A photosystem I reaction center was isolated from *Chlamydomonas reinhardii* chloroplasts. It consists of four different polypeptides with \( M_r \approx 70,000 \) (subunit I), 19,000 (subunit II), 10,000 (subunit III), and 8,000 (subunit IV). In the presence of salts, the purified reaction center was active in cytochrome 552 photooxidation. Short term labeling experiments with \([^{35}S]\)sulfate revealed that subunit III contains no cysteine or methionine. Subunits I and IV were shown to be chloroplast translation products, while subunit II appears to be synthesized on cytoplasmic ribosomes. The site of synthesis of the subunits to the proton-ATPase complex was studied. A differential effect of cycloheximide on the assembly of photosystem I reaction center and the proton-ATPase complex was indicated.

The green alga *Chlamydomonas reinhardii* has been extensively used in genetic and biogenesis studies of photosynthesis (1-4). Ohad and his co-workers (2, 5) conducted a detailed study of the development of the photosynthetic membranes during greening of the cells. The onset, upon illumination of the activity of the two photosystems, was worked out. Moreover, the presence of polypeptides of cytoplasmic and chloroplast origin in partially purified preparations of photosystems I and II was indicated (6).

A photosystem I reaction center was isolated from higher plants (7-10). The purified reaction center was shown to consist of six different polypeptides designated as subunits I to VI in order of decreasing \( M_r \approx 70,000 \) (I), 25,000 (II), 20,000 (III), 18,000 (IV), 16,000 (V), and 8,000 (VI). Subunits III and IV switch their positions in Tris/glycine/sodium dodecyl sulfate slab gels and subunit VI splits into two apparent protein bands (10). The role of subunits I and III in the photobiological activity of the photosystem I reaction center have been worked out, and the subunit structure within the chloroplast membrane has been proposed (8, 9).

In this article, we report on the purification of the photosystem I reaction center from *Chlamydomonas* chloroplasts. The subunit structure and some biochemical properties of this reaction center and the photosystem I reaction center from higher plants are compared. The sites of synthesis of individual subunits are studied by labeling with \([^{35}S]\)sulfate in the presence of protein synthesis inhibitors. The effect of polypeptide(s) originating in the cytoplasm on the assembly of the reaction center is indicated.

**EXPERIMENTAL PROCEDURES**

**Materials**—Most of the chemicals were purchased from Sigma.

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at 0 °C, the suspension was centrifuged at 200,000 × g for 60 min. The supernatant was used for the preparation of the proton-ATPase complex as previously described (15). The pellet was homogenized in a solution containing 25 mM Tricine (pH 8) and 2% Triton X-100 to give a final chlorophyll concentration of 0.5 mg/ml. It was then centrifuged at 10,000 × g for 10 min and the supernatant was kept at −20 °C until used. A sample of ~10 ml was thawed and centrifuged at 10,000 × g for 10 min and the supernatant was applied to a DEAE-cellulose column (1 × 12 cm) equilibrated with a solution containing 20 mM Tris-Cl (pH 8) and 2% Triton X-100. The reaction center was eluted with a linear NaCl gradient of 0 to 300 mM (20 ml in each chamber) in a solution containing 20 mM Tris-Cl (pH 8) and 2% Triton X-100. The tubes of the first green peak (containing more than 0.2 mg of chlorophyll/ml) were pooled and fractions of 1 ml were applied to linear sucrose gradients of 5 to 30% sucrose in a solution containing 20 mM Tris-Cl (pH 8) and 0.2% Triton X-100. The gradients were centrifuged in an SW-41 rotor at 35,000 rpm for 15 h at 2 °C. The lower green band was collected and used promptly or stored frozen at −70 °C. When desired it could be concentrated on a small DEAE-cellulose column as previously described (9).

The purification of the photosystem I reaction center is summarized in Table I. The purified preparation contained ~75 chlorophyll a molecules per/1 P700, and it enriched ~25-fold over the chloroplast membranes. The absorbance spectrum of the isolated reaction center is nearly identical to the one isolated from higher plants. Light-induced P700 oxidation was readily observed in the purified preparation, but NADP photoreduction could not be detected either in the purified reaction center or in the chloroplast membranes. The regularity of the donor site of the photosystem I reaction center was examined by following the light-induced cytochrome 552 oxidation. Fig. 1 shows that under conditions in which high rates of cytochrome 552 photooxidation were recorded with the photosystem I reaction center from higher plants (8), the Chlamydomonas reaction center was not active. However, the inclusion of 200 mM NaCl or 8 mM MgCl2 or lowering the pH to 5 induced the light-dependent cytochrome 552 photooxidation activity of the Chlamydomonas photosystem I reaction center. Recent studies in our laboratory have shown that the Swiss chard photosystem I reaction center that was depleted of subunit III is analogous to the Chlamydomonas reaction center. The regularity of the acceptor site of the reaction center is currently being studied by low temperature EPR spectroscopy.

The Chlamydomonas photosystem I reaction center was reconstituted into phospholipid vesicles together with purified proton-ATPase complex from lettuce chloroplasts (13). Upon illumination in the presence of N-methylphenazonium methosulfate, phosphorylation rates above 10 µmol of ATP/mg of chlorophyll/h were recorded.

On sodium dodecyl sulfate gels, the reaction center dissociated into four major bands that were designated as subunits I, II, III, and IV with Mr, ~70,000, 19,000, 10,000, and 8,000, respectively (Fig. 2). The stoichiometry between subunits I, II, III, and IV was found to be 2:1:1:1 in three different experiments. This was deduced from the amount of 14C that

1 R. Nechashtai and N. Nelson, J. Bioenerg. Biomembr., in press.
was incorporated into the protein bands and the above mentioned molecular weights of the polypeptides.

