Plant Physiol. (1990) 94, 278–283
0032-0889/90/94/0278/06/$01.00/0

Received for publication February 22, 1990
Accepted May 29, 1990

Determination of Oxygen Emission and Uptake in Leaves by Pulsed, Time Resolved Photoacoustics[1]

David C. Mauzerall

The Rockefeller University, New York, New York 10021

ABSTRACT

Pulsed, time resolved photoacoustics has sufficient sensitivity to determine oxygen emission and uptake by single turnover flashes to leaves. The advantage over previous methodologies is that when combined with single turnover flashes the kinetics of the thermal and the gas signals can be resolved to 0.1 millisecond and separated. The S-state oscillations of oxygen formation are readily observed. The gas signal from common spongy leaves such as spinach (*Spinacia* sp.), Japanese andromeda (*Pieris japonica*), mock orange (*Philadelphus coronarius*) and viburnum (*Viburnum tomentosum*), after correction for instrumental rise time, show a lag of only 1 millisecond and a rise time of 5 milliseconds in the formation of oxygen. Thus a recent proposal that the formation of oxygen requires over 100 milliseconds cannot be true for choroplasts *in vivo*. The rapid emission is correlated with structure of the leaf. At low light flash energies a rapid gas uptake is observed. The uptake has slightly slower kinetics than oxygen evolution, and its magnitude increases with damage to the leaf. The pulse methodology shows that the uptake begins with the very first flash after dark adaption, and allows the detection of a positive signal (oxygen) on the third flash. These observations, the long wavelength of excitation (695 nanometers) and the magnitude of the signal support the contention that the gas uptake is oxygen reduction by electrons from photosystem I. These results show that important physiological aspects of a leaf can be studied by pulsed, time resolved photoacoustics.

The photoacoustic effect is caused by the thermal expansion of a fluid when the energy of absorbed photons is degraded to heat (17). The expansion is detected by a microphone in the gas phase or a hydrophone in the liquid phase. The 50-fold larger thermal expansion of a gas over a liquid makes the former measurement proportionately more sensitive, but limits its time resolution to that of the microphone and cell, typically about 30 μs. Only that part of the absorbed photon energy that is degraded to heat in the measured time period is detected by this methodology. Photoacoustics offers the possibility of obtaining the reaction enthalpy, *i.e.* thermodynamic data on systems as complex as an intact leaf (12, 16). The discovery that oxygen can also be directly measured as a gas pressure change distinct from the usual photothermal effect (4, 23) has opened further possibilities. However, the inherent complexity of photosynthetic systems coupled with the limited data obtained in the usual photoacoustic methodology using modulated light has made progress slow. A complete analysis would require a dense set of both amplitude and phase measurements over a range of at least three orders of magnitude in frequency. Since the measurements are made one frequency at a time, it is not surprising that such data assets are rare (23). Further, the analysis of such a data set with multiple relaxation times having both positive and negative amplitudes is itself very difficult and may lead to ambiguous results. By contrast, the analysis of the photoacoustic response to a single short pulse of light is relatively straightforward. The signal to noise ratio is typically sufficient to obtain the complete kinetic trace of the signal in a single measurement over the accessible time range of 30 μs to 100 ms. In addition to this enormous advantage of obtaining all of the accessible time data (*i.e.* reciprocal frequency) in one measurement, the use of classical single turnover flashes (so short the system can only turnover once [8]) provides the further advantage of identifying and distinguishing processes related to oxygen formation. The slower oxygen pressure signal is easily resolved from the faster photothermal signal because molecular diffusion is typically 100-fold slower than thermal diffusion in a liquid. The oxygen yield oscillations following single turnover flashes are readily measured (6). In this paper it will be shown that a millisecond lag and the 5 ms rise time of the escape of oxygen from chloroplasts, following single turnover flashes to leaves, can be determined. An almost equally rapid oxygen uptake can also be observed at low flash energies and is assigned to PSI.

MATERIALS AND METHODS

Leaves

Healthy leaves of Japanese andromeda (*Pieris japonica*), mock orange (*Philadelphus coronarius*), viburnum (*Viburnum tomentosum*), and ilex (*Ilex crenata*) were picked from plants on the Rockefeller campus and used within a few minutes. Fresh spinach (*Spinacia* sp.) was obtained from local markets. Leaf discs were 1.3 cm in diameter. At least six samples of each kind were used on four different occasions. The leaves contained between 10 and 40 μg/cm^2 total Chl.

