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PHOTOACOUSTIC DETECTION OF PHOTOSYNTHETIC ACTIVITIES IN ISOLATED BROKEN CHLOROPLASTS

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Summary

Methodology and demonstration how to utilize the photoacoustic technique in photosynthesis research are presented. Photoacoustic signals were obtained from suspensions of isolated broken chloroplasts. In the presence of strong, continuous (non-modulated) background light the signals were normally larger than without the background light. The effect of the background light was saturable and was absent when non-active (e.g. heat-treated) samples were used, showing that the normal smaller signal in the absence of background light is a genuine reflection of the loss of heat due to the competing photochemistry. The effect of the background light is to close the reaction-centers and hence to inhibit the photochemical process. The percent difference of the photoacoustic signal (± background light) is taken as a measure of the photochemical activity ('photochemical loss').

Initial results demonstrate the wavelength dependence of the 'photochemical loss'. As expected there was a 'red-drop' decrease of the 'photochemical loss' for $\lambda > 690$ nm, when the cofactor methyl viologen was present. Surprisingly, however, there was a 'red-rise' increase for $\lambda > 690$ nm when no cofactor was present. These findings indicate that under the last conditions there is an unsuspected photoactivity of PS I which was not detected hitherto by the conventional techniques. The dependence on the background light intensity confirms this result. This photoactivity can be explained tentatively as a cyclic electron flow around PS I, present without any added cofactor.

Initial results on the modulation frequency dependence in the presence of electron acceptors are also demonstrated.

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Abbreviations: PS, photosystem; DCIP, 2,6-dichlorophenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

Introduction

Photoacoustic spectroscopy is a method based on the detection of periodic heat production generated by absorption of periodically modulated radiation in a given sample. In most cases of solid or liquid samples the heat diffuses to the sample/gas boundary and causes pressure modulation (sound) in the closed volume of gas surrounding the sample, which is picked up by a microphone. The resulting signal is analyzed by a phase-sensitive detecting device (lock-in amplifier) for its amplitude and phase [1,2].

Most of the attempts in exploiting photoacoustic spectroscopy were in gain-in information on the spectral and thermal properties of the sample under study [3,4]. Several reports showed its usefulness also in studying photochemical processes with a particular emphasis on measurements of fluorescence quantum yields [5,6] and relaxation processes in gases [7,8] as well as for the studies of photo-voltaic devices [9]. The usefulness of the method to study photochemical processes stems from the fact that if a photochemical intermediate stores a certain amount of energy the photoacoustic response will be less when compared to that which is expected from an equivalent non-photochemical system. Such 'photochemical loss' depends on the photochemical quantum yield, the energy of the intermediate (compared to the photon energy) and also on the modulation frequency [7,8,10].

The use of photoacoustic methods in studying the detailed kinetic steps of photochemical and particularly energy-storing photobiological reactions was envisaged in a recent publication [10]. In this case the photoacoustic response is scanned as a function of the modulation frequency. The information gained contains the relative energy changes and kinetic decay constants of the intermediates formed successively following the primary photochemical act. Fig. 1 gives, in a very simplified manner, the correlation between a diagram of a photochemical reaction scheme and the expected signal-dependence on the modulation frequency (cf. Ref. 10). This diagram is idealized and simplified for a case where the kinetic constants are widely separated in magnitude, thus giving a clearer correlation between the photochemical scheme and the resulting photoacoustic signal. A different type of idealization is that the strong dependence on the frequency due to the heat transfer from the sample to the gas phase and its transduction to sound was not accounted for. A suitable method to avoid these factors, is to compare the photoacoustic signal to a suitable reference of identical thermal and light absorption parameters as that of the sample, but without activity. The ratio of the heat productions of the sample and the reference at the site of light absorption remains the same also in the resulting acoustic signals, and reflects the effect of the photochemistry only.

