PHOTOACOUSTIC SPECTROSCOPY OF CHLOROPLAST MEMBRANES; LISTENING TO PHOTOSYNTHESIS

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1. Introduction

We report here on the use of photoacoustic spectroscopy (PAS) to study the energy conversion process in photosynthesis. In PAS a sample, situated in a gas-filled chamber containing a microphone, is illuminated by light modulated at an acoustic frequency. Part of the exciting radiation is converted into heat which is tranferred to the gas phase and induces pressure waves which are detected by a microphone.

Since its revival several years ago [1], PAS has been used especially to study the optical and thermal properties of solids and solutions [2,3]. Less attention has been given to using the technique for the study of energy conversion processes for which it is very well suited as it measures directly the thermal energy generated upon absorption of radiation by the sample [2,4]. In photosynthesis this thermal energy is the wasted part (together with fluorescence) of the absorbed radiation. Therefore, the photoacoustic and the optical absorption spectra of photosynthetically active samples should differ by that amount of the absorbed energy that is converted into chemical energy (named here photochemical loss)*. Also inhibited photosynthetic samples should give photoacoustic signals larger than those obtained with normal samples because now there is no photochemical loss.

A theoretical treatment of the influence of photochemical processes on PAS signals, and the quantitative dependence of such effects on the modulation frequency, will be presented elsewhere (S. Malkin and D. Cahen, submitted). For any photochemical process a decrease of the photoacoustic signal with increase in quantum yield for photochemistry and increase in wavelength is expected (for reactions starting from the first excited singlet). Furthermore by varying the modulation frequency information can be obtained on kinetic parameters and on the energy content of intermediates, using both the in-phase and quadrature components of the signal. It can be shown that for a given angular frequency, ω, the photochemical loss is due to intermediates having decay rate constants equal to or higher than ω. Then, as the frequency increases, more primary steps are sensed and the photochemical loss increases.

The results described below, show that such differences are indeed observed and thus indicate the feasibility of using PAS in the study of energy conversion processes.

2. Experimental

In our experiments we used lettuce chloroplast membranes, prepared according to [5] except for final resuspension in an ethylene glycol and Bovine Serum Albumin containing medium for preservation, in a home-built differential photoacoustic spectrometer [6]. PAS signals that can be obtained from aqueous samples (such as the chloroplast suspension) tend to be much lower than those from equally absorbing solid powdered samples. We tried several methods to increase the signal level and best results were obtained when the chloroplast suspension was adsorbed on

* The effect of fluorescence should also be considered generally, but is marginal here

cotton wool. This method has the dual advantage of decreasing the gas (air) volume in the cell and increasing the sample surface area, both of which are factors in determining the PAS signal strength [7,8]. In this way the absolute background signal (wet cotton wool alone) is increased as well but, notwithstanding increased acoustic damping, the signal itself increases much more, so that the signal to background ratio is at least two times better than when the aqueous suspensions are put directly in the quartz cell or on a millipore filter paper as a holder, or when whole leaves are used. Furthermore because the higher signal level allows operation at lower amplifications a noticeable improvement in signal to noise ratio is obtained.

The photosynthetic activity of the cotton wool-adsorbed samples was checked separately by fluorescence induction measurements and found not to be impaired for the duration of the experiments (~ 20 min). The effect of light intensity on the PAS signals was investigated and, within the experimental error, linearity was observed. This is reasonable since, at 4×10^{-9} Einstein/cm^2 · s, the average incident light intensity used, the chloroplast membranes are far from saturation [9].

3. Results and discussion

Figure 1 shows the optical absorption and photoacoustic spectra of chloroplast membranes, *normalized at 440 nm* and adjusted to the same concentration, to facilitate comparison between the different samples.

At 680 nm the PAS signal is 10–15% less than the absorption signal, while at 720 nm, where the quantum yield for photosynthesis is very low [11], normalization at 440 nm causes the PAS signal to be larger than the absorption signal. The proportionality between the PAS and optical absorption signal for solids has been derived by Rosencwaig and Gersho [12]. In case of biological samples the heterogeneity of the system and the presence of water complicates the situation, but even here such a proportionality should still hold, as was shown in a general way by Aframowitz et al. [13]. Therefore the difference between the two kinds of spectra must be related to photochemical activity.