Photoacoustic Measurement

A simplified, two-piece photoacoustic cell was built based on the design of Bults *et al.* (4). Two pieces of Plexiglas (2 inches × 1½ inches × ½ inch) were held together with knurled

[1] This work was supported by the National Science Foundation Grant DMB87-18078, and the National Institute of Health Grant GM25693.

screws and sealed by an O ring of ⅞ inch diameter held in a groove. One piece had a ⅝ inch chamber, 0.8 mm thick. A small hole was drilled to the microphone (Knowles No. 1785) which was surrounded by silastic cement in a larger hole. The microphone amplifier was directly driven by a 1.3 V mercury battery. Under these conditions the output is the derivative of pressure with time, not the pressure itself. This is an example of the usefulness of the pulse methodology. To reach such a conclusion, quite obvious in the pulse response, requires analysis of the absolute phase of a modulated light signal. The above claim was proven in several ways. By changing the pressure in the cell with a connection to a syringe and observing the output, by theoretical fit of the response, and by observing the pressure pulse in a similar cell with a pressure transducer (Piezotronic 103A). The output of the microphone was AC coupled through a high impedance amplifier, (Ithaco 1201) with suitable low (1–10 kHz) and high (0.1 Hz) pass filtering. The gain was usually 20- to 100-fold. The output was digitized by a fast, 16 bit voltmeter (Keithley 194A). The data were digitally transferred to a computer (Hewlett Packard 320) for averaging, display, storage, and analysis. All data collection, transfer, and analysis programs were written by the author.

The light source was a flash lamp-pumped dye laser (Candela LFDL-2). The present experiments were done with the dye Oxazine 720 in methanol, having output at 695 nm. This wavelength corresponds to a photon energy ideally close to the trapping energy level. Thus, no excess heat is lost immediately following absorption. Moreover, the sixfold excess absorption by PSI over PSII at this wavelength (N Greenbaum, D Mauzerall, unpublished data) ensures that PSII is fully active. The laser output, of 1 μs width and 2 mJ energy, was attenuated in decadic steps by stable grey filters of aqueous transition metal salts (15) and continuously by a refraction attenuator (Newport 935-5). The pulse frequency was 3 Hz. The light pulse was led via bifurcated fiber optics to just above the photoacoustic cell. The second light input was a heat-filtered tungsten lamp used to saturate the photosynthetic system. The leaf or disc was placed in the thin cell chamber and the cell sealed by strong tightening of the screws. This isolated the sample from the room noises. The cell was isolated from vibrations by a floating table (Newport TXM-3) and by viscoelastic supports. Nothing touched the cell aside from the soft wires to the battery supply and thence to the amplifier.

RESULTS

Analysis of the Microphone Signal

Typical responses of the microphone to dark adapted leaf discs illuminated by the short flashes are shown in Figure 1. The biphasic nature of the signal is a consequence of the microphone responding to the time derivative of the pressure change in the cell (6). Filtering to 1 kHz in the amplifier averages the impulse resonance response (see below). The response to the first flash is purely the photothermal-acoustic effect since no oxygen is formed. The amplitude of the signal is proportional to that fraction of the absorbed photons which

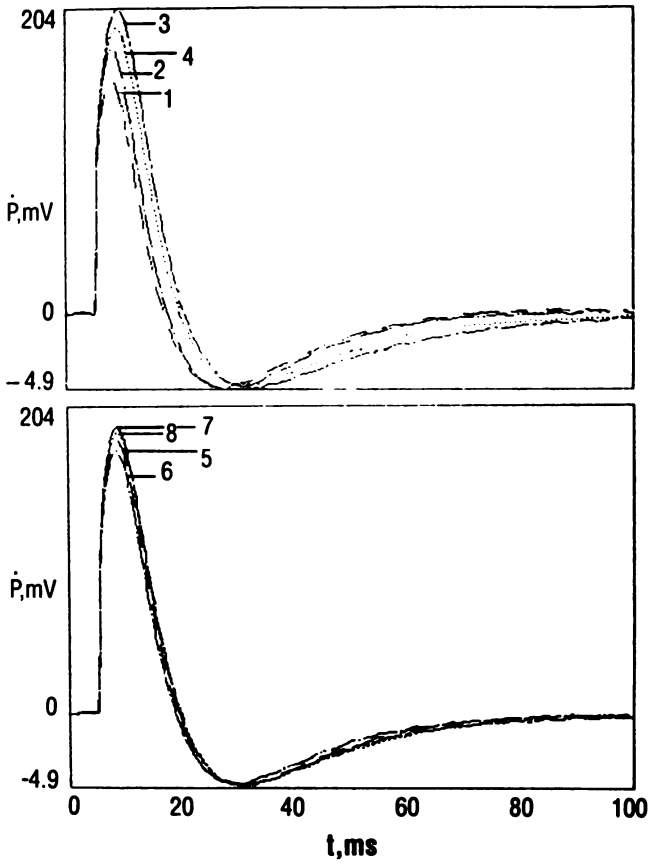

Figure 1. Rate of pressure change *versus* time following a series of eight saturating flashes (numbered) spaced 0.3 s apart to a dark adapted leaf of Japanese Andromeda (*Pieris japonica*). Similar data were obtained with other leaves: spinach (*Spinacia* sp.), Viburnum (*Viburnum tomentosum*), Ilex (*Ilex crenata*), and mock orange (*Philadelphus coronarius*). The signals could be repeated many times following 5 min dark adaption.

