On the use of the open photoacoustic cell technique for studying photosynthetic O₂ evolution of undetached leaves: Comparison with Clark-type O₂ electrode

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The leaf photosynthetic O₂ evolution detected by the open photoacoustic cell (OPC) technique is compared to the measurements using a Clark-type O₂ electrode detector. *In vivo* and *in situ* measurements were carried out with undetached maize leaves. From the correlation between these two methods the possibility of a calibration is proposed for the oxygen evolution OPC signal.

I. INTRODUCTION

Oxygen evolution measurements in photosynthetic systems have been made over several decades. In Ref. 1 there are described several methods for detection of oxygen in photosynthesis through the end of the sixties. After that, the improvements of the Clark-type O₂ electrode method,^{2,3} resulted in important contributions to the oxygen detection in photosynthesis studies until the present time.⁴ With the advent of the photoacoustic (PA) and related photothermal techniques in the early seventies (see Refs. 5 and 6, for a review on the subject), a new PA method for evaluating the O₂ evolution from leaves has been proposed.⁷ Even though both the O₂ electrode and this early PA method have proved to be very useful in the study of oxygen evolution, they lack, however, the capability of performing *in vivo* and *in situ* measurements. As originally proposed, the measurements with both techniques are carried out on isolated chloroplasts or pieces of detached leaves.

In contrast to these methods, the recently proposed open photoacoustic cell (OPC) technique⁸ has proven^{9,10} to be able to conduct *in vivo* measurements on undetached leaves. In this article we present a quantitative analysis of the photosynthetic O₂ evolution of undetached leaves using the OPC detection technique. A comparison with the conventional Clark-type O₂ electrode method is made and a correlation between the two techniques is established.

II. EXPERIMENT

A. Experimental setup

The OPC experimental setup is schematically shown in Fig. 1. It consists basically of a modulated monochromatic

excitation light beam and a strong dc white light for saturating photosynthesis.⁷ The photosynthesis excitation beam is generated by a 1000 W Xenon lamp which after being mechanically modulated by a light chopper (PAR, model 191) is focused onto a monochromator centered at 680 nm. The dc, nonmodulated saturating light source is obtained from a direct current operated 250 W halogen lamp which is heat filtered by a 6 cm thick water filter. Both the modulated and the saturating light beams are combined in a bifurcated fiber-optic light guide and projected onto the leaf. The intensity calibrations were carried out with a calibrated silicon photodiode.

The OPC detection unit consists of mounting the leaf sample directly onto a cylindrical electret microphone, as schematically shown in Fig. 2(a). The exploded view of the OPC setup for the *in vivo* and *in situ* measurements is shown in Fig. 2(b). The front sound inlet of the electret microphone [see Fig. 2(a)] is a 3 mm diameter hole, and the front air chamber adjacent to the metallized face of the electret diaphragm has a diameter of 7 mm and is roughly 1 mm long. The PA signal in the OPC configuration is essentially due to the periodic heating of the sample by the absorption of modulated light. The resulting periodic heat flow from the sample to the air in the OPC chamber produces pressure fluctuations at the chopping frequency which, in turn, causes diaphragm deflections. The diaphragm deflection generates a voltage across the resistor R which is sensed by a lock-in amplifier (PAR, model 5210). The lock-in amplifier is controlled by a microcomputer which reads the resulting OPC signal amplitude and phase every 3 s at the same time that graphical records are produced by a plotter (HP-7090) connected to the microcomputer.

For the Clark-type O₂ electrode measurements, the experimental setup consists basically of the same optical illumination system as in the OPC configuration. The O₂ detection unit is schematically shown in Fig. 3. It consists of an

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FIG. 1. Block diagram of the experimental arrangement.

acrylic cylindrical body in which the oxygen detector is inserted, leaving a 0.58 cm^3 volume air chamber between the electrode and the outer face of the acrylic body. At the top of the cylindrical cell there is a 3 mm diameter hole on top of which the leaf is placed similarly to the case of OPC mea-

FIG. 3. Schematic design of the O_2 sensor.

