Properties of Chloroplasts Isolated from Siphonous Algae

EFFECTS OF OSMOTIC SHOCK AND DETERGENT TREATMENT ON INTACTNESS

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ABSTRACT

Chloroplasts isolated from the siphonous green alga Caulerpa simpli-
culosa (Turner) C.Ag. were shown to be resistant to dissolution by the
nonionic detergent Teric-10 at concentrations as high as 0.3% (v/v) when
treated at 0 C. There was little release of stromal enzymes under these
conditions. These chloroplasts were disrupted by osmotic shock as shown
by measurement of the release of both glucose-6-phosphate dehydrogenase
and NADP-dependent glutamate dehydrogenase into the suspending me-
dium. Ribulose-1,5-bisphosphate carboxylase, an accepted marker for chloro-
estromal protein in higher plants, was largely retained in the
disrupted chloroplast following the osmotic shock. This is considered to be
due to the location of a significant proportion of enzyme within the
pyrenoid, which protects it from dissolution and causes it to behave as
though it were an insoluble protein.

It has been reported that chloroplasts isolated from the algal
species Caulerpa are resistant both to dissolution in detergent and
to disruption by osmotic shock (1, 6). Similar properties were
found to be characteristic of chloroplasts isolated from Codium
fragile (18). Previous work (7) suggested that isolated chloroplasts
prepared from Caulerpa simpli culosa were damaged by osmotic
shock, since greatly increased rates of ferricyanide-dependent Hill
reaction were noted following suspension in hypotonic media.
Similar results were obtained with chloroplasts from Codium
vermiforme (15).

In this paper we give details of experiments which provide a
more quantitative measure of siphonous algal chloroplast damage
resulting from both osmotic shock and detergent treatment. By
measuring the activity of the enzymes glucose-6-P dehydrogenase
and NADP-dependent glutamate dehydrogenase it was shown
that most of the enzymes were released from the chloroplast
following osmotic shock but not by detergent treatment. However,
the enzyme ribulose-1,5-bisphosphate carboxylase, an accepted
marker for chloroplast stromal protein in higher plants (19), was
largely retained within the chloroplast following osmotic shock.
This appears to be due to the location of a part of this enzyme
within the chloroplast pyrenoid.

MATERIALS AND METHODS

Preparation of Chloroplasts. Chloroplasts were isolated from
fronds of Caulerpa simpliculosa (Turner) C.Ag. collected from depths
of 1 to 3 m and held for periods of up to a month in an illuminated
aquarium at 15 C. The photosynthetic rate of tissue segments
varied from a maximum of 60 μmol of O₂ hr⁻¹ mg⁻¹ of Chl a+b
(spring and early summer) to a minimum of 5 μmol of O₂ hr⁻¹

mg⁻¹ of Chl a+b (midwinter). Immediately before chloroplast
preparation, the alga was illuminated in a shallow tray of sea
water for 30 min at a quantum flux of 500 to 700 μE m⁻² s⁻¹
(400–700 nm). The material was then blotted dry, chopped into 1-
to 2-cm pieces, and ground in breaking medium for 1 min
while contained in a muslin bag. The extruded suspension was then
wring out of the bag and poured through two layers of Miracloth.
The breaking medium contained: NaCl, 700 mm; MgSO₄, 50 mm;
KCl, 10 mm; MnCl₂, 1 mm; Na₂EDTA, 2 mm; Tricine-NaOH
buffer (pH 7.5), 50 mm; sodium isocitrate, 5 mm; DTT, 1 mm;
and BSA, 0.1% (w/v). Two hundred ml of breaking medium (10
°C) were used per 80 to 90 g of tissue. The crude suspension of
chloroplasts was centrifuged at 1,000g for 1 min, the pellet surface
washed, and then resuspended in a suspending medium identical
in composition to the breaking medium except that BSA and DTT
were omitted. Resuspension was achieved using a Dounce-type
homogenizer with a loosely fitting pestle. These suspensions con-
tain almost exclusively intact chloroplasts, contaminated only by
some starch grains and the occasional diatom which was present
as an epiphyte on the frond. Where pea chloroplasts were used for
comparison they were prepared by the method of Mitchell and
Stockey (13).

Chloroplast suspensions, containing between 0.3 and 0.5 mg of
Chl in 2 ml, were then diluted slowly with an equal volume of
suspension medium containing the detergent, Teric-10, to yield
the final concentration of detergent desired. After 10-min incu-
bation of the suspension in an ice-water bath in darkness, with
gentle swirling, the chloroplasts were centrifuged from the sus-
pension at 1,000g for 90 sec. The pellet was surface-washed with fresh
suspension medium (detergent-free) and then resuspended in a low
osmotic strength medium, minus NaCl, or in the case of the pea
chloroplasts, sorbitol. After osmotic shock the chloroplast pellet
was separated by centrifugation at 750g for 10 min. Controls
were treated in identical fashion except that detergent was omitted
from the first suspension medium. Prior to assay of the enzymes,
each fraction was sonicated for 15 sec to disrupt any particular
material present. The protocol is summarized in Figure 1.

