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PHOTOACOUSTIC MEASUREMENTS OF THE ENERGY-CONVERSION EFFICIENCY OF PHOTOSYNTHESIS IN THALLI OF THE GREEN ALGA *BRYOPSIS MAXIMA*

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The efficiency of photosynthetic energy conversion in thalli of the green alga *Bryopsis maxima* was studied with the photoacoustic technique. Photosynthetic O_2 evolution did not interfere with the photoacoustic measurements in this material, most probably owing to its coenocytic cellular organization. The energy yield (defined as the fraction of absorbed photon energy that is stored in photosynthetic products or intermediates relative to the total absorbed photon energy) was estimated from the photoacoustic signals by applying the background-illumination method to obtain a reference without the photochemical capacity (Lasser-Ross, N., Malkin, S. and Cahen, D. (1980) Biochim. Biophys. Acta 593, 330–341). With the monitoring light modulated at 60 Hz, photon energy is mainly stored by redox changes in electron-transport chains because the energy yield was strongly reduced by 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea and heat treatment of the thalli, whereas KCN, an inhibitor of CO_2 reduction, had no effect, and because a significant lowering of the energy yield occurred in the presence of methyl viologen but the effect of the Photosystem I acceptor was largely reversed on the addition of an uncoupler, methylamine. The maximum energy yield of 0.4 that was obtained with a saturating background light and with a sufficiently weak monitoring light modulated at 100 Hz is explained in terms of electron transfer from electron-donor pools to acceptor pools of the two photosystems with the quantum yield close to unity. A lowering of the modulation frequency decreased the energy yield, indicating that less energy is stored in more stable intermediates.

Introduction

Light energy absorbed by photosynthetic pigments in green plants is either converted to chemical energy or dissipated as heat or light (fluorescence). Only a small percentage of absorbed photons are re-emitted as fluorescence even with the closed reaction center of PS II [1], whereas the fraction of absorbed light energy that is dissipated as heat is expected to vary to larger extents depending upon the state of reaction centers. Thus, measurement of the heat production provides important information concerning the efficiency of photon utilization in photosynthesis.

The photoacoustic spectroscopy measures the periodic heat generation in a system which is irradiated with modulated light. Heat produced in the sample is transferred to the gas phase and induces an acoustic wave which can be monitored by a sensitive microphone [2]. The application of the photoacoustic technique to photosynthetic systems has been described in details by Malkin and co-workers from the theoretical as well a methodological points of view [3–7].

An important advantage of the photoacoustic

Abbreviations: DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; PS, Photosystem.

spectroscopy is that it monitors spectral and photochemical characteristics of plant leaves, which are not amenable to optical spectroscopy due to their strong light scattering properties [6–9]. There are, however, two technical difficulties. First, the monitoring light modulated at low frequencies produces a nonthermal pressure wave due to photosynthetic O_2-evolution, which strongly interferes with measurement of the heat production in intact leaves [6]. Second, because chloroplasts are present at various depths inside leaves and because the periodic heat generation deeper inside the sample is detected with decreased modulation frequency of the monitoring light, different populations of chloroplasts contribute to the photoacoustic signals measured at different modulation frequencies [5,8,10].

In the present work, we applied the photoacoustic spectroscopy to the thalli of the coenocytic green alga *Bryopsis maxima* with a simpler cellular organization. A feather-like thallus of 10–20 cm long is a single giant tubular cell bearing numerous lateral branches. Chloroplasts are located in a parietal cytoplasmic layer between the cell wall and the huge central vacuole extending throughout the thallus [11]. Thus, the photoacoustic signal from the same population of chloroplasts can be measured irrespective of modulation frequency of the monitoring light. In addition, contribution of the modulated photosynthetic O_2 evolution to the signal was found to be practically negligible in this material, probably owing to the unique localization of chloroplasts inside the cells. The efficiency of the photochemical energy conversion was determined under various conditions, and reaction steps responsible for the energy conversions are discussed.