FIG. 2. (a) Cross section of the OPC apparatus. (b) Exploded view of the OPC operation for *in vivo* and *in situ* measurements.

surements. Here, we note that care has been taken to make sure that the leaf sensing area was the same in both experiments. Two thin ducts were connected to this chamber so that different gas mixtures saturated with water vapor could be blown into the chamber. The electrode used was a Clark-type Pt/Ag/AgCl₂ electrode manufactured by Radelkis (OP934-5). The filling solution was Radelkis-type "O-107" solution and the active membrane was an O_2 permeable $12 \mu\text{m}$ thick polypropylene membrane (Radelkis). The measurements were carried out with the adapter Radelkis-type OP-960 for the polarization of the working electrode of the sensor (polarizing voltage: -0.65 V) and for the conversion of the cell current to a voltage signal. The latter was measured with digital pH meter Radelkis-type OP-271. (The 8.3 mV voltage corresponds to $1 \times 10^{-9} \text{ A}$ current intensity).

Under illumination, photosynthesis is excited and O_2 is evolved from the leaf to the gas chamber. The oxygen diffuses through the polypropylene membrane and is reduced at the platinum electrode. The oxygen partial pressure is determined by a pH meter connected to the electrode.

The oxygen partial pressure, P_{O_2} , of a gas mixture at $^{\circ}\text{C}$ temperature, is obtained from the equation¹¹

$$P_{\text{O}_2} = \{(B - A)[\text{O}_2]\} / 100, \quad (1)$$

where B is the atmospheric pressure in mm Hg, A is the water vapor pressure in mm Hg, and $[\text{O}_2]$ is the oxygen content in vol. %.

B. Plant material

Approximately 20-day-old maize seedlings, grown in pots, were used for *in vivo* and *in situ* measurements. The undetached leaf was held flush against both the OPC and

FIG. 4. Calibration of the O_2 sensor vs O_2 partial pressure.

oxygen detector with the help of vacuum grease. In all experiments, the leaf upper epidermis was facing the light.

III. RESULTS AND DISCUSSION

The first measurements we have carried out were the oxygen sensor calibration. This was done by flushing air through the O_2 detector chamber with known oxygen concentrations. The correlation between the measured pH voltage and the O_2 partial pressure in the gas mixtures used is summarized in Fig. 4. Denoting by pH the pH meter output voltage, in MV, and P_{O_2} , the oxygen partial pressure, in mm Hg, the data best fit yielded

$$P_{O_2} = -1.884pH + 4025.7. \quad (2)$$

Having calibrated the oxygen sensor we have then carried out the first experiments of O_2 evolution in undetached leaves. Two points got our attention. First that the amount of oxygen evolved is quite low and, secondly, that the process is relatively slow. To overcome these difficulties we have adopted the same procedure as the one used by previous authors,^{7,12} namely, we have made some small holes in the sensing area of the undetached leaves, using a surgical needle. The result was a quicker and stronger oxygen diffusion from the leaf to the electrode chamber. Figure 5 shows the typical time dependence of the oxygen evolution as given by the pH meter voltage. The solid line in this figure represents the data linear regression, namely,

$$pH(mV) = -0.065t + 2059.4. \quad (3)$$

Combining Eqs. (2) and (3), and the O_2 partial pressure, in mm Hg, dependence with time can be written as

$$P_{O_2} = 0.122t + 145.8. \quad (4)$$

Equations (4) and (1) yielded the following results for the percent O_2 concentration rate of change in the electrode chamber

$$\frac{d[O_2]\%}{dt} = 0.122 \frac{100}{(B-A)}. \quad (5)$$

FIG. 5. Typical measurement of oxygen evolution from undetached maize leaf using the O_2 sensor showed in Fig. 3. The intensity of the excitation light 680 nm was 32 W m^{-2} , modulated at 17 Hz.

This percent rate of change of the O_2 concentration can be converted into the absolute O_2 concentration rate of change using the manufacturer's calibration constant, K (Ref. 11)

$$\frac{d[O_2]}{dt} = K \frac{d[O_2]\%}{dt}, \quad (6)$$

where $K = 0.079 \mu\text{g/cm}^3$.