Enzyme Assays. RuBPC² (EC 4.1.1.39) was assayed by the
method of Wishnick and Lane (21), G6PDH (EC 1.1.1.49) by the
method of Kuby and Noltmann (11), and GDH (NADP) (EC
1.4.1.3) by the method of Gayler and Morgan (5). Protein was
measured by a modification of the Lowry method (12) and Chl
was measured in 90% acetone using the equations of Jeffrey
and Humphrey (10). Where detergent concentrations were suffi-
ciently high to solubilize chloroplast thylakoids, the Chl was measured
after extraction into diethyl ether after saturating the aqueous
phase with NaCl (9).

Chemicals. BSA, Cohn fraction V, was obtained from Com-
monwealth Serum Laboratories and free fatty acids removed prior

² Abbreviations: G6PDH: glucose-6-phosphate dehydrogenase; GDH
(NADP): NADP-dependent glutamate dehydrogenase; RuBPC: ribulose-
1,5-bisphosphate carboxylase.

1 This work was supported by the Australian Research Grants Com-
mittee.
RESULTS

Chloroplasts from *C. simpliciuscula* prepared as described have CO<sub>2</sub>-dependent Hill reaction rates which vary from 8 to 25 μmol of O<sub>2</sub> hr<sup>-1</sup> mg<sup>-1</sup> of Chl. The addition of ferricyanide (1 mM initial concentration) to these chloroplasts increased the rate of O<sub>2</sub> evolution by a maximum of 25%.

When these chloroplasts were suspended in 0.1% (v/v) Teric-10 solution, it was found that a maximum of 17% of the Chl was solubilized (Table I). When pea chloroplasts were subjected to the same treatment 78% of their Chl was solubilized by the detergent. Chloroplasts from *Caulerpa* which had been removed from the detergent and then resuspended in hypotonic medium underwent some further fragmentation, but more than 50% of the Chl originally present remained in fragments large enough to sediment after 10-min centrifugation at 750g. The corresponding figure for pea chloroplasts was 15%. Washing in Teric-10-free medium followed by osmotic shock had little effect on the fragmentation of the chloroplasts, and 99% of the Chl remained in the *Caulerpa* chloroplasts and 85% in the pea chloroplasts.

At the highest concentration of Teric-10 used (0.1%, v/v) 77% of the RuBPC activity was retained in the *Caulerpa* chloroplast pellet (Table II). After resuspension in hypotonic solution, 63% of the initial RuBPC activity remained associated with the chloroplast fragments. Pea chloroplasts, on the other hand, released more than one-third of their total RuBPC activity after the initial centrifugation and resuspension. Incubation with 0.1% (v/v) Teric-10 released 93% of the enzyme activity from the chloroplast, and suspension of both detergent-treated or control pea chloroplasts in hypotonic solution caused complete loss of the RuBPC activity from the chloroplast fragments. *Caulerpa* chloroplasts, however, were much more resistant to damage by detergent and on the basis of the retention of a large proportion of their RuBPC activity, they appeared to resist osmotic shock as well. When the distribution of two other enzymes was examined a different picture emerged. G6PDH has been shown to be located within the chloroplast (14) as has GDH(NADP) (5), although both enzymes are also found in cytoplasm.

When chloroplasts prepared from *C. simpliciuscula* were washed twice with fresh suspension medium (BSA-free) the total activity and the specific activities of both enzymes in the wash medium fall rapidly (Table III). Immediately following osmotic shock, the total amount of enzyme activity appearing in the suspending medium was 10-fold that present in the preceding wash, and the specific activity was almost five times that of the activity which remained associated with the chloroplast pellet (Table III). The results obtained with these two enzymes confirmed that there is a

![FIG. 1. Protocol for preparation and detergent treatment of chloroplasts.](image)

**TABLE II.** Distribution of RuBP Carboxylase activity in chloroplasts after detergent treatment and osmotic shock treatment of chloroplasts.

<table>
<thead>
<tr>
<th>FRACTION</th>
<th>CAULERPA</th>
<th>PEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 0.1% Teric</td>
<td>0.2</td>
<td>17.4</td>
</tr>
<tr>
<td>Control 0.1% Teric</td>
<td>0.7</td>
<td>29.7</td>
</tr>
<tr>
<td>Control 0.1% Teric</td>
<td>99.1</td>
<td>52.8</td>
</tr>
<tr>
<td>Control 0.1% Teric</td>
<td>15.3</td>
<td>37.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>% RuBP Carboxylase Activity in Fraction</th>
<th>0</th>
<th>0.01</th>
<th>0.06</th>
<th>0.10</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. simpliciuscula</td>
<td>7.1</td>
<td>6.8</td>
<td>12.5</td>
<td>14.1</td>
</tr>
<tr>
<td>Supernatant after detergent treatment</td>
<td>21.2</td>
<td>21.3</td>
<td>22.4</td>
<td>22.8</td>
</tr>
<tr>
<td>Supernatant after osmotic shock</td>
<td>71.8</td>
<td>71.9</td>
<td>65.2</td>
<td>63.1</td>
</tr>
<tr>
<td>Final pellet</td>
<td>14.4</td>
<td>15.2</td>
<td>16.3</td>
<td>17.0</td>
</tr>
</tbody>
</table>

