Analysis of the Subunit Structure of Protochlorophyllide Holochrome by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis

Received for publication June 11, 1976 and in revised form February 28, 1977

Ora D. Canaan and Kenneth Sauer
Laboratory of Chemical Biodynamics and Department of Chemistry, University of California, Berkeley, California 94720

ABSTRACT
The subunit structures of protochlorophyllide holochrome (PCH) and chlorophyllide holochrome (CH) were studied by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. PCH from leaves of dark-grown (Phaseolus vulgaris var. red kidney) is a polymeric pigment-protein complex of approximately 600,000 daltons. It is composed of 12 to 14 polypeptides of 45,000 daltons, when examined prior to and immediately following photoconversion. The protochlorophyllide or chlorophyllide pigment molecules are associated with these polypeptides. Subsequent to photoconversion, the absorption maximum of newly formed chlorophyllide shifts from 678 nm to 674 nm upon standing in darkness. Following the 678 to 674 spectral shift, the chlorophyllide is associated with a polypeptide with a molecular weight of 16,000 daltons. In addition, sucrose gradient centrifugation of PCH and CH under nondenaturing conditions indicates that during the course of the dark spectroscopic shift, the 600,000 dalton CH undergoes dissociation into a small chlorophyllide protein. The dissociation of CH, the change in the molecular weight of the chlorophyllide polypeptide from 45,000 to 16,000 daltons, as well as the dark spectroscopic shift are temperature-dependent and blocked below 0 °C. It was also found that each holochrome molecule of 600,000 daltons contains at least four protochlorophyllide pigment molecules.

The assembly of the photosynthetically active membrane of chloroplasts is triggered by Chl synthesis. This process takes place when dark-grown seedlings are subjected to illumination. It involves a photoreduction of PChlide to Chlide at a protein site. The photoconversion can be studied using an extracted pigment-protein complex called PChlide holochrome. The biochemistry and physiology of PCh have been extensively reviewed by Boardman (4), Kirk (14), and more recently by Rebeiz and Castelfranco (19). Bogorod et al. (5) have presented evidence that the 600,000 dalton PChlide-protein complex from dark-grown bean leaves undergoes dissociation following photoreduction, thus producing Chlide attached to a small protein.

Henningsen et al. (11) observed the change of a 63,000 dalton mol wt photoactive Chlide subunit to a 29,000 dalton Chlide subunit following the dark spectroscopic shift in a dark-grown barley leaf homogenate.

Guignery et al. (9) demonstrated in Zea mays L. leaves that PChlide is associated with two polypeptide chains of 21,000 and 29,000 daltons. The study also showed that Chl is associated with four polypeptide chains of 21,000, 25,000, 29,000, and 70,000 daltons.

The exact number of PChlide chromophores/protein macromolecule is still a matter of controversy. CD measurements performed on PCh suggest a dimer of interacting PChlide molecules as the basic unit (17, 21). This spectroscopic result is in agreement with structural information obtained by Schopfer and Siggelman (20), who found at least two molecules of PChlide/550,000 dalton mol wt protein. On the other hand, fluorescence spectroscopy studies at −196 °C performed by Kahn et al. (12) indicate a basic aggregate of at least four chromophores/unit.

Thorne (24) suggested an aggregate of 20 chromophores. His estimate was based on studies of energy transfer between PChlide and Chlide in etiolated bean leaves at −196 °C.

In this paper we examine the subunit structure of PCh purified from dark-grown bean leaves. The fate of the pigment-protein complex after photoconversion and after the completion of the dark spectroscopic shift (22) is studied both on the macro-molecular level and the polypeptide level. The results provide further evidence for the dissociation of the nascent CH. The number of PChlide molecules attached to the protein complex is determined for the most purified PCh preparation.