Materials and Methods

B. maxima was harvested from a coast near Choshi, Chiba, stored in laboratory and exposed to light (10 h)-dark (14 h) cycles for 2 days as described previously [12]. The thalli were kept in aerated sea water for 2 h in the dark before measurement. Where indicated, additions were made at least 30 min prior to measurement to ensure their penetration into the cells.

A photoacoustic apparatus similar to that described by Rosencwaig [2] was used. The sample cuvette has an inner dimension of $15 \times 20 \times 10$ mm (depth) and is connected through a channel to an electretcondenser microphone (Matsushita-Tushin, WM034AY). Signals from the microphone were amplified by an AC amplifier with a suitable bandwidth and fed into an Ithaco lock-in amplifier (Dynatrac 391A). Pieces of thalli (2–5 cm long) were placed in the photoacoustic cuvette so as to cover most of its bottom surface. The samples were illuminated through an upper glass window of the cuvette with red light (620–700 nm), which was modulated by a mechanical chopper. An additional strong continuous illumination (430–750 nm) was provided through the same window by means of a half mirror. Appropriate combination of filters were used for the monitoring and continuous background illumination. Light intensity was measured with a calibrated thermopile.

Results

Illumination of dark-adapted *B. maxima* thalli with red light modulated at 60 Hz induced a photoacoustic signal, which decreased to some extent, first rapidly then slowly, before it reached a steady state (Fig. 1, trace a). The signal arises mostly from the algal thalli because the empty photoacoustic cuvette gave only a small constant signal (trace a′). Trace a also shows that the addition of strong continuous background light to the modulated monitoring light induced a rapid rise in the signal level, which was often followed by a small and slow rise. When the continuous light was turned off, the signal decreased rapidly and then slowly.

The addition of strong continuous background light to the modulated monitoring light is introduced by Malkin and Cahen as a means to obtain a reference with similar optical and thermal properties but no capacity for the photochemical energy conversion [3]. The signal increases on addition of the background illumination because a fraction of absorbed modulated light that is photochemically converted to chemical energy is totally dissipated as heat when the photochemical system is saturated. Only the rapid rise was taken into consideration in the following experiments, be-

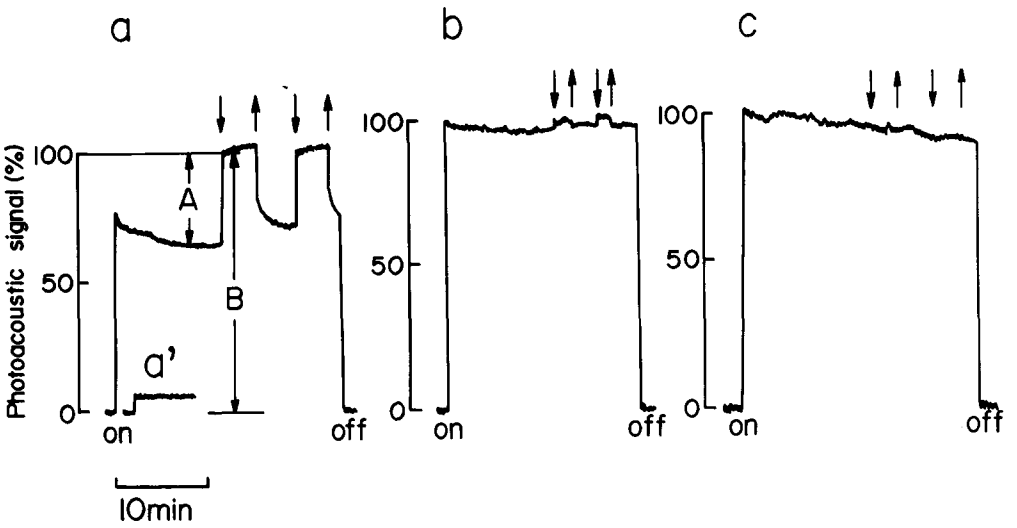

Fig. 1. Photoacoustic signals from thalli of *B. maxima*. Measurements were started by turning the modulated monitoring light of 7 W/m^2 on. Modulation frequency was 60 Hz. Downward and upward arrows indicate on and off of the continuous background light of 298 W/m^2, respectively. a, No addition; b, 20 μM DCMU; c, thalli were treated at 80°C for 5 min. a' shows a signal from the cuvette without sample. A and B, see text.

