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Photoacoustic detection of energy conversion in a Photosystem II submembrane preparation from spinach

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Photoacoustic spectroscopy has been used to monitor the energy conversion in Photosystem II submembrane fractions isolated from spinach. The extent of detected photochemistry was studied as a function of the modulated light intensity. Half of the reaction centers were saturated at an intensity of 0.5 $W \cdot m^{-2}$. However, the energy storage yield extrapolated to a modulated light intensity of zero was comparable to the yields reported for chloroplasts or algae. It is suggested that the molecular component responsible for the energy storage detected by photoacoustics at a modulation frequency of 35 Hz is located at the level of electron transport.

Irradiation of a sample with a modulated light beam allows monitoring the conversion of light energy into heat by photoacoustic spectrometry [1]. In the case of photosynthetic material, the energy storage in chemical intermediates decreases the thermal yield. Photosynthetic activity can thus be estimated by comparison of the thermal emission of a photochemically active sample and an inactive one [2]. Energy storage can be evaluated in intact organisms such as leaves [3,4], algae [4–7] or photosynthetic bacteria [8]. Isolated PS II activity was studied in leaves by monitoring the oxygen evolution as part of the acoustic signal [3], while PS-I-mediated electron transport activity was detected as energy storage in chloroplast (DCIP to $NADP^+$) and in cyanobacteria (TMPD to $NADP^+$) [4,9]. Cyclic PS I was also observed in these organisms [4,9]. However, the study of isolated photosystems is often simplified by the use of a submembrane fraction representative of the photosystem under study. Whether or not these preparations can be used for photoacoustic measurements is of crucial interest. In this report, we show the photoacoustic detection of energy storage by PS II submembrane fractions isolated from spinach.

PS II submembrane fragments were isolated from spinach chloroplasts by Triton X-100 solubilization as described [10]. Photoacoustic measurements were performed at 35 Hz and 680 nm (maximum of Chl absorption in the red) with a photoacoustic spectrometer described previously [11]. For these experiments, 100 μl of a PS II sample containing 2 mg $CHl \cdot ml^{-1}$ was adsorbed on a nitrocellulose filter (HA type, Millipore Corp.,

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Abbreviations: PS, Photosystem; DCIP, 2,6-dichlorophenolindophenol; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine; Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

25 mm diameter, 0.45 nm pore size) and introduced into the cell described elsewhere [12], after cutting the disk to the appropriate size. Visual inspection showed that most of the aqueous solution was adsorbed in the filter, while the membrane particles remained on the surface. This procedure minimizes the path of heat diffusion through the aqueous media, and improves the signal-to-noise ratio.

In the following experiments, a continuous actinic beam is used to saturate the photochemistry, and leads to a total conversion of the absorbed modulated light into heat [3,9]. The acoustic signal produced by a PS II sample increased upon addition of the nonmodulated background light as described [4]; however, the phase angle of the signal remained unchanged (Fig. 1A). The fact that this increase represents the inhibition of the modulated photochemistry by the actinic beam is substantiated by the absence of this effect with a heat-treated sample (Fig. 1B). A loss of the effect of the actinic beam was also observed in the presence of 200 μm calmidazolium, a compound previously shown to inhibit the water-splitting system in these preparations [13] or 10 μm DCMU (not shown). The detection of energy storage in the absence of any artificial acceptor or donor can be explained by the presence of some intrinsic electron acceptor (quinones) in an amount sufficient to sustain the electron transport or by the autooxidation of some intermediates of the electron-transport chain. At the removal of the actinic beam, 5–6 s are required for the acoustic signal to return to the initial level (Fig. 1A) probably reflecting a slow reoxidation of the electron acceptor sustaining the energy storage.

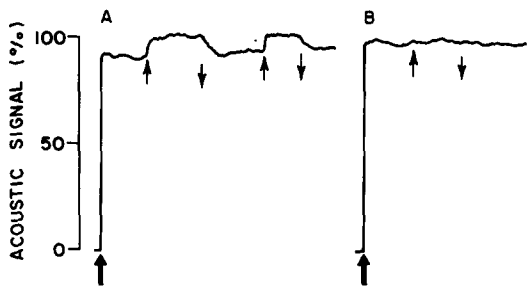

Fig. 1. Acoustic signal from a sample of PS II submembrane fraction at 35 Hz and 680 nm. ↑, onset of modulated beam (1.25 $W \cdot m^{-2}$); ↑, onset of actinic beam (75 $W \cdot m^{-2}$, 400–700 nm); ↓, terminating actinic beam. (A) Intact sample; (B) sample heated 5 min at 50°C. See details in the text.