MATERIALS AND METHODS
PREPARATION OF HOLOCHROME
Bean seedlings (Phaseolus vulgaris var. red kidney) were grown on vermiculite 12 ± 2 days in the dark at 22 °C. The leaves (50–100g) were harvested and ground in a buffer containing 10 mM tris-HCl (pH 8.5), 2 mM MgSO₄, 1 mM EDTA, and 25% (v/v) glycerol. In some cases, Triton X-100 (in final concentration of 0.06%, v/v) was added to the extracting solution and is referred to as buffer + Triton. All manipulations were done in a cold room (4 °C) under a green safelight. Two different methods were used for preparation of PCh.

Method 1. The leaves were ground in buffer + Triton in a Waring Blender during four 60-sec intervals, separated by 5 min. The homogenate was filtered through six layers of cheesecloth and centrifuged at 78,000g in a Beckman (model L-2) ultracentrifuge (No. 30 rotor) at 0 °C for 1 hr. The supernatant was treated with a 50% PEG-6,000 solution to a final concentration of 17% and centrifuged at 48,000g (−2 °C) in a Sorvall RC-2B centrifuge for 1 hr. The precipitate was redissolved in buffer.

1 This work was supported by the United States Energy Research and Development Administration.
2 Abbreviations: PCH: protochlorophyllide holochrome; CH: chlorophyllide holochrome; RuDP-Case: ribulose diphosphate carboxylase; SDBS: sodium dodecyl benzene sulfate; Kd: kilodaltons; Bis: N,N'-methylene-bis-acrylamide; TEMED: N,N,N',N'-tetramethylethlenediamine; CD: circular dichroism.
Triton and clarified by centrifugation at 48,000g for 30 min. Before redissolution, the precipitate was stored with a layer of fresh buffer + Triton for about 1 hr to facilitate the resuspension. The clarified supernatant was adjusted to 0.2 M KCl and applied to an equilibrated hydroxylapatite column (7.5 × 20 cm). The column was washed with 2 to 3 liters of tris buffer containing 0.2 M KCl. We found that tris buffer containing 0.1 M K-phosphate (pH 8) was sufficient to elute the PCH. The flow rate was maintained at 10 ml/min. All fractions with an absorption maximum at 639 nm containing PCH were combined. This partially purified pigment-protein complex was used immediately or stored at −20 °C. This procedure was similar to the one reported by Schopfer and Siegelman (20). The partially purified holochrome was very stable, and maintained its photoconversion activity for at least a week at 4 °C.

Method 2. This procedure was adapted from Akoyunoglu et al. (1). Etiolated leaves were homogenized in tris buffer (same as above) and filtered through six layers of cheesecloth, followed by centrifugation at 48,000g for 30 min in a Sorvall RC-2B centrifuge (SS-34 rotor). The pellet was extracted three times with tris buffer, collecting supernatants S1 through S3 and a fourth time with tris buffer + Triton. The supernatant from the fourth extraction, S4, was used directly for polyacrylamide gel electrophoresis or was centrifuged on a sucrose gradient. The supernatants S1 to S4 were assayed for protein content and PCH activity. In some of the experiments, the harvested leaves were frozen and kept at −90 °C for several weeks. The leaves were not thawed prior to homogenization but ground to a fine powder with a mortar and pestle while frozen on dry ice. The powder was transferred to a Waring Blender, and all subsequent steps were the same as with fresh leaves.

The S1 is of high purity, but not very stable. It loses its activity within several hr at 4 °C. The S1 preparation was used immediately for further experiments or made 50% with respect to glycerol (v/v) and kept at −20 °C. The S1 PCH was immediately diluted to produce S1 CH. Absorption spectra of the various preparations were measured at 22 °C in a 1 cm cuvette using a Cary model 14 spectrophotometer.