cause the reaction centers will be closed very rapidly on illumination with the strong background light used. The slower components of the rise and decay kinetics may be ascribed to a long-term adaptation of the thalli to varied light conditions.

The ratio of the signal increase induced by the background illumination (A in trace a) to the total signal with the background light (B) is called the energy yield because the ratio represents the fraction of absorbed light energy which is stored in chemical intermediaries relative to the total absorbed light energy. An energy yield of 0.35 was estimated from trace a. In this estimation, the total signal size was not corrected for the small constant signal from the empty cuvette because the constant signal must have been largely reduced when the bottom surface of the cuvette was covered by the thalli.

The energy yield was usually determined with fresh dark-adapted samples because, once illuminated with the background light, the original signal level was restored slowly (see trace a). Due to difference in quantity and geometry of thalli in the cuvette, absolute signal intensity cannot be compared between different samples.

Lasser-Ross et al. [4] and Malkin et al. [5] showed that DCMU abolishes the increasing effect of the background light on the photoacoustic signals from broken chloroplasts and intact leaves, respectively. Only a small reversible increase occurred on the addition of the background light in thalli which had been incubated in sea water containing 20 μM DCMU prior to measurement (trace b). As the poison blocks electron transport from PS II, the residual increase may be ascribed to cyclic electron transport around PS I. In fact, heating the thalli at 80°C for 5 min totally eliminated the response to the continuous illumination (trace c). The results indicate that the efficient conversion of light energy to chemical energy requires the cooperation of the two photosystems.

Bults et al. [6] showed that photosynthetic O_2 evolution, which is partly modulated at a frequency equal to that of the monitoring light, interferes with the photoacoustic measurement of the photon utilization in intact leaves. The modulated O_2 evolution will cause an apparent decrease in the energy yield because it elevates the signal level without the background illumination but not when photosynthesis is saturated by the background illumination. Especially, at low modulation frequencies of the monitoring light, the background light

induces a decrease in the signal level, indicating that the magnitude of the pressure wave due to O_2 evolution is larger than that of the pressure wave due to heat production [6].

The thalli used here were photosynthetically competent. The modulation frequency of 60 Hz is the optimal for the detection of the negative effect of the background illumination in the intact tobacco leaves [6]. Nevertheless, we have never observed a negative effect of the background illumination under various experimental conditions. Obviously, the modulated O_2 evolution is less significant in the algal thalli than in intact plant leaves.

We studied the effect of KCN on the energy yield in order to examine the contribution of the modulated O_2 pressure to the photoacoustic signal from the *Bryopsis* thalli. Fig. 2 shows that thalli, of which photosynthetic O_2 evolution had been suppressed by incubation with 10 mM KCN for 30 min prior to measurement, gave an energy yield identical to that in the unpoisoned thalli. This indicates that the modulated O_2 evolution is negligible in the *Bryopsis* thalli and that the energy conversion takes place at reaction steps not related to the CO_2 reducing cycle under the experimental conditions used.