In fig.2A the raw photoacoustic spectra at 770 Hz

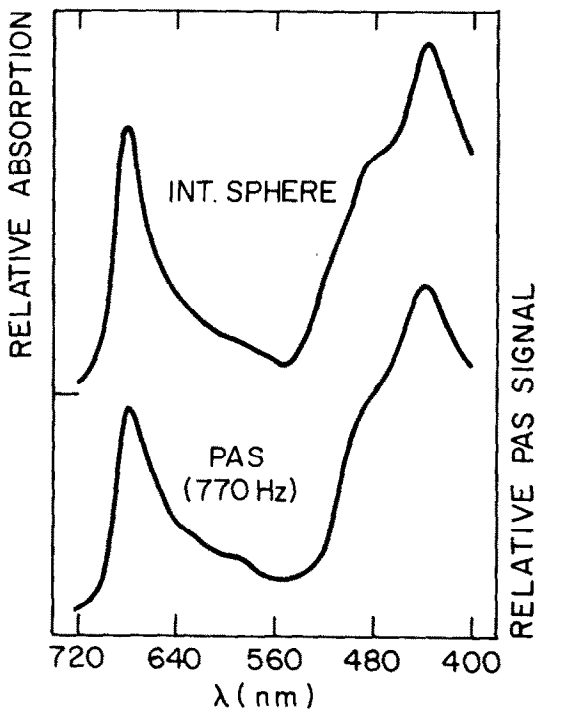

Fig.1. Absorption and photoacoustic spectrum of lettuce chloroplast membranes. The absorption spectrum was obtained using both a home-built integrating sphere and the Shibata [10] technique on a CARY-17 spectrophotometer, with essentially identical results. A suspension containing 32 μg chlorophyll/ml, buffered at pH 7.8, was used. For the photoacoustic spectrum a suspension containing 150 μg chlorophyll/ml, was used. No external electron acceptor was added. When methylviologen, as acceptor, is added, the (relative) photoacoustic signal decreases further, if compared to the optical absorption spectrum at 680 nm and normalized at 440 nm. A 450 W Xenon arc lamp, monochromator, focussing lenses and light guide were used for illuminating samples for PAS. Detection was by Knowles Electronics BT-1753 microphones, Princeton Applied Research CR-4 low noise amplifier and JB-6 lock-in amplifier.

(not corrected for the variation of incident light intensity with wavelength) of normal and DCMU-poisoned* chloroplast membranes are compared. The coincidence in part of the spectral range is somewhat fortuitous, but nevertheless important, as it shows that differences between the separately investigated

* DCMU = 3-(3,4-dichlorophenyl)-1,1-dimethylurea, an electron transport inhibitor

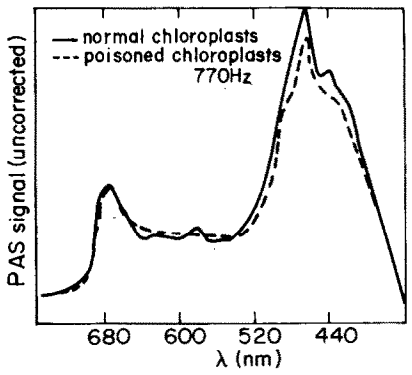

Fig.2A

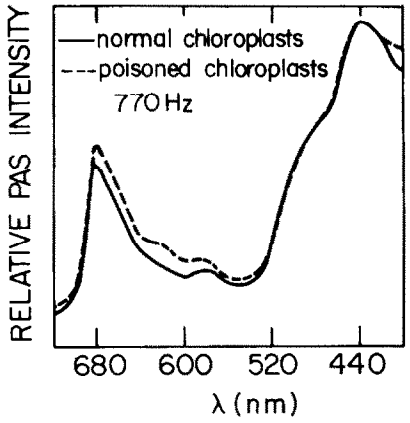

Fig.2B

Fig.2. Photoacoustic spectra of photosynthetically active and poisoned chloroplast membranes. In the case of active membranes the suspension of fig.1 was used, to which methylviologen was added to a final concentration of 20 mM. DCMU was used to poison the chloroplasts by adsorbing them on cotton wool, which previously had been immersed in a DCMU-saturated methanol solution and allowed to dry. This was necessary in order to avoid the presence of methanol vapour in the cell, as it was found that even small quantities affect the photoacoustic parameters. (A) Shows the raw photoacoustic signal, obtained using the differential technique to suppress the background (empty cell + wet cotton wool) signal. (B) Shows these spectra, corrected for the variation of light intensity with wavelength using a carbon black sample.