The fact that in all the preparations of the reaction center, the same subunit composition was obtained strongly suggests that we are dealing with a tight protein complex which is typical for a functional unit in the chloroplast membrane. The designation of its various subunits was done according to the order of decreasing molecular weights and their relation to the subunits of photosystem I reaction center from higher plants will be pointed out in the discussion. Subunit I was dissociated into two apparent protein bands (Fig. 2). This phenomenon was observed previously in photosystem I preparations from Chlamydomonas chloroplasts (3, 4, 6), and in purified photosystem I reaction center from higher plants (8, 9). Fig. 3 depicts one-dimensional fingerprinting (18) of the upper and lower bands of subunit I. It is evident that the fingerprints are similar and that the two polypeptides resemble each other.

The synthesis and assembly of individual subunits in the photosystem I reaction center was followed by short term labeling with [35S]sulfate with subsequent isolation of the reaction center. Fig. 4 shows the autoradiography of photosystem I reaction centers isolated from cells grown in the presence of [14C]carbonate or [35S]sulfate. All four subunits of the reaction center were labeled by the [14C]. Since only subunits I, II, and IV were labeled with [35S]sulfate, subunit III probably does not contain any cysteine or methionine.

Fig. 5 shows autoradiographs of purified photosystem I reaction centers and proton-ATPase complexes that were prepared from [35S]sulfate-labeled cells in the absence or presence of protein synthesis inhibitor. Chloramphenicol prevented the synthesis of subunits I and IV of photosystem I reaction center and subunits α, β, ε, and III of the proton-ATPase complex. Subunit II of the photosystem I reaction center and the γ, δ and subunit II of the proton-ATPase complex were synthesized and assembled into the complexes in the presence of chloramphenicol. The presence of cycloheximide abolished the synthesis of subunit II of the photosystem I reaction center and the γ, δ, and II subunits of the proton-ATPase complex. While preincubation with cycloheximide for 1 h completely prevented the assembly of labeled subunits into the photosystem I reaction center (Fig. 5, lanes 4), labeled α and β subunits in the amount of up to 40% of the control were incorporated into the proton-ATPase complex.

**DISCUSSION**

A photosystem I reaction center containing four different subunits has been isolated from Chlamydomonas chloroplasts. Subunit I resembles that of higher plants in $M_r = 70,000$, its stoichiometry with the other subunits (2:1), and even its appearance on sodium dodecyl sulfate gels as a diffuse band or even resolved into two distinct bands (7–10). A question has been raised as to whether these two bands are
two different polypeptides, or whether they are related to each other (3, 6, 9). The one-dimensional fingerprinting experiment (Fig. 3) clearly indicated a resemblance between the two polypeptides; it is quite likely that their different mobilities on the gel are due to posttranslational modification of the polypeptide. Attempts to detect sugar moieties covalently bound to subunit I failed, and therefore, the nature of the modification is not known.

The number of chlorophyll $\alpha$ molecules and $P_{300}$ pigment are comparable to the higher plant reaction center (7, 8). However, the Chlamydomonas reaction center is inactive in NADP photoreduction. This might be due to the lack of a polypeptide analog to subunit III of the photosystem I reaction center from higher plants (8, 9). We do not yet know whether such a subunit was dissociated from the complex during its preparation or whether the Chlamydomonas photosynthetic system replaces its function by a proton gradient across the chloroplast membranes.

It is also possible that one of the secondary electron acceptors is missing from the preparation. Since subunit III lacks cysteine, it cannot serve as "bound ferredoxin"; only subunits II and IV are candidates for this function.

Short term labeling experiments with [$^{35}$S]sulfate were employed for the study of subcellular sites of synthesis of chloroplast proteins. In vivo labeling of Chlamydomonas cells in the presence of specific inhibitors revealed that subunits $\alpha$, $\beta$, $\epsilon$, and III (the chloroplast proteolipid) (see Refs. 15 and 19) of the proton-ATPase complex are chloroplast products while the $\gamma$, $\delta$, and subunit II of CF$_{1}$ have been synthesized in a cycloheximide sensitive system. These findings are in line with previous in vivo studies in higher plants (10, 20). The biological significance of synchronizing the synthesis of the proton-ATPase subunits has been discussed elsewhere (10, 15). In the photosystem I reaction center, the synthesis of subunits I and...
IV was inhibited by chloramphenicol, while the synthesis of subunit II was inhibited by cycloheximide. Therefore, subunit II appears to be synthesized on cytoplasmic ribosomes, whereas subunits I and IV appear to be chloroplast translation products. It was previously observed that a polypeptide corresponding to subunit I from photosystem I reaction center is made inside the chloroplasts of *Chlamydomonas* and higher plants (21, 22).

The effect of preincubation with cycloheximide on the incorporation of ^35^S into the various protein complexes is of special interest. The preincubation completely prevented the incorporation of ^35^S into assembled photosystem I reaction center while appreciable amounts of ^35^S were observed in the chloroplast translation products of the assembled proton-ATPase complex. This must be due to the existence of pools of cytoplasmic products as precursors outside the chloroplasts, or as mature subunits inside the organelle (15). The assembly of photosystem I reaction center was abolished by blocking the protein synthesis on cytoplasmic ribosomes. It is tempting to suggest that subunit II is responsible for this property and that this subunit serves as a template for the assembly of photosystem I reaction center. However, it was recently shown by Girard *et al.* (23) that several nuclear genes are responsible for the functional assembly of photosystem I in the chloroplast membrane.

It is quite clear that photosystem I reaction centers from higher plants and *Chlamydomonas* resemble each other in several respects. It is also likely that one or more of the missing three subunits of the *Chlamydomonas* reaction center might be loosely bound polypeptides that were lost during its preparation.

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