is converted to heat previous to this time. It is inversely proportional to the heat capacity of the sample. The rise time of the pressure pulse is determined by the distribution of heat in the sample, by its thermal conductivity, by the cell design, and in this case largely by the microphone. The fall time is determined by the thermal and pressure losses in the cell. In this case it is largely the loss in the microphone which has a cutoff at 10 Hz.

The sound waves of the traditional photoacoustic signal are produced when the energy input is much faster than the thermal condition time. The latter is determined by the thermal conductivity and the spacial distribution of the energy input, *e.g.* the optical depth, of the sample. Since the sound waves are largely determined by surface effects in the sample and by resonances in the cell and in the microphone, they contain less useful information for the present measurements and are best averaged out with a low pass filter. The useful information is in the observed pressure changes, both thermal and oxygen, and the term photobarics is actually more appropriate than photoacoustics. Unfortunately, this term has already been used to distinguish the gas signal from the thermal

signal (23). I believe it should be used to distinguish the resonant (acoustic) signal from the thermal/gas pressure (baric) changes.

Oscillations of Flash Yield of Oxygen

A series of saturating single turnover flashes to a dark adapted leaf produce a similar response on the first two flashes, a large increase on the third, and cyclically varying increases on successive flashes. Typical data are shown in Figure 1. This extra signal has been proven to be oxygen by several criteria (6). These include saturation by increasing flash energy, the lag of two flashes, and damped cycle of four following dark adaption characteristic of the S state cycle of oxygen evolution, the loss of oscillations by S state mixing at low (3% of saturation) continuous light, the total loss of the slow signal with saturating continuous light, and the inhibition of the signal increase by imbibing DCMU, by heat and by infiltration of the leaf with water. The latter proves the extra signal is a gas phase signal because the diffusion time to the gas phase is strongly slowed by filling the leaf's internal gas space with water. Many of these criteria were repeated on the present leaves with similar results, so the data are not presented.

The pressure changes caused by the photothermal effect and by oxygen emission arise in two different ways. For this constant volume system, the pressure increases because of an increase of temperature in the former case, and because of an increase in number of moles of gas in the latter. Thus, the two effects can be separated by simple subtraction of the pressure-time curves. Were one effect to cause the other, they would have to be separated by deconvolution. It is the use of single turnover flashes (<1 μs) to dark adapted leaves that allows this simple subtractive procedure to produce useful results. Subtracting the pressure change observed on the first flash from that observed on succeeding flashes produces the curves shown in Figure 2. Assuming the photothermal effect is constant throughout (see below), these different curves represent the rate of formation of oxygen. The maximum yield on the third flash and the oscillation of period four is apparent. By converting this digital signal to analog form and driving a speaker, one can literally hear the sound of oxygen.

The assumption that the thermal signal is constant can be supported by three arguments. First, the flashes are 'fully saturating,' *i.e.* well over 90% of the photons absorbed must be degraded to heat. Thus, any change in photosynthetic efficiency as a function of flash number must have a small effect on the total heat output. Second, succeeding flashes after the third show close to the same lag time, indicating a constant subtraction is correct. It is important that the amplitude during the lag period does not go negative. This implies that the photosynthetic efficiency does not increase after the first flash. Finally, the good 'isosbestic' points seen at 28.5 ms on all the oxygen curves following the second (Fig. 2) are a proof that the waveform does not change, justifying the subtractive procedure. The response of the second flash does not show this isosbestic. Moreover, its integral often extrapolates to near zero time (Fig. 3), indicating that a part or all of this small signal is thermal, not oxygen. This would indicate that the photosynthetic efficiency may be somewhat less on the second flash. Whether this represents an oscillation of two via the quinone acceptor (28, 29) remains to be determined. If it is so, its amplitude on the fourth flash must be already smaller, since little deviation is seen (Fig. 2).