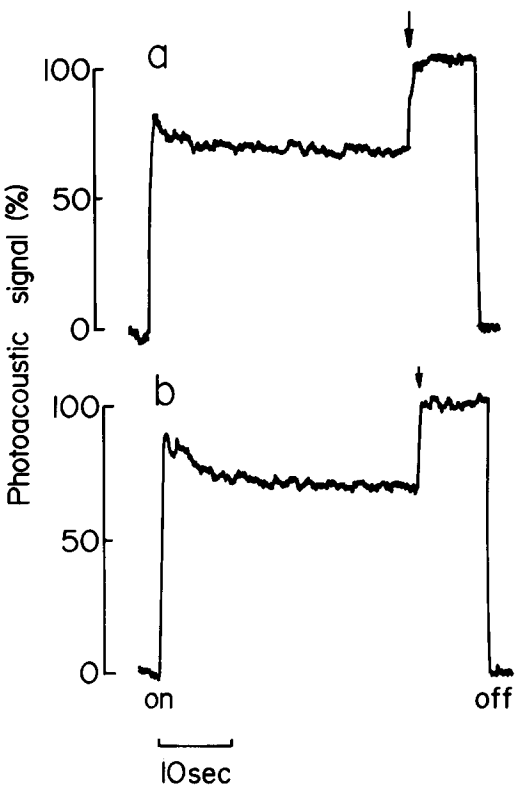

Fig. 2. Effect of KCN on photoacoustic signals. Measurements were started by turning the monitoring light (7 W/m^2, 60 Hz) on. Arrows indicate when the background light of 585 W/m^2 was turned on. a, No addition; b, 10 mM KCN. The energy yields were 0.31 in both a and b.

The following experiments were carried out to explore the nature of chemical intermediates, in which absorbed light energy was stored. Fig. 3 shows the energy yield as a function of the background light intensity. Data were collected from thalli, which had incubated under three different conditions. Note that the continuous light used (100% intensity) was saturating under all the conditions. However, the energy yield was markedly affected by prior treatments of the thalli. The energy yield was considerably lowered in thalli which had been kept in sea water containing 10 mM methyl viologen (closed circles). Methyl viologen inhibits CO_2 reduction by competing electrons at the reducing site of PS I. The methyl

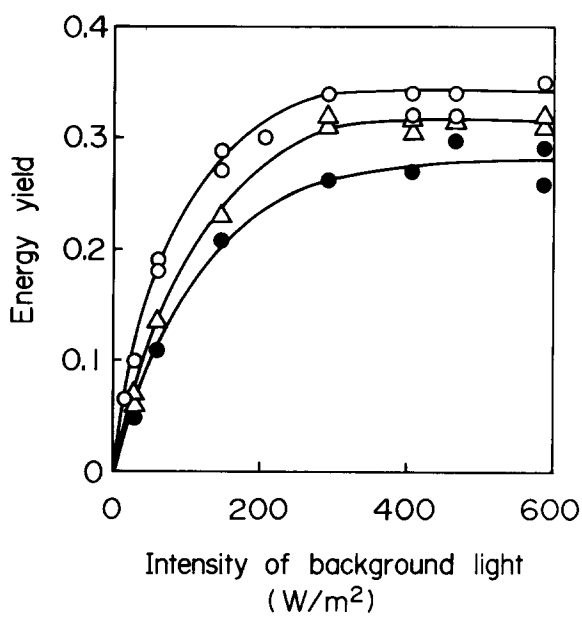

Fig. 3. Dependence of energy yield on intensity of the background light. Intensity and modulation frequency of the monitoring light was 7 W/m^2 and 60 Hz, respectively. Intensity of the background light was varied with neutral density filters. ○, No addition; ●, 10 mM methyl viologen; △, 10 mM methyl viologen plus 100 mM methylamine.

viologen Hill reaction itself does not give rise to net O_2 evolution because the reduced dye is rapidly oxidized with O_2. This cannot explain the lowered energy yield, however. On the contrary, the introduction of the Hill reaction without O_2 evolution would increase the energy yield, provided that the contribution of the modulated O_2 evolution is significant. The methyl viologen Hill reaction may cause net O_2 uptake when H_2O_2 accumulates. The lowered energy yield cannot be explained by the occurrence of the modulated O_2 uptake because it would again result in an apparent increase in the energy yield.