Our preliminary work with chloroplasts [11] convinced us that the photoacoustic method may be used as a tool to study photosynthesis. The problem for a systematic research was to find a suitable non-photoactive reference. Making two sets of measurements, one for active and the other for inhibited chloroplast suspensions, was not practical, since the fluctuating experimental conditions give rise to fluctuations in the photoacoustic signals which are of the same order of magnitude as the expected effects (i.e. $\simeq$10%). It would be pre-

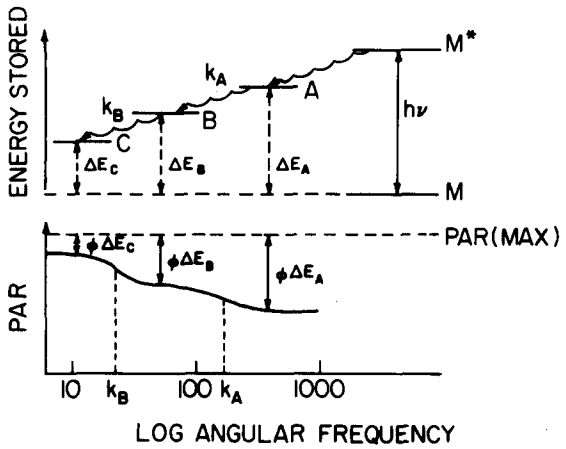

Fig. 1. Top: (read from right to left). An energy diagram for a photochemical reaction. Absorption of a photon in a molecule M leads to an excited state M *. Intermediates A, B, C are formed in succession. For the particular case of this diagram it is assumed that A is formed with a quantum yield ϕ and the other intermediates follow from A with a yield of 1 (i.e. no branching). Bottom: The corresponding type of dependence of the photoacoustic signal as a function of the modulation frequency. The 'relative photochemical loss' is the difference between the maximum photoacoustic signal (obtained from a suitable reference and normalized to 1; cf. text) and the signal of the sample. In Fig. 7 the 'relative photochemical loss' for chloroplasts is plotted against the modulation frequency. The plateaus correspond to the different intermediates A, B, C and the corresponding relative photochemical losses are equal to the energy stored in each intermediate ($\phi\Delta E_i$) divided by the energy of the absorbed photon. The points of half transition between the plateaus occur at angular frequencies equal to the rate constants of decay of the corresponding intermediates. PAR, photoacoustic response.

ferable to find a method where the sample itself can serve alternately as its own reference in situ.

We show in this work, for the particular system of isolated chloroplasts, how to suitably reference the photoacoustic signal. The photosynthetic system has a high ratio of antennae pigments per reaction centers (around 500) and a rate limiting time of the order of 10 ms. Hence, the photochemical reaction becomes saturated at a reasonably achievable light intensity (approx. 200 nano-einstein $\cdot$ s $\cdot$ mg^{-1} chlorophyll). Application of such bright and constant (non-modulated) light (which by itself should not contribute to the signal), on top of the modulated light, will cause a severe drop in the macroscopic quantum yield of the photochemical system. This will enhance the conversion of light to heat and hence the photoacoustic signal. The maximally enhanced signal represents 100% conversion of light to heat and can serve as a suitable reference for the photochemically active chloroplasts (i.e. without the background light). The heat conductance and the specific heat of the chloroplasts are presumably only negligibly affected by such treatment. Thus the sample and reference both should have identical behaviour for that part of the signal vs. frequency response which originates from all the other factors besides photochemistry. Any change between the sample and reference of the photoacoustic signal vs. frequency behaviour can be traced directly to the photochemistry. The application of a constant background light and measuring its effect on the modulated response from photosynthetic systems was done in the past, mainly in measuring fluorescence [12] and oxygen evolution [13]. The photoacoustic method

applied here is different from the flash measurements of Callis et al. [14] and of Ort and Parson [15] where a microphone in close contact with the suspension was used; in the last method photochemically induced volume changes have an important contribution and were measured. In our case heat production is sensed primarily.

Experimental procedure

The details of the photoacoustic system were reported elsewhere [16,17]. The cuvette was made from transparent lucite in the form of a drawer of dimensions 5.7 × 4.2 × 1.3 mm inner diameter, which was fitted into a suitable chamber of quartz and which communicated through a small channel with a microphone. The signal from the microphone was amplified and fed into a lock-in amplifier (Brookdeal, Ortholoc 9502). The optical system consisted essentially of a 450 W Xenon arc, a mechanical chopper, monochromator and a fiber optics light guide to deliver the resulting monochromatic chopped light onto the cuvette through the top of its chamber. Another light source (100 W incandescent) was used for applying the strong constant backgrouns light. Its output was filtered by a cut-off yellow filter (>450 nm) and a standard heat absorber taken from a slide projector. This gave a band of wavelengths between 450 and 720 nm. The light was delivered onto the cuvette by a second light guide. The whole arrangment is sketched in Fig. 2.