**TABLE I.** Distribution of chlorophyll after detergent treatment and osmotic shock treatment of chloroplasts.

The activity of RuBPC activity in the total *Caulerpa* chloroplast preparation was 7 μmol CO<sub>2</sub> fixed g<sup>-1</sup> protein hr<sup>-1</sup> and for pea chloroplasts 115 μmol CO<sub>2</sub> fixed g<sup>-1</sup> protein hr<sup>-1</sup>. All other conditions as for Table I.
TABLE III. Distribution of Glucose-6-P Dehydrogenase and Glutamate Dehydrogenase in Caulerpa chloroplast preparations before and after osmotic shock.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total Chlorophyll mg</th>
<th>Total Activity a</th>
<th>Specific Activity mg⁻¹ Protein</th>
<th>Total Activity b</th>
<th>Specific Activity mg⁻¹ Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original Supernatant</td>
<td>3.91</td>
<td>1724</td>
<td>.b</td>
<td>232</td>
<td>.b</td>
</tr>
<tr>
<td>First Washing</td>
<td>0.68</td>
<td>193</td>
<td>55</td>
<td>167</td>
<td>49</td>
</tr>
<tr>
<td>Second Washing</td>
<td>0.04</td>
<td>24</td>
<td>26</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Released after Osmotic Shock</td>
<td>0.02</td>
<td>334</td>
<td>99</td>
<td>98</td>
<td>29</td>
</tr>
<tr>
<td>Pellet after Osmotic Shock</td>
<td>5.00</td>
<td>151</td>
<td>18</td>
<td>7</td>
<td>0.6</td>
</tr>
</tbody>
</table>

a Activity in units of umol substrate transformed min⁻¹.
b Since the original supernatant contains added RSA, specific activity in terms of protein cannot be given.

TABLE IV. Distribution of Glucose-6-P Dehydrogenase and Ribulose-1,5-Bisphosphate Carboxylase following detergent treatment of Caulerpa chloroplasts.

Conditions as for Table I. The activity is corrected for the amount of enzyme released in the absence of detergent (6% maximum). The control rate for RuBPC activity was 2.8 umol substrate min⁻¹ g⁻¹ protein and for G6PDH 350 umol substrate min⁻¹ g⁻¹ protein.

<table>
<thead>
<tr>
<th>Teric Concentration % v/v</th>
<th>Enzyme Activity Remaining in Pellet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RuBPC</td>
</tr>
<tr>
<td>0</td>
<td>70</td>
</tr>
<tr>
<td>0.04</td>
<td>70</td>
</tr>
<tr>
<td>0.1</td>
<td>70</td>
</tr>
<tr>
<td>0.3</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>G6PDH</td>
</tr>
<tr>
<td>100</td>
<td>66</td>
</tr>
<tr>
<td>100</td>
<td>76</td>
</tr>
<tr>
<td>100</td>
<td>72</td>
</tr>
</tbody>
</table>

component of their activity specifically associated with the chloroplast, and showed that this component was released after osmotic shock.

Since RuBPC did not behave in the same way as the other two stromal enzymes after osmotic shock, it could not be used as a marker for chloroplast stroma in this species. However, when the distributions of RuBPC and G6PDH were compared following incubation of the Caulerpa chloroplasts in Teric-10 it was found that the greater part of the total activity of both enzymes was retained within the chloroplast over the range of detergent concentrations (Table IV). This supports the conclusion that these chloroplasts are not disrupted by detergent concentrations more than three times higher than those which completely solubilize pea chloroplasts under the same conditions.

These results were further supported by ultrastructural studies (unpublished) which showed no apparent damage to chloroplasts following Teric-10 treatment, but massive disruption of the plastid following osmotic shock, with loss of chloroplast envelopes and stroma. If the osmotically shocked chloroplast was treated with Teric-10 (0.1%, v/v) the thylakoids were dissolved and the only recognizable structures remaining were pyrenoids. These pyrenoid preparations contained 30% of the total RuBPC activity initially present in the intact chloroplast, suggesting that a significant portion of the enzyme is located within this structure.

DISCUSSION

These results confirm that C. simplicissima chloroplasts are sensitive to disruption by osmotic shock and that they lose their stromal proteins as a result of this treatment. In this they resemble not only higher plant chloroplasts, but also the majority of algal chloroplasts which have been examined (7, 15, 16). Chloroplasts of Caulerpa geminata, Harvey (formerly Caulerpa sedoides [R. Brown in Turner] C.Ag.) (6) and Codium fragile (18) have been reported to resist disruption following suspension in hypotonic media, but neither ultrastructural nor biochemical studies were reported. In our experience it is difficult to find membranes impossi...