Equation (6) gives us the rate of change of the O_2 evolution per unit volume in the sensor active chamber. Multiplying this by the sensor active volume we can get the total rate of oxygen evolution, which divided by the illuminated leaf area gives us finally the rate of oxygen evolution from the leaf by unit area, R . This is the quantity we shall use to relate with the OPC signal dip.

In Fig. 6 we show a typical plot of O_2 evolution moni-

FIG. 6. Typical OPC detector measurement of the oxygen evolution from an undetached maize leaf. When the background white light is applied the negative effect is obtained. The intensity of the modulated light at 680 nm was 10 W m^{-2} and the continuous background light intensity ($\uparrow$ on, $\downarrow$ off) was 200 W m^{-2} . The modulation frequency was 17 Hz.

FIG. 7. A typical OPC signal transient, observed when the undetached maize leaf is exposed to the excitation light. The intensity of the modulated light 680 nm was 20 W m^{-2} . Other conditions were the same as in Fig. 6, except the leaf.

toring by the OPC signal. The arrow in this figure represents the instant when the continuous white light, with an intensity of 200 W m^{-2} , is switched $\uparrow$ on ($\downarrow$ off). It should be pointed out that care has been taken to make the OPC measurements when the steady state of the O_2 evolution has been reached. A typical transient curve is shown in Fig. 7. The reason for the dip in the PA signal when the continuous light is switched on, which is known in the PA literature as the negative effect,^{7,12} is the following. The PA signal in response to the modulated photosynthetic excitation at 680 nm is the result of two main contributions: One being the usual photothermal component, resulting from the periodic heat flow from the leaf to the OPC chamber, as described earlier. The second one being the so-called photobaric component,¹² resulting from the periodic oxygen evolution accompanying the modulated photosynthesis. The modulated O_2 flow into the OPC chamber, produced during photosynthesis, induces a pressure fluctuation in the OPC chamber. The dip in the OPC signal shown in Figs. 6 and 7 represent the decrease of the OPC signal due to the canceling of the photobaric contribution by the continuous white light illumination. This dc illumination saturates the photosynthesis, so that the applied modulated excitation light is converted totally into heat and fluorescence. As the background light induces a continuous photosynthesis, the modulated excitation light does not affect photosynthetic process and no modulated oxygen is produced. This, therefore, results in a decrease of the OPC amplitude signal (negative effect) and an increase of the photothermal component (positive effect). At low modulation frequency the net effect is negative and, as the modulation frequency is increased the net effect becomes small until it becomes positive.^{7,12} The oxygen evolution measurement is based upon the negative effect amplitude.¹⁰

In Fig. 8 the correlation between the rate of oxygen evolution by unit area, R , as measured by the O_2 sensor and by the negative effect of the OPC signal, ΔS , is presented. The solid line in this figure represents the data linear regression expressed by

FIG. 8. Correlation between the O_2 sensor signal and OPC signal. Three different maize plants were used with three different modulated light intensities at 680 nm. Other conditions were the same as in Fig. 6.

$$R(\mu\text{g m}^{-2} \text{ s}^{-1}) = 0.078\Delta S(\mu\text{V}) + 0.97. \quad (7)$$

We note in Fig. 8 that there is a small deviation from zero in this correlation at the origin. This is attributed to the fact that we were working at the limit of the oxygen sensor. In fact, as mentioned before, in the oxygen evolution measurements using the O_2 sensor, we had to punch small holes on the leaf in order to improve the signal level. In contrast, in the OPC measurements, the leaf sample was left intact since we had a much better signal-to-noise ratio. A second reason for this discrepancy could be the argument that we had not fully saturated the photosynthesis with the continuous white light during the OPC measurements. This argument can be ruled out since it is known that a continuous white light of 200 W m^{-2} is sufficient to saturate photosynthesis. In any event, the above result clearly shows us that there is a correlation between the OPC signal dip and the oxygen evolved from the leaf. Therefore, once calibrated as indicated above, the OPC technique may offer us a unique capability for performing *in vivo* and *in situ* photosynthetic measurements on intact leaves. Furthermore, being a minimum-volume PA technique,⁸⁻¹⁰ the OPC method presented in this article may be readily used for the development of a compact plant efficiency measuring device based upon the monitoring of oxygen evolution.

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