Lag and Risetime of Oxygen Formation

The data of the thermal response from the first flash and of the oxygen response from the second to fourth flash is integrated to produce the pressure change and is replotted in Figure 3, top, on a fourfold expanded time scale. Subtraction of the response to the first flash from that to succeeding flashes gives the detailed time course of the emission of oxygen (Fig. 3, bottom). The time course of the thermal pressure change (curve 1) is also replotted on the appropriate scale. The advantage of the pulsed, time resolved photoacoustic methodology is now apparent: The complete kinetics of oxygen emission are obtained from a single sequence of μs flashes. The time and effort required to obtain similar data (30 μs time resolution, S/N > 100) by the modulated light method is incalcuable. A 1 ms lag is seen in the oxygen evolution curve on extrapolating the slope at the inflection point to zero pressure change. This lag is general for this kind of leaf: a range of 0.5 to 2 ms is observed in leaves from spinach

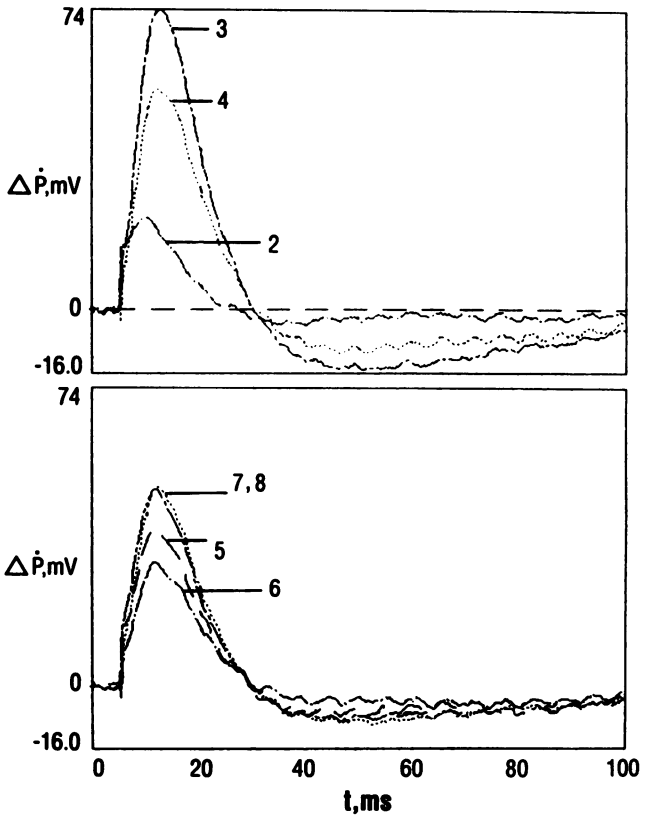

Figure 2. Rate of oxygen emission *versus* time from the Andromeda leaf obtained by subtracting the response of the first flash from that of succeeding flashes shown in Figure 1. The response to the second flash is larger than average.

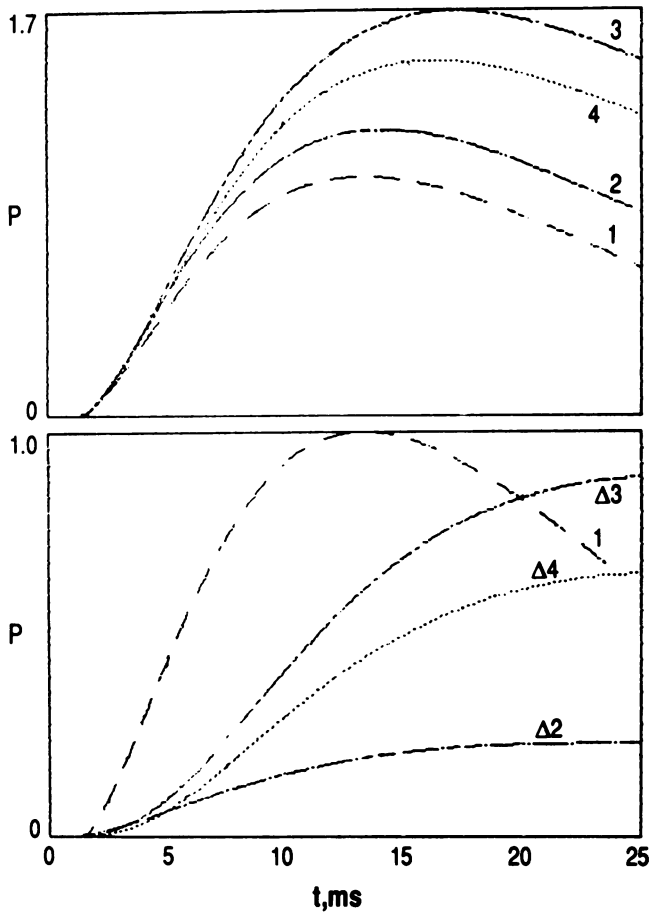

Figure 3. Top, Pressure response *versus* a fourfold expanded time scale to the first four flashes obtained by integration of data similar to that of Figure 1. Bottom, Pressure response *versus* time on expanded amplitude and time scales for the first flash (1, photothermal effect) and for the difference between the succeeding three flashes and the first (Δ3 and Δ4, oxygen effect). The Δ2 response may be a mixture of thermal and oxygen effects.