Interestingly, the methyl viologen-induced decrease of the energy yield was found to be largely reversed upon addition of an uncoupler, methylamine (triangles). The uncoupler had no effect on the energy yield of untreated thalli (data not presented). The results strongly suggest that the efficiency of energy conversion depends on electron transport, which is regulated by the proton gradient formed across the thylakoid membranes.

The energy yield decreased with increasing intensity of the modulated monitoring light, because the monitoring light itself serves as an actinic light [4–6]. We checked that the monitoring light of 7 W/m^2, which was used throughout the present work, gave the maximal energy yield.

The modulation frequency of the monitoring light is an important parameter to determine the efficiency of energy conversion [3]. In plant leaves, not only the magnitude of signals but also the sign of changes in signals caused by the background illumination vary with the modulation frequency [6]. In contrast, Fig. 4 indicates that the background light increased the signal amplitudes in *Bryopsis* thalli at all modulation frequencies examined. The maximum energy yield of 0.4 was obtained between 100 and 200 Hz. The energy yield decreased monotonously with decreasing modulation frequency from 100 to 3 Hz in thalli. This cannot be ascribed to increasing contribution of the modulated O_2 evolution at lower frequencies because thalli, which had been treated with methyl viologen plus methylamine, showed a parallel decrease in the energy yield.

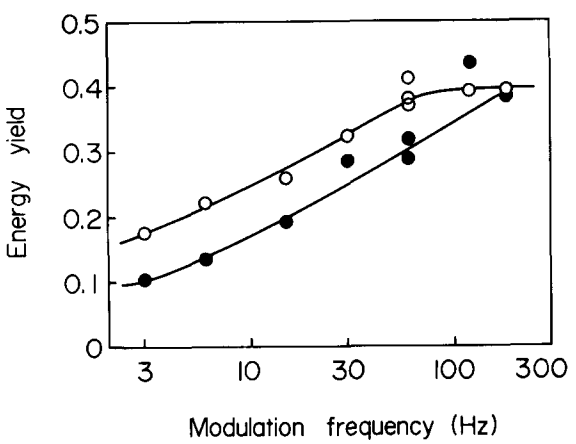

Fig. 4. Dependence of energy yield on modulation frequency of the monitoring light. Intensities of the monitoring and the background light were 7 and 585 W/m, respectively. ○, No addition; ●, 10 mM methyl viologen plus 100 mM methylamine.

Discussion

The present work demonstrates that *B. maxima* is a particularly suitable material for photoacoustic spectroscopy. The contribution of the modulated gas exchange to the photoacoustic signal is much less significant in the thalli as compared with leaves of higher plants, where the modulated O_2 evolution strongly interferes with the measurement of heat production by this technique. This can be related to the coenocytic cellular organization of the alga. The unique structure of the multinucleous algal cells has been described in detail for *Bryopsis hypnoides* [11]. A large central vacuole occupies most of the volume of the thallus, leaving a thin layer of the cytoplasm appressed to the cell wall. The cytoplasm is divided into two distinct layers; chloroplasts are located only in an inner layer of the cytoplasm immediately adjacent to the central vacuole and hence are separated from the outer gas phase by the cell wall and an outer chloroplast-free layer. Less is known about the ultrastructure of *B. maxima* used here, but chloroplasts appear to distribute more uniformly in the cytoplasmic layer (about 20 μm thick) between the cell wall (2.5–4 μm thick) and the central vacuole [13]. Thus, O_2 produced in chloroplasts has to diffuse a longer distance to reach the cell surface in the *Bryopsis* thalli than in leaves, where the minimum diffusion distance is about 1 μm [6,14]. The amplitude of the modulated O_2 concentration

field is attenuated at a distance x from chloroplasts by a factor of e^{-x} [6,14]. It is highly likely, therefore, that the oscillation of the O_2 pressure is damped out during the diffusion to the outer gas phase in the *Bryopsis* thalli. At any event, the absence of a large modulated O_2 pressure signal enables us to measure the efficiency of the photochemical energy conversion in this material with the photoacoustic technique.