In the case of photosynthetic materials evolving oxygen, the total acoustic signal is a composite of the thermal emission and the oxygen evolved by the sample [3]. An inhibition of the photochemistry will increase the thermal yield, but cancel the oxygen participation to the overall pressure wave created in the sample cell. The positive effect of the background light on the acoustic signal (Fig. 1) indicates that the thermal participation overcame the negative effect created by the inhibition of modulated oxygen evolution. This situation encountered at very low modulation frequency (35 Hz) in a preparation containing only the PS II oxygen-evolving photosystem, shown to be devoided of any detectable PS I proteins [14], reinforces the proposition made earlier from measurements with cyanobacteria and chloroplast layers that the oxygen participation to the pressure wave is negligible compared to the thermal signal, even if the oxygen-evolution yield is high [4]. The predominance of the oxygen component in leaves was explained by a time delay and by a serious damping of the thermal wave that travels all the way through the leaf material (including a weakly pigmented epidermis layer of 10–30 μm), compared to the oxygen wave that is detected early through mechanical vibrations from the leaf [4].

The photoacoustically detected energy storage is usually defined as the relative photochemical loss, ϕ'_r [2,4]:

$$\phi'_r = \frac{Q_m - Q_c}{Q_m} \cdot 100,$$

where Q_m is the maximum acoustic signal obtained in the presence of the background light and Q_c the control signal. In Fig. 2, ϕ'_r is plotted against the modulated light intensity, I. We can see that the photochemical loss is strongly dependent on the light intensity, and that the modulated beam alone saturates the photochemistry. These data can be analysed as a linear relationship between the reciprocal of ϕ'_r and I [8] (Fig. 2). An I_{50} (light intensity saturating half of the reaction centers) of 0.5 $W \cdot m^{-2}$ can be interpolated from Fig. 2. This value is one order of magnitude below

the value obtained for linear electron transport in chloroplast (5–8 $W \cdot m^{-2}$, not shown). The measured I_{50} represents a rather low turnover rate of the reaction center, P-680. This may reflect the enhancement of PS II electron transport rate by the electron demand of PS I in intact thylakoid membrane. In the absence of this electron sink, a slower turnover rate is achieved by P-680. No exogenous electron acceptors were present for these experiments. Attempts to restore higher rates of PS II turnover by addition of electron acceptors was unsuccessfull. Most of the solubilized material is probably removed from the membrane particles due to adsorption of the liquid medium by the filter as stated earlier. Damaging effects by the isolation procedure could also account in part for the low I_{50}. However, high rates of DCIP photoreduction and oxygen evolution in the presence of 2,5-dichlorobenzoquinone were recorded from this type of preparation [14].

The above discussion implies that only very low intensities of the modulated beam can give a proper measurement of the photochemical loss. From Fig. 2, it is possible to extrapolate ϕ_r' for $I = 0$. This maximum value of ϕ_r' (22%) represents the true photochemical loss at the modulation frequency used (35 Hz). Two facts indicate that the material used here does not allow generation of a proton gradient [15]: (a) it is considered to be constituted of open membrane fractions; (b) it lacks the cytochrome *b–f* complex that is necessary for proton translocation by the plastoquinone pool [16]. It thus appears that the detected photochemical loss occurs at the level of electron transport. During photoacoustic measurements, only the events that proceed faster than the time corresponding to the modulation period will participate in the thermal yield. The energy storage measured should represent the reduced form of an acceptor having a lifetime longer than 4.5 ms (at a modulation frequency, ω, of 35 Hz, so $1/2\pi\omega \approx 4.5$ ms). Candidates for this function are the plastoquinone pool (present to some extent in our preparation [15]) and autooxidation products of the electron-transport chain. The steps preceding the reduction of plastoquinone in the course of electron transport initiated by the charge separation at the reaction center, P-680, are rather fast events that would dissipate in a time shorter than 4.5 ms.

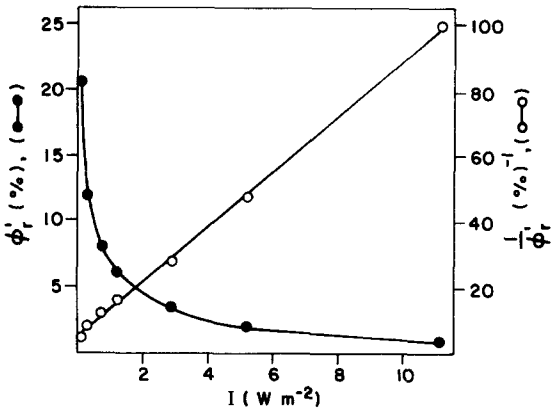

Fig. 2. Dependency of the relative photochemical loss and the reciprocal of the relative photochemical loss on the modulated light intensity at 35 Hz and 680 nm. Conditions are as in Fig. 1.

This investigation represents the first demonstration that photochemical energy storage with submembrane fractions can be detected efficiently by photoacoustic spectroscopy. In this case, fractionated systems would represent a useful tool for the photoacoustic study of energy conversion.

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