GEL ELECTROPHORESIS

Gel electrophoresis was carried out following the Weber-Osborn (25) procedure modified by Neville (18). SDS gels contained 10% and 5% in the separating and stacking gel, respectively. Two different sets of experimental conditions were used. The first set provided for the analysis of the peptide composition of the holochrome. In this set, the gel measured 0.5 cm in diameter, 8 cm high and contained 0.1% SDS. Electrophoresis was carried out in 0.1% SDS at room temperature for 1 hr at 1.5 mamp/gel followed by 2 hr at 3 mamp/gel. Samples containing 0.05 to 0.1 mg protein in 8 ml urea, 1% β-mercaptoethanol, 2% SDS, and 10% glycerol were boiled for 2 min and loaded on the gels. After electrophoresis, the gels were stained overnight with 0.1% Coomassie brilliant blue (Blr-Richmond, Calif.) in 5:1:5 methanol-acetic acid-water. Destaining was achieved in 5:1:5 methanol-acetic acid-water by diffusion. A trace of the stained gel was obtained by scanning at 600 nm using a Gelscan densitometer (Gelman Instrument Co., Ann Arbor, Mich.). The second set of experimental conditions was used for locating the pigment on the gel. For these conditions, a gel column 1.3 cm in diameter and 8 cm high was used. The sample contained 1 mg protein in 8 ml urea, 2% SDS, 1% β-mercaptoethanol, 10% glycerol. It was incubated for 15 min at 22 °C and loaded on the gel. After electrophoresis at 4.5 hr for 4.5 hr at 3 mamp/gel followed by 4.5 hr at 6 mamp/gel, the gel was frozen and sliced into 3.8-mm sections. Typically, two identical gels were combined. Each section was dispersed by extrusion from a syringe through 125 mesh nylon bolting cloth (Turtex/Com-
and peaks B3, C3 had very similar mobilities and mol wt to those of the large (58 Kd) and small (14 Kd) subunits of RuDP-Case, respectively. Peaks B2 and C2, corresponding to 40 Kd mol wt (Table I), did not appear in the analysis of RuDP-Case. The ratio of staining intensity of peaks B1 and B3 to B2 was smaller than the ratio of C1 and C3 to C2. From this result we conclude that the three polypeptides do not belong to one protein. The 40 Kd polypeptide (peak 2) appeared to be enriched in fraction B compared to that from fraction C from the sucrose gradient. The ratio between the areas of peaks B1 and B3 (presumably the large and small subunits of RuDP-Case, respectively) to the area of peak B2 (shown later to be the polypeptide of PCH) is about 1:1. This indicates that PCH can be no more than 50% pure in fraction B.

To identify peak 2 as the major polypeptide of PCH, an attempt was made to locate the pigment on the gels. A sample of irradiated S0 containing 200 µg of protein (five times more than the usual amount) was analyzed on polyacrylamide gel. Following electrophoresis the gel was scanned at 675 nm. Two peaks of Chlde absorption were observed (Fig. 2). The major peak, D1, had a mobility of 0.4 corresponding to a mol wt of 40 Kd. The minor peak, D2, had a mobility of 0.8 corresponding to a mol wt smaller than 10 Kd. The mobility of the major peak D1 was very similar to the mobility of peaks B2 and C2 (Table I). These results suggest that peak D1 is composed of a 40 Kd polypeptide associated with the Chlde molecule. The minor peak D2 appears to be a proteolytic product of PCH.

**Fig. 1.** SDS-polyacrylamide gel electrophoresis of PCH after purification by differential solubility and sucrose gradient centrifugation. Sample contained 40 µg protein. Ten per cent stacking gel was stained by Coomassie blue and scanned at 600 nm. Peaks observed are marked C1 through C3.

**Table I**

<table>
<thead>
<tr>
<th>Gel</th>
<th>Peak 1</th>
<th>Peak 2</th>
<th>Peak 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCH Fraction B</td>
<td>0.228</td>
<td>0.385</td>
<td>0.785</td>
</tr>
<tr>
<td>PCH Fraction C</td>
<td>0.226</td>
<td>0.397</td>
<td>0.731</td>
</tr>
<tr>
<td>RuDP-Case</td>
<td>0.225</td>
<td>---</td>
<td>0.74</td>
</tr>
</tbody>
</table>

**Fig. 2.** SDS-polyacrylamide gel electrophoresis of CH in an irradiated S0 supernatant. Sample contained 200 µg protein. Ten per cent stacking gel was scanned at 675 nm for Chl absorption before fixation. Peaks observed are marked D1 and D2.

