Study of Energy Storage Processes in Bundle Sheath Cells of Zea mays

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ABSTRACT

Photochemical energy storage in isolated bundle sheath cells from Zea mays was examined. Photoacoustic spectroscopy was used in this study to monitor energy storage processes. The presence of methyl viologen or addition of substrates which activated carbon fixation, prevented energy storage processes through the electron transport system. The energy storage was inhibited completely by dibromothymoquinone (DBMIB) and DCMU, inhibitors of noncyclic electron flow. However, the reductants such as dihydrobiocyt and ascorbate increased the energy storage. It was concluded that photosystem (PS) I may be mediated by some electron donor(s) other than water and that PSII only partially participates in PSI reduction. It is postulated that the role of PSII is to regulate PSI electron transport and prevent its overoxidation. In the presence of high level of malate, photoacoustic spectroscopy indicated a low energy storage which may be due to induction of energy utilization in carbon assimilation.

In C₄ plants there are two separated compartments for photosynthetic carbon metabolism. One is located in mesophyll chloroplasts and the other in BS¹ cell chloroplasts. However, those separated compartments depend functionally on each other. The primary step of CO₂ fixation in C₄ plants occurs via phosphoenolpyruvate carboxylation in mesophyll cells and subsequently results in malate production. Then, malate, as an intermediate, is transported to BS chloroplasts and decarboxylated resulting in formation of pyruvate and CO₂. The reductive pentose-phosphate cycle located in BS chloroplasts refixes the CO₂ provided by malate decarboxylation (8). The fixation of 1 molecule of CO₂ into the reductive pentose cycle requires 3 molecules of ATP and 2 molecules of NADPH (14). However, PSI activity in maize BS chloroplasts is much lower than in mesophyll chloroplasts (25, 29). Therefore, an input of PSI electron transport cannot provide an adequate level of ATP and NADPH for reductive carbon metabolism (13, 17). It has been postulated that the requirement for ATP in BS chloroplasts is mainly met by cyclic photophosphorylation through PSI activity (8). In some reports it has been shown that PSI makes an insignificant contribution to linear electron transport and NADP reduction (10, 13).

There is a proposal that NADP reduction by malate stimulates cyclic electron flow and consequently photophosphorylation. In this hypothesis, malic acid serves as an important reducing intermediate for PSI (16). However, the possible mechanism by which some other substrates may reduce PSI is still not clear. In this report we use photoacoustic spectroscopy to monitor energy storage in BS cells chloroplasts. The results describe the types of effects of different reductants and oxidizing substrates on photochemical activity of BS chloroplasts.

MATERIALS AND METHODS

Maize (Zea mays) was grown in a soil-sand peatmoss mixture at 25°C under high pressure sodium lamps (Philips) with light intensity of 45 to 50 mW/cm². Leaves from 4 to 6-week-old plants were used to isolate strands of BS cells. BS strands were purified by a slightly modified mechanical maceration-filtration technique reported earlier (9, 10, 21). Leaves were sliced with a razor blade into segments of 1 cm in length and 1 mm in width. Using a Waring Blender, as previously described (15), 20 g of leaves were blended in 200 ml of grinding medium for 30 s. Grindings were repeated four times at the highest possible speed. Then, the homogenate was filtered through nylon nets of 500, 250, and 80 mm pore size (B.S. Thompson Co., Ltd., Ville Mont-Royal, Québec, Canada) in series. Finally, the fraction obtained on the 80 mm net was washed and resuspended in the resuspending medium, giving 100 μg Chl ml⁻¹. As described earlier (16), the grinding medium consists of 0.35 m sorbitol, 4 mM MgCl₂, 2 mM KH₂PO₄, 10 mM sodium ascorbate, and 20 mM Hepes-KOH (pH 6.4). The resuspending medium contains: 0.35 m sorbitol, 4 mM MgCl₂, 2 mM KH₂PO₄, 5 mM K₂SO₄, and 20 mM Tricine-KOH (pH 8.2), instead of 10 mM Tricine. Each preparation was examined with a light microscope in order to confirm that it was not contaminated with mesophyll cells. Isolation of BS cells strands was performed at 5°C and the sample was kept in the resuspending medium in the dark at room temperature (i.e. 22°C). Such isolated BS cells showed good stability over a 4 h period based on reproducible photoacoustic signal. Chl concentration was determined by the Arnon method (1).

The photoacoustic spectrometer previously described (6) was slightly modified for our experiments. It consists of a light beam, produced by a 1000 W xenon lamp, passing through a monochromator (Schoeffel Instrument Corporation). The light beam emerging from the monochromator (700 nm) was modulated to 75 Hz and reflected by a mirror into the acoustic cell.