'Broken' chloroplasts from tobacco, grown in the green house, were prepared as described before [18]. The final pellet was resuspended in a medium containing 30% ethylene glycol, 20 mM sucrose, 20 mM Tris-HCl buffer (pH 7.8) and 125 mM KCl, and was used as such in the experiments. This medium was optimal for preservation of photosynthetic activities in cold storage [19] and was found to give stable results in the photoacoustic experiments, compared to

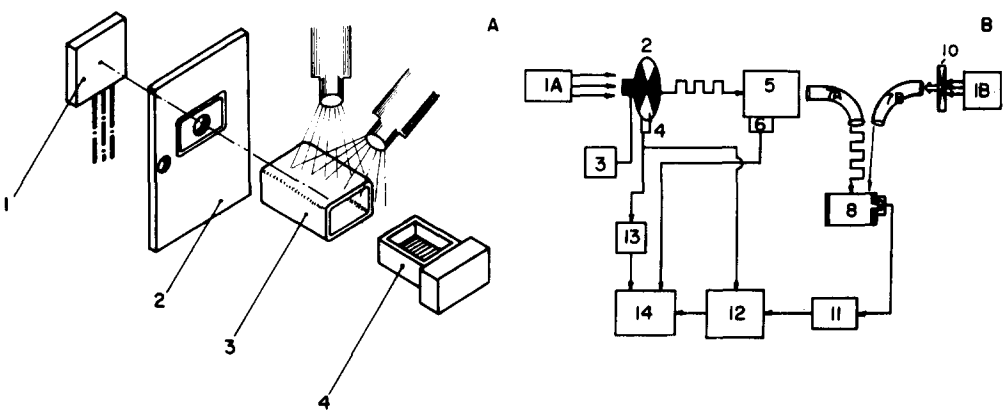

Fig. 2. Schematic sketch of the experimental system (cf. Ref. 16). A. View of the photoacoustic cell. 1, microphone; 2, wall with communication channel; 3, cover; 4, sample holder. B. Control and detection system. 1A, 1B, light sources including all filters from the modulated and background beams, respectively; 2, chopper; 3, chopper control; 4, source for reference signal for lock-in amplifier and frequency meter; 5, monochromator; 6, monochromator wavelength drive; 7A, B, light guides for the modulated and background light; 8, photoacoustic cell; 9, microphone; 10, fast mechanical shutter for background light; 11, preamplifier; 12, lock in amplifier; 13, frequency-voltage converter and meter; 14, *X-Y-t* recorder.

other conventional media, the reason being mainly that chloroplasts did not tend to settle down in this medium. The photoacoustic response is presumably very sensitive to such settling down, which changes the distance for the heat diffusion through the liquid from the chloroplasts to the liquid/air interphase (cf. Ref. 3).

We experimented for the optimal way to introduce the chloroplasts into the cuvette. The problems involved were rooted mainly in the small dimension of the cell which required introduction of a very small volume (approx. 10—25 μl). A straightforward placement of a concentrated suspension gave a noisy and unstable signal. The reason for that was presumably 2-fold: the adsorption of the suspension onto the walls and some persistent settling down of the chloroplasts. Best results were achieved when a piece of filter paper or soft tissue paper was used as a support at the bottom of the cuvette. In this case the suspension effectively impregnated the paper and the signal was satisfactorily stable. We checked that the photosynthetic activity of chloroplasts under such conditions remains intact. This was conveniently carried out in a different set-up where chlorophyll *a* fluorescence transients were measured. A comparison of such fluorescence transients and also the response of the fluorescence intensity vs. the exciting light intensity for a regular diluted chloroplast sample and chloroplasts impregnated into a tissue of filter paper, showed that the activity of the chloroplasts placed on the paper was not changed significantly. Such experiments were done also to establish in a preliminary way the range of light intensities to be used for the modulated light and the background constant light in the photoacoustic apparatus (however, see below).

Results and Discussion

The main theme of this communication is to establish that the method of referencing by constant strong background light indeed works properly and gives meaningful results. A further step is to outline the behaviour of the photoacoustic response vs. the modulation frequency and the wavelength.

Fig. 3a shows the basic experiment. A photoacoustic response is shown as a result of application of modulated light to chloroplasts. When a strong background constant light was added, the signal increased by about 10—20%. It decreased again when the background light was switched off. Such an experiment could be repeated several times, but eventually, after several such cycles, the difference of responses between the on and off periods became smaller, indicating that the chloroplast activity died off. For this reason we used results of the first cycle only, in dark-adapted chloroplasts.