(*Spinacia* sp.), Japanese andromeda (*Pieris japonica*), mock orange (*Philadelphus coronarius*) and viburnum (*Viburnum tomentosum*).

The total rise time to half amplitude, including the lag of the oxygen signal, is about 7 to 9 ms for the various leaves studied here. The half-rise time of the thermal signal is about 4 to 6 ms for the various leaves. These times are slowed by the inherent response time of the microphone-cell combination (see conclusion). Both responses are corrected for the decay times in the microphone. The faster decay time of the thermal signal (about 20 ms) than of the oxygen signal (about 40 ms) is clearly seen (Fig. 3). This is because the thermal signal decays by both thermal and pressure losses but the oxygen signal only via the latter.

The sensitivity of the photoacoustic cell is worthy of comment. A leaf disc containing 30 μg of Chl will emit, given an Emerson-Arnold number of 2000, 15 pmol of O_2 when illuminated with a saturating, single turnover flash. As can be seen in Figure 3, on integration, the signal to noise ratio may approach 100. Thus, the noise level is about 200 femtomoles of O_2. This corresponds to a relative change in pressure of 2×10^{-8}. These numbers also agree with the absolute sensitivity calibration of the cell. Thus, most if not all of the emitted O_2 is measured in the cell.

Oxygen Uptake

At low flash energies, well into the linear region of the light saturation response, a rapid negative signal is often seen (Fig. 4, top). This cannot be carbon dioxide uptake for several reasons. First, carbon dioxide is reduced independently of photochemistry requiring only NADPH and ATP, both present in (potentially) large pools. Second, if carbon dioxide uptake and oxygen evolution were correlated and equally rapid, no gas phase signal would be seen. Third, the negative signal is too large, amounting in the extreme cases to about three times that of oxygen formation at this pulse energy. Therefore, the signal is caused by oxygen uptake. The reaction is very rapid and is caused purely by excitation of Chl (695 nm). The time scale of the uptake is only 50% slower (13 ms) than that of oxygen evolution (9 ms). The negative signal increases with the time the leaf is left in the cell and with damage to the leaf. It is minimal in Ilex (*Ilex crenata*) and

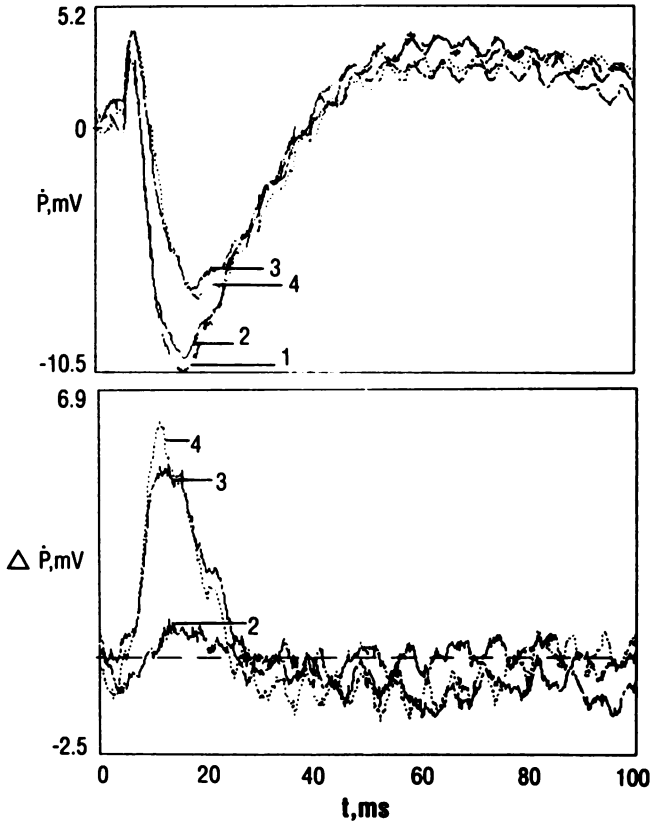

Figure 4. Top, Rate of pressure change *versus* time following a series of four weak flashes (about 40 μJ) spaced 0.3 s apart to a dark adapted leaf of mock orange (*Philadelphus coronarius*). This sample showed nearly the maximum observed negative signal. Bottom, Rate of oxygen emission *versus* time (curves 3, 4) obtained by subtracting the response of the first flash from that of succeeding flashes shown in the top half of this figure.