samples, are observed directly. In fig.2B these spectra are shown, corrected and normalized at 440 nm. At 680 nm the DCMU-poisoned chloroplasts give a signal that is ~10% higher than that of the normal chloroplasts. The work of Emerson and Lewis shows that at wavelengths below 580 nm a decrease of 20–40% in the quantum yield is observed for *Chlorella* [14]. Extrapolating to our chloroplasts, this might explain why we observe the largest differences in PAS signals around 680 nm (the largest possible photochemical loss). This, also, is the reason for normalizing the spectra at 440 nm, where the photochemical loss effect is minimal due to low quantum yield at short wavelength. (Direct comparison between different samples was not possible practically because of insufficient reproducibility upon replacing samples.)

Figure 3 illustrates the differences obtained when a lower modulation frequency is used. The spectra were normalized at 440 nm as before, and the relative differences between them are larger than in fig.2, with the DCMU-poisoned sample giving a ~ 20% stronger signal at 680 nm than the normal one.

At first glance this result seems to contradict PAS theory for the photosynthetic system, because as DCMU is known to inhibit photochemical activity, the DCMU-spectrum should reflect the normal absorption spectrum at any modulation frequency. Then, the higher the modulation frequency, the larger the difference between normal and inhibited photoacoustic signals should be (larger photochemical loss). The reason for this becomes clear when we compare the normalized, DCMU, PAS signals to the absorption signals (fig.1). Taking the 440 nm signal as unity in all spectra, only at 72 Hz the ratio of the normalized, DCMU, PAS signal at 680 nm to the absorption signal is one (fig.3). At 770 Hz this ratio is only 0.8 (fig.2). (In qualitative agreement with the theory the ratio for normal samples at 770 Hz is lower than at 72 Hz: 0.7 vs. 0.8.) Thus it seems that the inhibitory effect

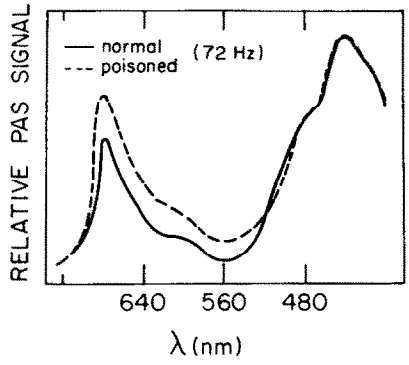

Fig.3. As fig.2B, but at low modulation frequency.

of DCMU at 770 Hz is not complete under our conditions. This may be due to a cyclic electron transport involving methyl-viologen around photosystem I*, which persists in the presence of DCMU. At low enough frequency PAS senses the final (= initial) products of such a cycle (then all light is converted into heat), while at higher frequencies intermediate ones (e.g., those of primary charge separation) are sensed. In this last case a smaller PAS signal will result and differences ± DCMU will reflect only part of the photochemical loss of the normal system. The problem of incomplete inhibition by DCMU can be circumvented by using strong, continuous side-illumination to close the reaction centers, and our set-up is being adapted to accomplish this.

Experiments such as those reported here, were carried out on other biological energy converters as well (*Halobacterium halobium*) and studies on non-biological photovoltaic conversion systems are in progress. Also whole leaves have been used previously in PAS studies and with the proper sample preparation, to allow for good impregnation by additives and high signal strength, and improvement in the instrument sensitivity they can be studied directly by this method.

* At the modulation frequencies used here, the presence of a large pool of electron acceptors and their slow rate of oxidation effectively decouples the two photosystems, so that they contribute separately to the PAS signal. (S. Malkin and D. Cahen, submitted; see [15].)

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