The following experiments were done in order to establish that the effect of the background light is due only to the 'switch-off' of the photochemistry. Other effects which can be thought of, such as a possible change of the thermal parameters, which are induced by a change of temperature, were eliminated.

Fig. 3b shows, for example, that chloroplasts which were inactivated by aging or heating treatment do not give changes of the photoacoustic signal upon switching on and off the background light.

The effect of the background light was studied with chloroplasts suspended in several media: with no added electron acceptor (Figs. 3a and a′) and with

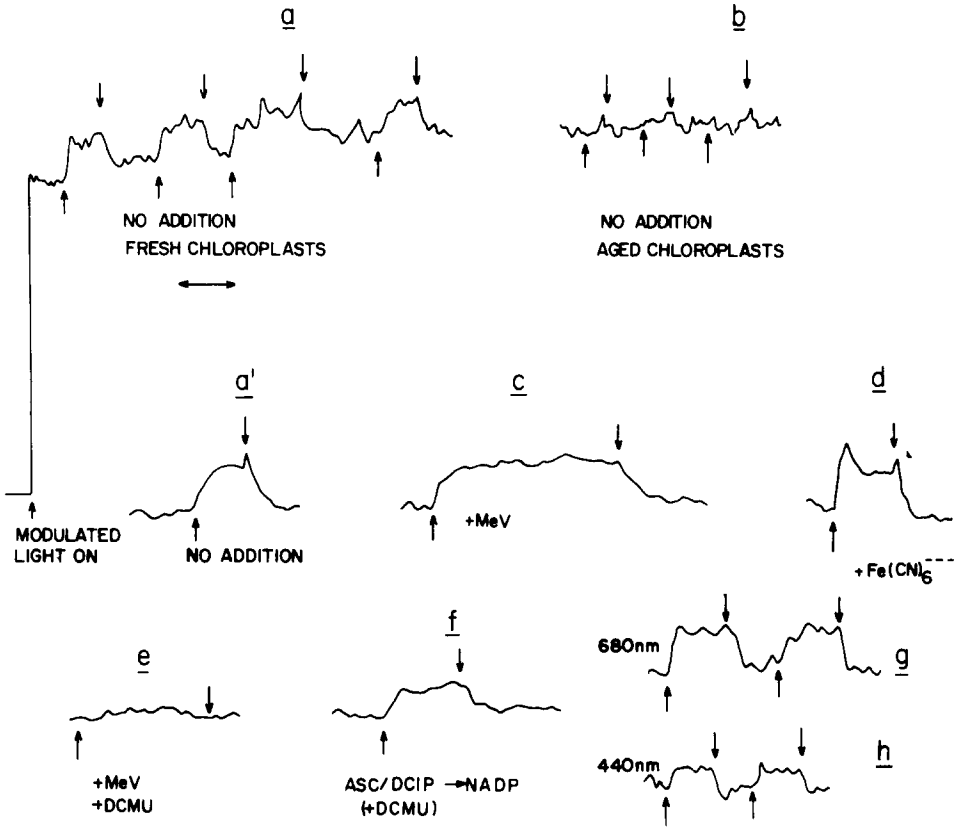

Fig. 3. Transients in the photoacoustic signal as a result of the applicaton of background light. Upward and downward arrows, background light on and off, respectively. Other details as indicated in the figure. Fig. 3a' is a control experiment for Expts. 3 c—f. Concentrations: chlorophyll, 3.5 mg/ml; methyl viologen (MeV), 10^{-3} M; $K_3Fe(CN)_6$, $5 \cdot 10^{-3}$ M; DCMU, $5 \cdot 10^{-5}$ M. For Expt. 3f: chlorophyll, 1.7 mg/ml; NADP, $2.5 \cdot 10^{-3}$ M; DCIP, $5 \cdot 10^{-3}$ M; Ascorbate, $5 \cdot 10^{-3}$ M; DCMU, $5 \cdot 10^{-5}$ M and saturating concentrations of ferredoxin and diaphorase. Total volume: 10 µl. Modulation frequency: 40 Hz. Wavelength of the modulated beam: 685 nm, except as indicated in Fig. 3g and h. Intensity of the modulated light ~ 5 W/m^2. Intensity of the background light ≈ 300 W/m^2.

electron acceptors methyl viologen and $Fe(CN)_6^{3-}$ (Fig. 3 c and d). In the last cases addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), a potent inhibitor of Photosystem II, inhibited the background light effect (Fig. 3e). With addition of 2,6-dichlorophenolindophenol (DCIP) with ascorbate as electron donor and NADP (plus ferredoxin) as an electron acceptor and with DCMU in the medium there was again the same background light effect although with a smaller amplitude (Fig. 3f). In some cases complicated transient effects were noticed (e.g. Figs. 3d and also Fig. a'), which could arise from a complex kinetics of reaction centers 'closure' and 'reopening'. However we did not eliminate the possibility of purely electronic artifacts. In this communication however we are interested only in steady-state measurements and will, therefore, ignore these transients.