An actinic light was supplied from a quartz halogen lamp (Oriel Corporation), and directed on the sample by using a fiber optic light guide. Modulated and actinic light intensities were, respectively, 1 mW cm⁻² and 30 mW cm⁻². The continuous actinic beam was used to saturate the photochemistry and induce
a total conversion of the absorbed modulated light into heat (4). A preamplified acoustic signal was sent to a phase lock-in amplifier (Htaco Dynatrac model 393) which was receiving a reference signal from the chopper. The output signal of the amplifier was computer processed (Apple II) or sent to a chart recorder. Then the photoacoustic spectra were normalized to the carbon black reference. The absorption spectra of BS cells were recorded using a spectrophotometer PYE UNICAM model SP 8-100 UV/VIS.

In the photoacoustic measurements, BS cells, containing 10 μg of Chl, were suspended in 3 ml of resuspending medium with 10% Ficoll (to avoid precipitation). PAS of leaves and BS cells were normalized at 550 nm. See "Materials and Methods."

FIG. 1. Spectra of BS cells, the optical density (O.D.) spectra was measured from cells in resuspending medium with 10% Ficoll (to avoid precipitation). PAS of leaves and BS cells were normalized at 550 nm. See "Materials and Methods."

RESULTS

Figure 1 shows the optical absorption and PAS of BS cells and maize leaf. A similarity was noticed between the PAS of BS cells and its optical absorption spectra. This similarity indicates that the pigment accessories system is responsible for the dissipation of absorbed light energy via heat. The PAS spectra of the maize leaf is different because of the leaves thickness and its pigment composition (5). The spectra for the leaf is in agreement to those previously reported for the maize leaf (22). Photoacoustic signal of BS cells increased upon addition of nonmodulated background actinic light and returned to the initial level after removal of actinic light beam (Fig. 2a). When the photochemistry is inhibited by heating the sample for 5 min at 50°C, the photoacoustic signal (heat dissipation) reaches a steady-state maximum without further changes in the presence of the saturating light beam (Fig. 2b). Photochemical energy storage expressed as the relative photochemical loss ϕ′ (7) is dependent on the light intensity. The reciprocal value of ϕ′ had a linear relationship with the modulated light intensity up to 2.5 mW cm⁻². However, further increase of the energy of the modulated light did not induce saturation (Fig. 3). This indicates an electron sink, that prevents light saturation of the BS photochemical reactions.

In the presence of electron donors or acceptors, we noticed different responses in the energy storage. DTT increased the energy storage signal, but MV completely eliminated the energy storage signal, even after addition of 10 mM ascorbate. After addition of 10 μM DCMU, the energy storage process was not detectable with a modulated light of 700 nm without addition of 10 mM ascorbate. In the presence of 10 μM DBMIB the signal was suppressed, but restored after addition of 10 mM ascorbate; whereas 20 μM DBMIB induced total inhibition (Fig. 4). However, energy storage in the presence of DCMU was detected between 400 and 690 nm of modulated light (Fig. 5) and this

Fig. 2. Photoacoustic signal from BS cells at 75 Hz and 700 nm. Short black arrows indicate the onset and terminating of the actinic beam, open arrow indicates the onset of the modulated light. Control sample without additives (a) and sample heated at 50°C for 5 min (b). For Q_c and Q_m, see "Material and Methods."
ENERGY STORAGE IN BUNDLE SHEATH CELLS

![Graph showing the relationship between photochemical loss and intensity of modulated light.](image)

**Fig. 3.** Relationship between photochemical loss, \( \Phi \) (energy storage) and intensity of modulated light (\( \Phi \)); reciprocal value of energy storage, \( 1/\Phi \) (—).

![Photoacoustic signals of BS cells incubated with additives.](image)

**Fig. 4.** Photoacoustic signals (at 700 nm) of BS cells incubated with additives. Arrows indicate the onset and terminating of the actinic background light. See "Materials and Methods" for details.

observation will be discussed later.

We also noticed that the addition of naturally occurring metabolites had different effects on the energy storage. Ascorbate increased the energy storage signal while malate had an inhibitory effect. The negative effect of 20 mM malate was overcome after the addition of 10 mM ascorbate. The presence of 4 mM R5P, 4 mM NaHCO3, and 0.5 mM NADP caused a decline in the energy storage process. However, treatment with 4 mM R5P had no significant effect on the signal (Fig. 6). Table I summarizes the effects of various additives used in the study. Two groups of effects were observed: the first group indicated quenching effects on the energy storage process as shown by malate, R5P + NaHCO3 + NADP, DCMU, DBMIB, and MV. The second group had caused an enhancement as seen with ascorbate and DTT.

These results show the additives have different effects on the oxidation reduction state of electron transport carriers in chloroplast. Therefore, the state of the chloroplast electron transport system appears to influence the rate of thermal dissipation of absorbed light energy by the light harvesting complex, as previously observed (LE Camm, unpublished data).