The maximum energy yield of 0.4 was obtained under the optimal conditions (i.e., with a saturating background light and a sufficiently weak monitoring light modulated at 100 Hz). Assuming the quantum yield of 1 and the average absorbed photon energy of 1.8 eV (680 nm), the energy stored in intermediates is about 0.7 eV.

Intermediates which store the photon energy at the modulation frequency of 60–100 Hz have lifetimes of 1.5–3 ms [3]. This indicates energy conversion at the electron transport step. In PS I, electron donors such as *P*-700, plastocyanin (cytochrome *c*-553) and cytochrome *f* and acceptors such as ferredoxin, NADP : ferredoxin oxidoreductase and NADP have midpoint potentials in the range of 0.3 to 0.5 [15,16] and -0.3 to -0.5 V [16], respectively. The midpoint potential of oxidants produced by PS II must be above 0.8 V, whereas that of plastoquinone is about 0.1 V [16,17]. Thus, the energy storage is well explained by electron transfer from the electron donor pools to the acceptor pools of the two photosystems. So, the photochemical reaction of the two photosystems proceeds with high quantum yields close to unity.

The following two observations are consistent with the above explanation. (1) The energy yield at 60 Hz was not affected by the KCN-treatment of the thalli, which causes a strong inhibition of CO_2 reduction. Clearly, the energy yield is not related to CO_2 reduction. Radmer and Kok [18] showed that photosynthetic electron transport to O_2 occurs in dark-adapted algae with inactive CO_2 reducing cycle. We suggest that electron transport proceeds with O_2 as electron acceptor even in the presence of KCN. Light-dependent O_2 uptake, like O_2 evolution, would not interfere with the measurement of the periodic heat production in this material. (2) The energy yield was considerably lowered in the presence of methyl viologen but further addition of methylamine restored a high conversion efficiency. Methyl viologen will mediate a rapid electron transfer from the reducing site of PS I to O_2 and thus accelerate non-cyclic electron transport on the one hand and reduce ATP utilization in the CO_2 reduction system on the other. Methylamine is an uncoupler which dissipates the proton gradient formed across the thyalkoid membranes. A simple explanation is, therefore, that even under illumination with the weak monitoring light, methyl viologen builds up the proton gradient to such an extent that electron transport is strongly suppressed. Because non-cyclic electron transport is regulated by the proton gradient at the plastoquinone region, the lowered energy yield is related to the accumulation of the PS II reaction centers with reduced electron acceptors and of the PS I reaction centers with oxidized electron donors. The energy yield will then be increased by methylamine, which accelerates electron transport and readjusts the steady redox states of electron carriers more favorably for the photon utilization.

The energy yield decreased with lowering modulation frequency of the monitoring light. This is expected because electrons or holes are transferred further and consequently the energy difference between oxidized and reduced products becomes less as the modulation frequency is lowered. The lowest energy yield of about 0.2 obtained at 3 Hz may be explained by the energy storage in products of the CO_2 reduction. The results agree reasonably well with the theoretical prediction based on a simpler model [3].

Recently, Kanstad et al. measured energy yields in leaves of several higher plants with photoacoustic spectroscopy and photothermal radiometry [7]. The conversion efficiencies in leaves are generally lower than those in *Bryopsis* thalli. Only tobacco leaves gave a value, at 311 Hz, comparable to the maximum efficiency in *Bryopsis*. The energy yield decreases with lowering modulation frequency in tobacco and bean leaves. However, a rather constant value of 0.26, which corresponds to the energy storage in the CO_2 reduction, was found in a wide range of the modulation frequencies from 2 to 622 Hz in Siberian pea and wheat leaves. It is surprising that little change in the stored energy occurs as the reaction proceeds from steps with a decay time of about 0.5 ms to steps with a decay

time of about 100 ms. At any event, these results suggest that situations are more complex in leaves of higher plants than in the algal thalli.

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