fresh spinach (*Spinacia* sp.), and maximal in Mock Orange (*Philadelphus coronarius*). This signal does not interfere with oxygen evolution since subtraction of the first flash from succeeding flashes shows the expected positive oxygen signal (Fig. 4, bottom) on the third and following flashes. Note that the tails of these signals alternate in sign with the early signals confirming that the response is inherently biphasic, *i.e.* caused by the transducer, not by the leaf. The oxygen yield on succeeding flashes monotonically rises to a constant level without oscillation since the flash energies are far below saturation. The fact that only oxygen evolution is observed at high flash energies means that the uptake saturates at a lower amplitude than that of oxygen emission. The maximum uptake signal observed was about one-quarter of the maximal oxygen emission signal. Taken together, this evidence supports the conclusion that the uptake is caused by reducing equivalents from PSI. The same conclusion as to source of the negative signal was reached by Havaux *et al.* (11) who studied heat shocked tobacco leaves with modulated light photoacoustics. They could not resolve the kinetics or the simultaneous formation of oxygen.

CONCLUSION

Under appropriate conditions the signal to noise ratio of the response of a leaf sample in the photoacoustic cell to a single turnover flash of light is large enough to determine the oxygen evolution to less than 3% error. This is sufficient to not only determine the S-state oscillations of the oxygen yield, but to determine their kinetics. It is found that there is only a 1 ms lag between the flash and the detection of the oxygen pressure change for 'spongy' leaves. This rather short time compares favorably with that observed with algae or chloroplasts directly on the platinum polarograph (1, 10, 13, 26). The reasons for this are worthy of analysis.

Diffusion of oxygen from its source in the thylakoid membranes of the chloroplasts to the detector must occur in both cases: either to the platinum surface or to the gas space in the leaf. This diffusion of oxygen implies an inherent 'lag' time between formation and detection which is determined by a diffusion constant (D) and a distance (x) squared:

$$t = x^2/2D \quad (1)$$

For simplicity we assume a one-dimensional model: a layer of chloroplasts, at a distance x from the gas space. If we assume the diffusion constant is the same as in water we can refer to the lag time in terms of an equivalent distance. Since this equivalent distance depends only on D, it is a useful parameter. For a lag of 1 ms this distance is calculated to be about 6 microns in the leaves and about 2 microns for chloroplasts in the polarograph (13). The former is consistent with the known morphology of the leaves (9). The reason for this small diffusion distance in leaves is that photosynthesis in higher plants depends on a trace contaminant of the atmosphere: carbon dioxide. For a leaf to grow rapidly, the gas exchange must be very rapid, and this is seen to be so by the measurement of oxygen: it can be used as a tracer for the diffusion of carbon dioxide. Thus, in addition to maximizing surface area for absorption of light in chloroplasts and to feeding (and cooling) the leaf by transpiration, the structure of a leaf also maximizes the rate of gas exchange.

A realistic complication is that not all the thylakoids are at the distance x from the gas space: a distribution of distances is required in the model. These distances will contribute to the lag and rise time of the oxygen signal. With the photoacoustic measurement one has a separate measure of this distribution via the photothermal effect. Thermal diffusion in water, which is essentially what the sample contains, is some 50-fold faster than molecular diffusion of small species such as oxygen. This is why the oxygen response lags the thermal response in Figure 3, bottom. Although the source distribution (chloroplasts) is the same for both effects, the diffusive path of the heat includes long paths (the body of the leaf) not seen in the oxygen signal since these paths require a time much longer than the loss time of the microphone. Moreover, these paths contain sinks for the oxygen, *e.g.* mitochondria. Attempts to fit both curves to different diffusion path lengths provided difficult. It appears that the measured thermal rise times are limited by the microphone response time to a pressure change in the cell. The measured pressure changes may be limited by flow of gas, not by the speed of sound. In that case the true oxygen rise time will be roughly the difference between the observed oxygen rise time, 9 ms, and the thermal rise time, 4 ms, *i.e.* about 5 ms. This explanation is verified by measuring the oxygen with a fast pressure transducer (our unpublished observations). The 5 ms rise time and 1 ms lag correspond to a distribution of equivalent distances between chloroplasts and gas space in the leaf of roughly 6 to 12 microns, consistent with observation (9). We are now attempting to fit such a distribution with one-dimensional diffusion equations (7, 27) and convolutions with the instrument response.