The difference of the steady-state levels between the switch-off and -on of the background light is taken to represent the effect of the 'photochemical

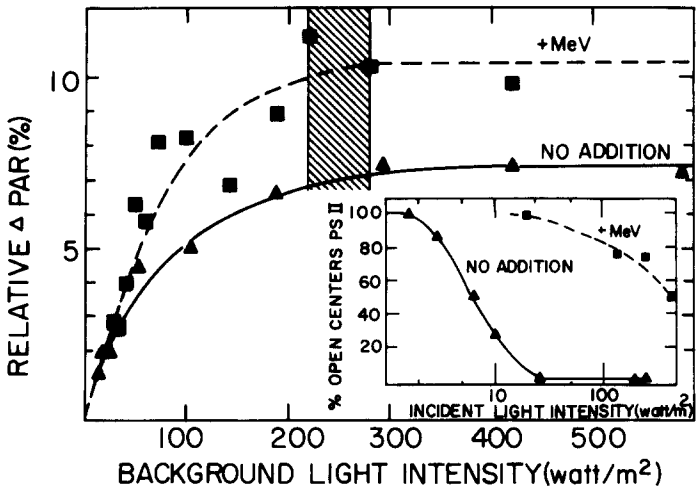

Fig. 4. The relative change in the photoacoustic response (ΔPAR) as a function of the background light intensity, for two cases: chloroplasts alone (▲) and with addition of methyl viologen (■). In calculating ΔPAR care was taken to subtract a background signal not due to the chloroplasts (mainly due to the window materials). This was done by taking a base line for the signal at 750 nm and comparing this to the signal from cell and holder filled with water, at 750 nm and the wavelength of interest. Concentrations: chlorophyl, 1—6 mg/ml, methyl viologen (MeV), 10^{-3} M. Modulated light 686 nm ~7 W/m^2; frequency, 40 Hz. The dashed region represents the range of background light intensity used normally. Insert: The fraction of open reaction centers of Photosystem II, from fluorescence experiments, as a function of the light intensity at 546 nm. The fraction of open reaction centers is taken here as $(F_{max} - F_{s,s})/(F_{max} - F_o)$, where F_{max} is the maximal fluorescence (+DCMU), F_o is the initial level in the fluorescence induction, $F_{s,s}$ is the steady-state level (cf. Refs. 21, 22).

loss'. Dividing this difference by the signal with the background light on gives the 'relative photochemical loss' which is the ratio of energy stored (per photon) to the energy of the photon.

The effect of varying the wavelength of the modulated light (430 nm vs. 680 nm, Figs. 3g, h) is also consistent with a true photochemical effect. As expected, the 'relative photochemical loss' at 430 nm is smaller than that at 680 nm, since excitation to a higher excited state means that a smaller fraction of the photon energy is stored by the same intermediate(s) (cf. Refs. 10, 11). Assuming a ratio of quantum yields (ϕ_λ) for photochemistry at 440 nm and 680 nm equal to 0.8 [20], the expected ratio of 'photochemical losses' at the corresponding wavelengths is $\phi_{430} \cdot 430/\phi_{680} \cdot 680 \approx 0.5$. The experimental ratio was indeed close to 0.5 (±20%).

Another indication that the background light effect on the photoacoustic signal is a genuine indication of photochemical loss is the property that the effect is saturated at higher intensity (Fig. 4), contrary to what is expected from a straightforward heating effect *. Such an experiment demonstrates also that care must be taken in selecting the intensities of both the modulated and background light. The modulated light intensity should be as low as possible so as to be in the 'light limiting' range (i.e. steady-state with maximal population

* The heating effect on the photoacoustic signal was actually calculated for the light intensities used by us, and found indeed to be extremely negligible.