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Table I

<table>
<thead>
<tr>
<th>Additive</th>
<th>Energy Storage Signal</th>
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</thead>
<tbody>
<tr>
<td>Control (No Additive)</td>
<td>Increase</td>
</tr>
<tr>
<td>10 mM Asc</td>
<td>Increase</td>
</tr>
<tr>
<td>200 ( \mu M ) MV</td>
<td>Increase</td>
</tr>
<tr>
<td>200 ( \mu M ) MV + 10 mM Asc</td>
<td>Increase</td>
</tr>
<tr>
<td>10 ( \mu M ) DCMU</td>
<td>Decrease</td>
</tr>
<tr>
<td>10 ( \mu M ) DCMU + 10 mM Asc</td>
<td>Decrease</td>
</tr>
<tr>
<td>10 ( \mu M ) DBMIB</td>
<td>Decrease</td>
</tr>
<tr>
<td>10 ( \mu M ) DBMIB + 10 mM Asc</td>
<td>Decrease</td>
</tr>
<tr>
<td>20 ( \mu M ) DBMIB + 10 mM Asc</td>
<td>Decrease</td>
</tr>
</tbody>
</table>

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DISCUSSION

In our study of BS cells of maize we found no energy storage in chloroplasts through the electron transport chain when MV was present as a strong oxidant (27). In earlier reports it was noticed that the treatment of BS cells with RuP, HCOO, and NADP stimulates photosynthetic carbon metabolism (10) and therefore increases the demand for NADPH and ATP. Consequently, a rapid turnover through electron transport can be expected. As it has been shown, under these conditions, an energy storage signal did not appear. We then infer the following: when the electron transport traps of the photosystems are open, heat dissipation is less favorable. The reduced electron transport system induced by the presence of ascorbate or DTT (11, 20) may provide reductant to PSI and poise cyclic electron flow leading to the energy storage via reduction of interelectron transport carriers. The stimulating effect of ascorbate on the energy storage signal indicates that an electron donor regulates PSI activity. Considerable amounts of ascorbate are found in stromal compartment of chloroplasts, in concentration from 15 to 75 mM (12, 26). Versatile roles of ascorbate in chloroplast functions appear to be very important (18). The recovered energy storage signal, in the presence of ascorbate after treatment with DCMU, indicates that PSI activity in BS plastid is highly dependent on some stroma containing reductant(s). The addition of ascorbate in the presence of a higher concentration of DBMIB does not recover the energy storage signal. The inhibitory effect of DBMIB shows that the electron donor is acting via PQ and Cyt f reduction (3, 24) and confirms that PQ and Cyt f are tightly associated as electron carriers to PSI.

We noticed that, in the presence of DCMU, the energy storage under modulated light higher than 690 nm was inhibited. However, under these conditions the energy storage in the range of 400 to 690 nm was decreased by 40% compared to the control sample. The experimental proofs support the belief that PSI activity in BS chloroplasts is low (23). However, it does not exclude a possible role of PSII electron transport in preventing the overoxidation of PSI (8).

Malic acid has been reported to stimulate photosynthetic carbon assimilation in BS chloroplasts and it also increases the demand for NADPH and ATP through donation of CO₂ to the reductive pentose-P pathway (2). In agreement with this, we observed no energy storage by electron transport in the presence of malate and therefore we suppose that the electron transport carriers were mostly in an oxidized state. This evidence is not in agreement with a proposed activation of cyclic electron transport by the increased supply of NADPH via malate decarboxylation (16). This discrepancy may arise due to activation of the reductive pentose-P pathway, increased supply of PGA from CO₂ fixation, and thereby a high demand for ATP and NADPH. A high consumption of NADPH will also cause a large increase in the ATP requirement from cyclic electron flow. This possibility is supported by the evidence that the energy storage signal is decreased in BS cell chloroplasts treated with a mixture of RuP, HCOO, and NADP, a treatment reported earlier to remarkably activate CO₂ fixation (10). The potential inhibitory effect of malate on PSII activity (8) and stimulatory effect on CO₂ fixation (2, 10) will consequently determine the redox state of PSI. Conditions may be favorable for electron carriers associated with PSI to be oxidized, especially if there is a deficiency of electron donors to PSI. Our data show that the suppressing effect of malate on the energy storage signal is eliminated in the presence of ascorbate. This indicates that in the presence of malate there is limited donation of electrons to PSI.

CONCLUSION

Photoacoustic spectroscopy proved to be a sensitive technique for monitoring the effects of electron donors or acceptors on the
photochemical activity in the BS chloroplasts. This study supports previous evidence that PSII activity in BS chloroplasts is partially responsible for PSI reduction. However, from this study we are not able to answer the following question: Is PSII activity in BS chloroplasts only a phylogenetic rudiment or does it still have some important functional role? We postulate that it may have a role to prevent overoxidation of electron carriers associated with PSII activity when other electron donors are insufficient. It is evident that PSI reduction may occur with some electron donor(s) other than water. Ascorbate is one of the candidates. Our investigation does not confirm malate oxidation as having a role in enhancing cyclic electron flow via poising the redox state of electron carriers associated with PSI. It appears that the energy storage from photochemistry in BS chloroplasts is only partially regulated by malate metabolism. Besides malate, other organic electron donors may interact with the electron transport chain. The use of photoacoustic action spectra may provide insight into the interaction of carbon metabolism with the photochemical generation of energy in BS chloroplasts of C₄ plants.

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LITERATURE CITED

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