**pigment extraction from gel after electrophoresis.** In many experiments it was difficult to measure the absorption of Chlide directly on the gel because of rapid diffusion of urea from the gel resulting in changes in light scattering by the gel. This problem was overcome by extracting the pigment from the gel slices and recording the fluorescence spectrum from each extracted fraction. A holochrome sample was prepared by treating the crude extract of etiolated leaves with PEG followed by hydroxylapatite chromatography (method 1). PCH was then photoconverted to CH absorbing at 680 nm. This preparation was denatured and dissociated into polypeptides by incubation in 2% SDS, 8 M urea, 1% β-mercaptoethanol. The absorption maximum shifted to a shorter wavelength and was located at 673 nm. Whether the incubation was carried out at 22 C for 15 min or by boiling for 2 min, there was only one polypeptide of 45,000 daltons with which the Chlide pigment was associated. However, the highest yield of the pigment-polypeptide complex was obtained by incubation of CH for 15 min at 22 C in the denaturing reaction mixture. The sample was then loaded on a 10% polyacrylamide gel, and electrophoresis was carried out at 4 C in the large gels (1.3 × 8 cm). The gel was sliced into 3.8-mm sections. Each section was extracted with acetone, and its Chlide content was measured by fluorescence, as described under "Materials and Methods." A schematic representation of the gel is shown in Figure 3A. One major peak of Chlide fluorescence was observed on the gel. Its location corresponded to a mobility of 0.28 ± 0.08 and mol wt of 45 ± 10 Kd as calculated from the calibration curve. In addition, there was a minor peak with mobility of 0.87 corresponding to a mol wt of about 6 Kd.

A typical fluorescence spectrum of the pigment obtained from the gel after acetone extraction is shown in Figure 4. In some experiments, the fluorescence of both PChlde at 630 nm and Chlide at 666 nm occurred at the same location on the gel (Fig. 4). The pigments were characterized by comparison with known fluorescence spectra of PChlde and Chlide in acetone and by
Plant associated with each band depict the position of the pigment; respective mobility and mol wt polyacrylamide gels before emission and excitation acetone.

Fluorescence intensity scale pure, the number of PChlide protein. Since fraction could not be estimated from the ratio of absorbance (4), this is equivalent to 0.13 g of protein/amol PChlide, which is about half of the value reported by Schopfer and Siegelman for their preparation of highest purity (20). Thus, an aggregate of mol wt of about 600 Kd contains at least four PChlide chromophores.

The molecular weight, mobilities, and absorption maxima of the different Chlide Polypeptides before and after the dark spectroscopic shift

Table II

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>Absorption maximum</th>
<th>Mobility</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>min</td>
<td>nm</td>
<td></td>
<td>daltons</td>
</tr>
<tr>
<td>1</td>
<td>679</td>
<td>0.3 (100%)</td>
<td>40,000</td>
</tr>
<tr>
<td>40</td>
<td>674</td>
<td>0.22 (5%)</td>
<td>50,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.86 (90%)</td>
<td>15,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.89 (9%)</td>
<td>10,000</td>
</tr>
</tbody>
</table>
corresponded to a mobility of 0.28 ± 0.08 and mol wt of 45 ± 10 Kd. However, after the dark shift, the pigment was found at a location corresponding to a mobility of 0.63 ± 0.03 and a mol wt of 16 ± 1 Kd. In a sample (not shown) where the dark shift was allowed to proceed only part way and the absorption maximum was at 677 nm before denaturation (673 nm after denaturation), about half of the Chlide fluorescence was associated with a mol wt of 45,000 and the other half with a mol wt of 16,000 daltons.