These measurements and their models are a good way to characterize the structures of a leaf. A loose, spongy leaf will have a short lag and rise time of O_2 evolution, while a thick, inviscid leaf will have a long lag and slow rise time. An indication of this effect is seen in the observed lag of about 5 ms in a wheat leaf (6) which has a more closed structure than that of tobacco (9).

Throughout this discussion we have assumed the stomata of the leaf are open. Using average dimensions (10 μ) and aerial density of stomata (10^4 cm^{-2}, see ref. 19) I estimate that the pressure equilibration time between inner leaf gas space and the atmosphere would be only 0.5 ms. What if the stomata are closed? At first thought no oxygen pressure changes will be observed, although the thermal photoacoustic effect will be hardly affected. However, there is a possibility of a 'drum' effect since the leaf structure may be somewhat elastic. Coating a leaf with grease only slowed the oxygen evolution by a small factor (4; Y Cha, D Mauzerall, unpublished results) suggesting the drum effect is large, *i.e.* oxygen formation can be 'heard' in a closed leaf. Further studies are required but the photoacoustic method shows great promise for the study of structure and physiology of leaves.

The claim that oxygen formation in isolated chloroplasts requires a hundred or more of milliseconds (22) is clearly wrong for chloroplasts in the intact leaf. Even if we assigned the observed rise time to half maximum of the oxygen signal,

5 ms, to its formation, totally neglecting diffusion distances and their inevitable distributions in real tissue, that time is much less than 100 ms. This oxygen rise time is not shortened by the loss time of the 10 Hz microphone since this loss is allowed for in the calculation of the rise time. Moreover, if a pressure transducer is used the oxygen signal has a rise time of about 4 ms and now remains for >1 s (our unpublished observations). Thus, the claim of Plyter *et al.* (22) must rest on a faulty measurement. Lavergne (14) has reached a similar conclusion based on the oxidation time of cytochromes in intact pale green mutants of *Chlorella sorokiniana* following irradiation by saturating, single turnover flashes.

Photodriven transient uptake of oxygen has been observed by modulated light photoacoustics on dark adapted spinach leaves on the time scale of 0.2 to 1 s with a high measuring beam intensity, 70 Wm^{-2} (16). Oxygen uptake at low light levels was observed in heat shocked (48°C) tobacco leaves (11). The authors ascribed the uptake to reduction of oxygen by electrons from PSI.

The general term photorespiration covers several mechanisms of light driven oxygen uptake or inhibition of uptake that are best separated by their time scales. The slowest is on the time scale of minutes and involves the oscillatory inhibition of mitochondrial respiration by chloroplast products (18, 24). 'Ordinary' photorespiration presumably occurs on a similar time scale and involves the oxidation of early carbon fixation products, *e.g.* glycollate (2). Separation of these reactions from the reduction of oxygen by elections from PSI is difficult (25). 'Chlororespiration' (3) is oxygen uptake by chloroplasts. Both photouptake (20) and photoinhibition of chlororespiration (21) have been observed on the time scale of about 0.3 s. The uptake observed in leaves (Fig. 4) is the most rapid (about 10 ms) and presumably represents the fast uptake of oxygen by the immediate reduction products of PSI such as ferrodoxin. Since the reaction of ferrodoxin with oxygen is expected to be very fast, the pulsed photoacoustic methodology may be the best way to study this component of photorespiration.

The above comments have been concerned only with the oxygen signal as observed with the photobaric methodology. The photothermal signal contains direct information on the thermodynamic efficiency of photosynthesis (5, 17). With some minor corrections, the measure of the thermal signal with and without saturating continuous light gives the thermodynamic efficiency, or quantum yield, of photosynthesis. These measurements will be the subject of coming publications.

ACKNOWLEDGMENT

We thank the Knowles Company for the gift of three microphones.