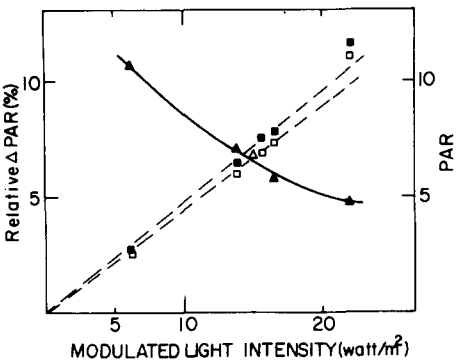

Fig. 5. The relative change of the photoacoustic response (ΔPAR) as a function of the modulated light intensity. Chloroplast with no added cofactor. Chlorophyll concentration 7 mg/ml. The background light intensity was saturating (~250 W/m^2). Modulated light, 686 nm; frequency, 40 Hz. The dotted line represents the total photoacoustic response as a function of the modulated light intensity plus (■) and minus (□) background light.

of opened reaction centers) while the background light intensity should be in the saturation range. The first requirement is against what one usually does in photoacoustic spectroscopy, which is to increase the light intensity as much as possible in order to maximize the signal/noise ratio. Indeed, in our present set-up we could reduce the modulated light intensity only to a point in which a compromise was made between a reasonable signal/noise ratio and maximal effect of the background light. An experiment which shows the extent of the 'relative photochemical loss' as a function of the modulated light intensity is also shown and confirms the above expectations (Fig. 5).

A quantitative correlation was attempted between the results shown in Figs. 4 and 5 and the results from the fluorescence intensity curves which presumably also indicate the degree of saturation in the reaction centers of Photosystem II [21] (cf. insert to Fig. 4). A direct comparison between the two experiments cannot be made accurately because of the difference of wavelengths used. However even a rough estimate shows that the results are not comparable. For the case of the photoacoustic experiment the half-saturation light intensities for both the cases of no addition and methyl viologen addition are about 55 watt · m^{-2}. (An estimate, taking into account the distribution of intensity over the wavelength range and the absorption spectrum of the chloroplasts shows that this number would be equivalent to an incident intensity of about 200 watt · m^{-2} or even more, of light wavelength 546 nm, where the fluorescence was excited). A drastically different situation is encountered for the fluorescence quantum yield saturation behaviour (insert to Fig. 4) where the half saturation intensities differ for the cases of no addition and methyl viologen addition, 6 and 400 watt · m^{-2} (incident), respectively.

It became clear from the above experiments that the photoacoustic and the fluorescence experiments do not monitor the same thing; obviously for the 'no-addition' case. The fluorescence experiments reflect the state of Photosystem II as it is balanced by a linear electron flow to Photosystem I (in the no-addition case, with oxygen acting as an electron acceptor) and the continuous excitation. The rate limiting step in the no-addition case was thought to

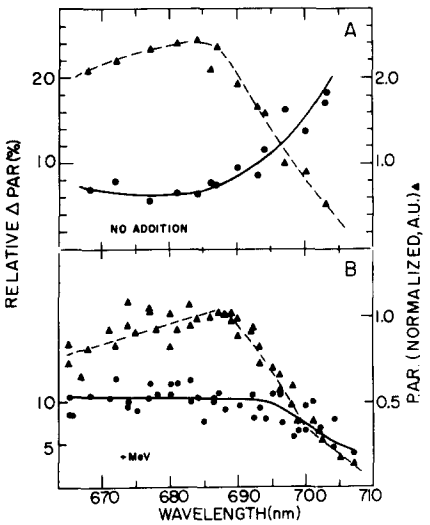

Fig. 6. The relative change of the photoacoustic response (ΔPAR, relative photochemical loss) (●——●) as a function of the modulated light wavelength. This curve closely represents the quantum yield spectrum, as it is proportional to the photochemical loss times the wavelength [10,11]; the relative change of the wavelength in the range of interest is about 6% only. (▲) The total photoacoustic response (▲- - - -▲), corrected for change of intensity by comparison to a signal from a sample of carbon black. This curve closely represents the absorption spectrum of the chloroplast sample; other conditions as in the above figures. A. Chloroplasts alone. B. With methyl viologen (10^{-3} M). Chlorophyll concentration, 6.5 mg/ml.

be at the reducing side of Photosystem I and saturated at low intensities (cf. Refs. 21, 22).