The results suggest that a polypeptide of mol wt 45 Kd, to which the Chlide molecule is attached, has been cleaved to yield a polypeptide of mol wt 16 Kd. The reaction leading to the decrease in mol wt of the 45 Kd polypeptide occurred during the same interval as the dark spectroscopic shift. Both were completed in about 40 min at 15 C and were blocked below 0 C.

A minor Chlide peak, consisting of 10% of the total Chlide on the gel and displaying a mobility of 0.86 ± 0.04 and mol wt smaller than 10,000 daltons, was also apparent in many experiments. It is probably a proteolytic product of the major subunit of the holochrome (45,000 daltons mol wt).

In many experiments, PChlide ran as a free pigment, with mobility identical to the bromphenol blue dye. Apparently, the pigment molecule is easily detached from the polypeptide.

**Absence of Dark Spectroscopic Shift in S4, Chlorophyllide Holochrome.** S4 supernatant was photoconverted and then incubated for 40 min at room temperature. It was then subjected to SDBS-gel electrophoresis. No Chlide could be detected on the gel after electrophoresis using the aceton extraction and fluorescence method. This is not surprising, because the S4 preparation is highly labile, and Chlide absorption is lost to a large extent within 1 hr at room temperature.

The absorption maximum of S4 CH immediately after photoconversion was located at 672 nm. The absorption maximum after partial photoconversion was also 672 nm. No corresponding long wavelength 678 nm form and no dark spectroscopic shift were observed for the S4 holochrome preparation. The S4 supernatant was analyzed by SDS-polyacrylamide gel electrophoresis for its protein composition. An identical electrophoretic pattern was seen for the following samples: (a) a dark sample at 4 C; (b) a dark sample incubated for 40 min at 22 C; (c) immediately after illumination at 4 C; (d) an illuminated sample incubated for 40 min at 22 C. Figure 6 depicts the electrophoretic pattern for the dark sample at 4 C. Two peaks were observed on the gels. The major peak in each gel was a polypeptide of mol wt 45 Kd, and the minor peak was a polypeptide of mol wt 60 Kd, according to the calibration curve. These peaks have been tentatively identified as the PCH polypeptide and the large subunit of RuDP-Case, respectively, as obtained previously for peaks C2 and C1 (Fig. 1). These results suggest that S4 CH does not exhibit the dark spectroscopic shift, nor does it display the change in mol wt of the 45 Kd Chlide polypeptide to a 16 Kd polypeptide.

Addition of glycerol to a final concentration of 50% had an effect on the absorption maximum of S4 CH. After photoconversion, the absorption maximum was found at 676 nm instead of 672 nm (not shown). In the presence of 50% glycerol the holochrome is more stable, and the photoactivity is retained for a much longer period. A similar effect of high concentration of sucrose on the spectroscopic properties and stability of holochrome homogenates was reported by Mathis and Sauer (17). The absorption maximum of the pigment may depend on the immediate environment of the pigment and thereby reflect a conformational change in the holochrome upon addition of glycerol to high concentration. The polypeptide composition of a high glycerol (676 nm) and a no glycerol (672 nm) form of the CH were identical and indistinguishable from that shown in Figure 6 for a dark S4 sample. Therefore, a difference in the absorption maximum of the pigment does not necessarily reflect a change in mol wt or composition.

**Dissociation of Chlorophyllide Holochrome Molecules of 600,000 Molecular Weight Simultaneously with Dark Spectroscopic Shift.** PChlide was prepared by polyethylene glycol precipitation and hydroxylapatite chromatography (method 1) and then centrifuged in a 10 to 70% sucrose gradient. Its distribution in the gradient is shown in Figure 7. The photoconvertible holochrome sedimented as a symmetrical peak with a sedimentation coefficient of 18S, based on the sedimentation velocity of two markers, thyroglobulin (sedimentation coefficient 19.2S) and apoferritin (sedimentation coefficient 17.6S). The inactive PChlide had an additional slower sedimenting shoulder. Approximately 70% of the inactive PChlide had a sedimentation coefficient of about 18S, and 30% had a sedimentation coefficient smaller than 10S.

