of NADP/mg of chlorophyll/h.

Photoreduction of NADP and photooxidation of cytochrome 552 by the reaction center, lacking Subunit III

Photoreduction and photooxidation activities were assayed as described under "Experimental Procedures." Reaction mixture for cytochrome 552 photooxidation was as described in the legend to Fig. 4. Reaction mixture for NADP photoreduction was as described in the legend to Fig. 5. PMS-S (1 μM) was added when indicated in the Table. Cytochrome 552 photooxidation is expressed as the amount of oxidized cytochrome 552 in steady state.

The NADP photoreduction activities and relative amounts of the subunits in the reaction center preparations, obtained by treatment of the DEAE-fractions with SDS prior to sucrose gradient centrifugation

The DEAE-fractions were treated with SDS in specific concentrations for 15 min at 0°C. The NADP photoreduction activity and subunit ratios in SDS-treated preparations are expressed as percentage of those in the control reaction center. Conditions for the NADP photoreduction reaction were as described in the legend of Fig. 5: 1 μM PMS-S was added to the reaction mixture where indicated. The subunit ratios were integrated from the scans of the SDS gels. Ectophosphorin in the presence of SDS was performed as described under "Experimental Procedures" and in the legend of Fig. 3. The control rate of NADP photoreduction with plastocyanin was 210 μmol of NADP/mg of chlorophyll/h and with PMS-S 30 μmol of NADP/mg of chlorophyll/h.

It may be seen that depletion in Subunits IV and V correlated with the inhibition of NADP photoreduction, when either reduced plastocyanin or PMS-S were the donors of
electrons for P800. Progressive depletion in Subunit VI was also observed in the SDS-treated preparations, but it was not followed quantitatively. On the basis of the results described in this section, it may be suggested that Subunits IV, V, and VI probably participate in NADP photoreduction on the reducing side of Photosystem I.

**Discussion**

Reaction center preparations, selectively depleted in the low molecular weight subunits, were obtained by means of SDS and Triton X-100 treatments and DEAE-cellulose chromatography or centrifugation in the sucrose gradients. The biochemical properties of the depleted preparations gave further insight into possible functions of the individual subunits of the purified reaction center in the overall photochemistry of Photosystem I.

We have observed that photooxidation of cytochrome f by the Photosystem I reaction center was completely dependent upon plastocyanin. This gives another piece of evidence in favor of the currently accepted scheme of the photosynthetic electron transport, in which plastocyanin is placed between cytochrome f and P800 (for reviews see Refs. 22 and 23). While most of the biochemical experimental evidence supports this scheme (14, 23, 24), some kinetic experiments indicate that both cytochrome f and plastocyanin, acting in parallel, may reduce P800 (25, 26).

The ability of PMS-S to serve as the electron donor for NADP photoreduction is probably due to its slow autooxidizability (20) and hydrophilic properties. We have observed that PMS-S concentrations of 1 to 2 μM were optimal for NADP photoreduction. At higher concentrations, the rates of reduction were progressively inhibited. This may indicate that PMS-S, when added in excess, became capable of trapping the electrons from the primary electron acceptor for Photosystem I.

It was previously suggested that Subunit I spans the lipid core of the thylakoid membrane (6, 7). This was based on the assumption that P800 pigment is situated in the internal side of the chloroplast membrane (21, 27) and that a specific antibody interacted with the same polypeptide on the external side of the membrane (6, 7). Purified Subunit I is free of bound ferredoxin but active in P800 photooxidation (6, 7, 12). Kinetic studies revealed that Subunit I contains the primary electron acceptor and its nature is under investigation. Two or three different clusters of bound ferredoxin were identified in chloroplasts Photosystem I preparations (10, 11, 28-30). Mild treatment with SDS diminished the EPR signal of bound ferredoxin with parallel loss of NADP photoreduction activity (12) and disappearance of Subunits IV, V, and VI (Fig. 5). Therefore, it was assumed that Subunits IV, V, and VI might be the bound ferredoxins that mediate electron transport from the primary acceptor to soluble ferredoxin in the external side of the thylakoid membrane. Nearest neighbor analysis revealed that Subunits III and V are in close position to Subunit I. Hence, Subunit V is positioned in contact with Subunit I on the reducing side of Photosystem I. Further studies are required to establish the role of each subunit in NADP photoreduction.

**Acknowledgment**—We would like to thank Mr. Chaim Julian for reading this manuscript.

**REFERENCES**


* N. Nelson, and B. Notsani, unpublished observations.
Subunit Structure of Chloroplast Photosystem I Reaction Center
Carmela Bengis and Nathan Nelson


Access the most updated version of this article at http://www.jbc.org/content/252/13/4564

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/252/13/4564.full.html#ref-list-1