The above discrepancy can be explained by assuming, in contrast to the paragraph above, that Photosystem I is rather not saturated at the intensities where Photosystem II is saturated. A possible explanation would be that Photosystem I performs, in addition to normal linear electron flow, a kind of cyclic electron flow involving a regeneration of its reaction centers back into an open form. In that case the photoacoustic results will reflect mainly the properties of the cycling Photosystem I as the detecting modulated light by itself is sufficiently strong to bring Photosystem II close to saturation. Although a similar cycle was demonstrated when adding suitable cofactors (e.g. phenazinemethosulphate) and was postulated for intact chloroplasts it was not suspected to occur in broken chloroplasts with no addition.

When methyl viologen or $Fe(CN)_6^{3-}$ are added the situation changes drastically. Methyl viologen accepts electrons readily at the reducing side of Photosystem I and therefore should compete strongly with electron cycle around Photosystem I. Both Photosystem II and I participate in an active linear flow of electrons from water to methyl viologen (and thence to oxygen). With the $Fe(CN)_6^{3-}$ it is probable that it accepts electrons in Photosystem II and blocks Photosystem I by rapidly oxidizing its primary donor. The photoacoustic signals in the no-addition case and in the plus $Fe(CN)_6^{3-}$ and methyl viologen cases reflect quite different events. In the last case the saturation properties of the fluorescence and the photoacoustic responses should become identical, as is, qualitatively at least, observed (Fig. 4, insert).

Further support to the above comes from comparing Fig. 6 A and B. These

figures show the relative 'photochemical-losses' plotted as a function of the wavelength in the red absorption band of the chloroplasts (between 665 and 710 nm), in order to follow the changes of the photochemical quantum yield. In this wavelength region there is a transition at about 690 nm where in the far-red side PS I absorbs preferentially. This gives rise to phenomena like 'red-drop' or 'red rise' in the quantum yield [23] depending on the reaction followed. Indeed Fig. 6b shows that for chloroplasts with methyl viologen as an added cofactor the 'photochemical-loss' decreases at longer wavelengths, demonstrating a clear 'red-drop' effect. On the other hand chloroplasts with no added cofactor demonstrate rather a 'red-rise' effect (Fig. 6a), which is quite puzzling if one regards only a linear electron flow to oxygen, which in this case should give also a 'red-drop' effect. A tentative explanation relies again on the assumption made above of cyclic electron flow around Photosystem I in the 'no-addition' case. In this case Photosystem II is probably blocked already by the modulated light at the short wavelength side and the 'photochemical-loss' is proportional to the fraction of light distributed to Photosystem I. At longer wavelengths the contribution to the 'photochemical loss' from Photosystem I increases because more light is channelled to this photosystem. Some contribution may arise from Photosystem II which may become partially opened for

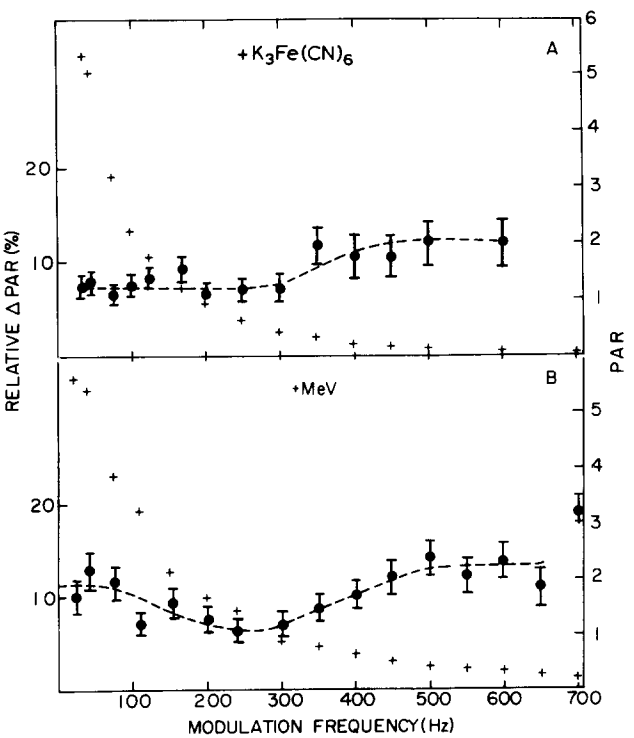

Fig. 7. (•) The relative change of the photoacoustic response (ΔPAR, relative photochemical loss) (•- - - - -•) as a function of the modulation frequency. A. Chloroplasts plus $K_3Fe(CN)_6$. B. Chloroplasts plus methyl viologen. Conditions similar to those in the above figures. The points (+) represent the total photoacoustic signal (with background light) as a function of the frequency, showing mainly the effect of heat diffusion and transduction to sound.

far-red excitation and receives also some small fraction of the light energy. The assumption of Photosystem I cyclic electron flow therefore naturally leads to a 'red-rise' effect. With addition of electron acceptors such as methyl viologen only linear electron flow is possible. At the far-red side of the spectrum the imbalance of the two photosystems closes the reaction centers and reduces the efficiency of photochemistry in both photosystems. Hence a 'red-drop' effect is expected. The conclusions made above are clearly tenative and await confirmation by a more thorough study of more systems and also with an improvement of signal/noise ratio.