Illumination for 2 min at an intensity of 90 ft-c at room temperature photoconverted all active PChlide to CH. The photoconverted sample was loaded on a sucrose gradient. CH appeared as a major peak with a sedimentation coefficient of 18S and a minor peak with a sedimentation coefficient smaller than 10S (Fig. 8).

CH was allowed to complete the dark spectroscopic shift during 40 min at 22 C, and the sample was analyzed by sucrose gradient centrifugation (Fig. 9). The 18S CH macromolecule dissociated almost completely to yield a new pigment complex with a sedimentation coefficient smaller than 4.6S. The inactive PChlide also appeared with a sedimentation coefficient smaller than 4.6S. In a control experiment, PChlide was incubated in the dark for 40 min at 22 C. The sample was analyzed by sucrose gradient centrifugation (Fig. 10). The photoconvertible holochrome sedimented as a symmetrical 18S PChlide peak. No dissociation into a small mol wt PChlide-protein complex was observed on the gradient. Inactive PChlide followed the same pattern. These results are summarized in Table III.

The sedimentation coefficient of PChlide was measured by
dissociates. The final product is a Chlide complex of mol wt smaller than 70 Kd, corresponding to a sedimentation coefficient smaller than 4.6S. The appearance of a small mol wt pigment-protein complex after photoconversion was also reported by Bogorad et al. (5).

**DISCUSSION**

RuDP-Case constitutes up to 50% of the total protein contained in etiolated bean leaf extracts (1). RuDP-Case has very similar physical-chemical properties to the holochrome. It has a sedimentation constant of 18.5S (13), while PCH extracted from etiolated bean leaves has a sedimentation constant of 18S (3). Moreover, both proteins bind to DEAE-cellulose in the pH range from 7 to 8, indicating a similar electrical charge distribution. It is very difficult to separate RuDP-Case and PCH by the usual column chromatography techniques (7). However, RuDP-Case is a soluble protein easily removed from mature chloroplasts by washing the thylakoid membranes with dilute buffers.

---

**Fig. 7.** Distribution of photoconvertible PCH and inactive PCH from etiolated bean leaves on a 10 to 70% sucrose gradient. Active PCH was photoconverted and monitored by Chlide fluorescence at 685 nm. Inactive PChlide fluorescence was monitored at 637 nm. Sample was loaded in darkness at 4°C. Numbers 0.62, 0.43 refer to relative distances of migration on the gradient.

**Fig. 8.** Distribution of CH and inactive PCH on a 10 to 70% sucrose gradient. Sample was irradiated for 2 min and incubated for 40 min at 22°C prior to centrifugation. Other experimental conditions are as in Figure 7.

**Fig. 9.** Distribution of CH and inactive PCH on a 10 to 70% sucrose gradient. Sample was irradiated for 2 min and incubated for 40 min at 22°C prior to centrifugation. Other experimental conditions are as in Figure 7.

**Fig. 10.** Distribution of photoconvertible PCH and inactive PCH on a 10 to 70% sucrose gradient. Sample was incubated in the dark for 40 min at 22°C prior to centrifugation. Photoconverted PCH was monitored by Chlide absorption at 674 nm. Inactive PChlide absorption was monitored at 630 nm.
A similar dark shift is reported in CH in vitro. Immediately after the phototransformation, the principal absorbance maximum is at 678 nm, owing to the newly formed chlorophyllide. The 678 nm peak shifts slowly toward a shorter wavelength with a slight decrease in absorbance. At 25 C, the shift is completed in 10 to 15 min. At the 12 C the shift requires 30 to 40 min for completion (20, and this work). We make the assumption that both the in vivo and in vitro dark shifts are due to the same process.