LITERATURE CITED

1. **Arnoson T, Sinclair J** (1976) Studies on the rate-limiting reaction of photosynthetic oxygen evolution in spinach chloroplasts. Biochim Biophys Acta **430:** 517–523
2. **Badger MR** (1985) Photosynthetic oxygen exchange. Annu Rev Plant Physiol **36:** 27–53
3. **Bennoun P** (1982) Evidence for a respiratory chain in the chloroplast. Proc Natl Acad Sci USA **79:** 4352–4356
4. **Bults G, Horwitz BA, Malkin S, Cahen D** (1982) Photoacoustic measurements of photosynthetic activities in whole leaves. Photochemistry and gas exchange. Biochim Biophys Acta **679:** 452–465
5. **Buschmann C** (1987) Induction kinetics of heat emission before and after photoinhibition in cotyledons of Raphanus sativus. Photosynth Res **14:** 229–240
6. **Canaani, Malkin S, Mauzerall D** (1988) Pulsed photoacoustic detection of flash-induced oxygen evolution from intact leaves and its oscillations. Proc Natl Acad Sci USA **85:** 4725–4729
7. **Crank J** (1975) The Mathematics of Diffusion, Chap 8. Clarendon Press, Oxford
8. **Emerson R, Arnold W** (1931) A separation of the reactions in photosynthesis by means of intermittent light. J Gen Physiol **15:** 391–420
9. **Esau K** (1964) Plant Anatomy, Ed 2, Chap 16. J. Wiley & Sons, New York, pp 700, 703
10. **Etienne AL** (1968) Etude de l'etape thermique de l'emission photosynthetique d'oxygene par une methode d'ecoulement. Biochim Biophys Acta **153:** 895–897
11. **Havaux M, Canaani O, Malkin S** (1987) Oxygen uptake by tobacco leaves after heat shock. Plant Cell Environ **10:** 677–683
12. **Jabben M, Schaffner K** (1985) Pulsed-laser induced optoacoustic spectroscopy of intact leaves. Biochim Biophys Acta **809:** 445–451
13. **Joliot P, Hofnung M, Chabaud R** (1966) Oxygen release by algae maintained under sinusoidally modulated light. J Chim Phys **63:** 1423–1442
14. **Lavergne J** (1989) Mitochondrial responses to intracellular pulses of photosynthetic oxygen. Proc Natl Acad Sci USA **86:** 8768–8772
15. **Ley AC** (1984) Effective absorption cross-sections in *Porphyridium cruentum*. Plant Physiol **74:** 451–454
16. **Malkin S** (1987) Fast photoacoustic transients from dark-adapted intact leaves: oxygen evolution and uptake pulses during photosynthetic induction-a phenomenology record. Planta **171:** 65–72
17. **Malkin S, Cahen D** (1979) Photoacoustic spectroscopy and radiant energy conversion: Theory of the effect with special emphasis on photosynthesis. Photochem Photobiol **29:** 803–813
18. **Mauzerall D, Greenbaum N** (1989) The absolute size of a photosynthetic unit. Biochim Biophys Acta **974:** 119–140
19. **Nobel PS** (1974) Introduction to Biophysical Plant Physiology. WH Freeman, San Francisco, pp 307–308
20. **Peltier G, Ravenel J** (1987) Oxygen photoreduction and variable fluorescence during a dark-to-light transition in *Chlorella pyrenoidosa*. Biochim Biophys Acta **894:** 543–551
21. **Peltier G, Ravenel J, Vermeglio A** (1987) Inhibition of a respiratory activity by short saturating flashes in *Chlamydomonas*: evidence for a chlororespiration. Biochim Biophys Acta **893:** 83–90
22. **Plyter JJ, Aalbers SE, Barends J-PF, Vos MH, von Gorkom HJ** (1988) Oxygen release may limit the rate of photosynthetic electron transport; the use of a weakly polarized oxygen cathode. Biochim Biophys Acta **935:** 299–311
23. **Poulet P, Cahen D, Malkin S** (1983) Photoacoustic detection of photosynthetic oxygen evolution from leaves. Quantitative analysis by phase and amplitude measurements. Biochim Biophys Acta **724:** 433–446
24. **Reid A** (1968) Interactions between photosynthesis and respiration in *Chlorella* I. Types of transients of oxygen exchange after short light exposures. Biochim Biophys Acta **153:** 653–663
25. **Robinson JM** (1988) Does O_2 photoreduction occur within chloroplasts in vivo? Physiol Plant **72:** 666–680
26. **Sinclair J, Arnason T** (1974) Studies on a thermal reaction associated with photosynthetic oxygen evolution. Biochim Biophys Acta **368:** 393–400
27. **Tucker JE, Mauzerall D, Tucker EB** (1989) Symplastic transport of carboxyfluorescein in staminal hairs of *Setcreasea purpurea* is diffusive and includes loss to the vacuole. Plant Physiol **90:** 1143–1147
28. **Vermeglio A** (1977) Secondary electron transfer in reaction centers of *Rhodopseudomonas sphaeroides*. Out-of-phase periodicity of two for the formation of ubisemiquinone and fully reduced ubiquinone. Biochim Biophys Acta **459:** 516–524
29. **Wraight CA** (1977) Electron acceptors of photosynthetic bacterial reaction centers. Direct observation of oscillatory behaviour suggesting two closely equivalent ubiquinones. Biochim Biophys Acta **459:** 525–531