To conclude the list of possibilities provided by the photoacoustic method we attempted a preliminary survey of the dependence of the relative 'photochemical loss' on the modulation frequency. This was done on two systems: chloroplasts plus methyl viologen and chloroplasts plus potassium ferricyanide. These experiments are still far from being perfect due to the unsatisfactory signal/noise ratios. However they were repeated several times and show consistently higher photochemical losses at higher frequency. We allowed ourselves to draw the curves between the points suggesting transition similar in appearance to what is expected from theoretical considerations [10]. Any conclusions from these curves should be considered very preliminary.

The results reported in this article demonstrate the lines along which we wish to develop the methodology of the photoacoustic approach in photosynthesis research, and the kind of information obtained, which otherwise is not readily available.

From the instrumental point of view we are, at present, able to get reasonable signals at very low incident intensities ($\sim 2\ W/m^2$), mainly due to the use of cells of very small free volume. However, this is perhaps not enough in some cases. Also we are limited presently to frequencies up to ~700 Hz (The total signal decreases below our detection limit at higher frequency). Further expansion of the work calls for the use of a different construction of a cell for easy and automated replacement of the sample and also possibly the use of a piezoelectric detector immersed in the suspension. This would enable the usage of much higher frequencies.

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References

1 Pao, Y.H. (1977) Optoacoustic Spectroscopy and Detection, Academic Press, New York
2 Rosencwaig, A. (1975) Physics Today 28, 23—29
3 Rosencwaig, A. (1978) Adv. Electron. Electron Phys. 46, 207—311
4 Adams, M.J. and Kirkbright, G.F. (1977) Analyst (London) 102, 281—282
5 Lahmann, W. and Ludewig, H.J. (1977) Chem. Phys. Lett. 45, 177—179
6 Adams, M.J., Highfield, J.G. and Kirkbright, G.F. (1977) Anal. Chem. 49, 1850—1852
7 Robin, M.B. (1977) in Optoacoustic Spectroscopy and Detection (Pao, Y.H., ed.), pp. 167—191, Academic Press, New York
8 Hunter, T.F. and Stock, M.G. (1974) J. Chem. Soc. Farady Trans. II, 70, 1022—1039
9 Cahen, D. (1978) Appl. Phys. Lett. 33, 810—811
10 Malkin, S. and Cahen, D. (1979) Photochem. Photobiol. 29, 803—813

11 Cahen, D., Malkin, S. and Lerner, E.I. (1978) FEBS Lett. 91, 339—342
12 Duysens, L.N.M. and Sweers, H.E. (1963) in Studies on Microalgae and Photosynthetic Bacteria, pp. 353—372, Jap. Soc. Plant. Physiol., University of Tokyo, Tokyo
13 Joliot, P., Joliot, A. and Kok, B. (1968) Biochim. Biophys. Acta 153, 635—652
14 Callis, J.B., Parson, W.W. and Gouterman, M.M. (1972) Biochim. Biophys. Acta 267, 348—362
15 Ort, D. and Parson, W.W. (1979) Biophys. J. 25, 355—364
16 Cahen, D., Lerner, E.I. and Auerbach, A. (1978) Rev. Sci. Instrum. 49, 1206—1209
17 Cahen, D. and Garty, H. (1979) Anal. Chem. 51, 1865—1867
18 Avron, M. (1960) Biochim. Biophys. Acta 40, 257—272
19 Farkas, D.L. and Malkin, S. (1979) Plant Physiol. 64, 942—947
20 Emerson, R. and Lewis, C.M. (1943) Am. J. Bot. 30, 165—178
21 Malkin, S. (1968) Biochim. Biophys. Acta 153, 188—196
22 Malkin, S. and Kok, B. (1966) Biochim. Biophys. Acta 126, 433—442
23 Hoch, G. and Martin, I. (1963) Arch. Biochem. Biophys. 102, 430—438