Immediately after photoconversion, the newly formed CH appears to have the same polypeptide composition (45,000 daltons) as the native PChlide macromolecule. After completion of the dark shift, the CH yields instead a 16,000 dalton polypeptide associated with Chlide. These results indicate that the polypeptide of 45,000 dalton mol wt has undergone a cleavage to yield a smaller Chlide polypeptide of 16,000 daltons. The time for completion of the reaction and the fact that it is blocked at 0 C are also characteristics of the dark spectroscopic shift.

The most plausible mechanism for the reaction leading to the change in the mol wt of the Chlide polypeptide is an enzymic one. We assume that an enzyme present in the crude chlorophyll homogenate is responsible for the cleavage. The fact that S4 does not have the capability of carrying out this reaction must be accounted for by postulate that this enzymic activity has been removed from the highly pure S4 supernatant. However, the possibility cannot be ruled out that PCh in S4 has been modified in such a way that, although it is photoconvertible, it does not undergo the cleavage reaction.

Henningsen et al. (11) isolated an active subunit of 63,000 daltons from barley leaves using the detergent saponin. Concomitant with the dark spectral shift from 678 to 672 nm a change in the apparent mol wt of the CH subunit from about 63,000 to 29,000 daltons was observed. In chlorohrome extracted from bean leaves with a high concentration of saponin, the spectral shift was extremely slow, and a corresponding change in the mol wt was not detected (11). Our experiments with S4-CH (iris/Triton extract), show the absence of both the spectral shift and the corresponding cleavage of the chlorohrome subunit. Apparently, the ability to carry out this reaction has been lost in this preparation.

Table III demonstrates that during the course of the dark shift, a major disintegration occurs at the macromolecular level. The 600,000 dalton CH with a sedimentation coefficient of 18S dissociates to a Chlide protein with a sedimentation coefficient smaller than 4.6S. This reaction has a specific light requirement, because it did not occur in a dark incubated sample. These results are in agreement with similar experiments conducted by Bogorad et al. (5). The observation that both active and inactive PChlide are associated with low mol wt protein may mean that not all of the PChlide of a chlorohrome particle needs to be photoconverted to bring about the dissociation.

Butler and Briggs (6) have previously attributed the Shibata shift in leaves to a pigment disaggregation. Pigment disaggregation during the Shibata shift was also implied from the studies of Schultz and Sauer (21) and Mathis and Sauer (17) on the CD spectrum of CH. They observed that a double CD signal, attributed to a dimer of Chlide molecules, disappeared progressively during the course of the dark shift. It was suggested that a dimer of Chlide molecules is dissociating into Chlide monomers. Another feature of the CD spectrum of CH immediately after photoconversion is a negative peak at 580 nm. This peak also vanishes in the course of the dark spectral shift (8, 17). These experiments have been interpreted as indicating a chlorohrome protein conformational change, which in turn affects the pigment-pigment interaction.

Our observations in this study together with the observations of others can be assembled in the following model. PCh with a mol wt of 600 Kd is photoconverted to a 600 Kd CH. The
photolytic hydrogenation of 2 carbon atoms in ring IV of PChlide to produce Chlal may necessitate proton donation from the protein, together with a simultaneous change in configuration of the protein molecule. This conformational change in the protein leads to an unstable 600 Kd CH oligomer. The 600 Kd CH oligomer can be stabilized in vitro either in a high concentration of sucrose or in a buffered solution below 0 C. Otherwise, the unstable CH complex dissociates into a small mol wt Chl protein. The dissociation leads also to pigment disaggregation, which is manifested in the Shibata shift and the loss of the characteristic CD Chlide dimer signal. Concomitantly, the 45 Kd Chlide polypeptide undergoes cleavage resulting in a 16 Kd Chlide polypeptide. The 16 Kd Chlide polypeptide may then be incorporated in vivo into the developing chloroplast membrane.

Acknowledgments — We appreciate the interest and great help of R. G. Alacher. We thank J. P. Thornber for communicating to us his method of pigment extraction.

LITERATURE CITED