FREQUENCY-DEPENDENT PHOTOACOUSTIC SIGNALS FROM LEAVES AND THEIR RELATION TO PHOTOSYNTHESIS

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1. Introduction

The photoacoustic technique allows us to follow the conversion of absorbed light into heat [1,2]. From measurements of the modulated heat release upon the absorption of intensity-modulated light, the extent of energy storage in photochemically induced intermediates and their decay times can be estimated [3–5], by comparing the signal from a photochemically active sample to that from a photochemically inactive reference. For photosynthetic systems an ideal reference is the sample itself, because, by exposing it to additional DC background illumination of sufficient intensity, its photochemistry is saturated, resulting in maximal modulated heat production and hence in a maximal photoacoustic signal. This procedure of referencing had been applied to isolated broken chloroplasts [4] and whole cells of *Rhodospirillum rubrum* [5]. We report here the application of this method of referencing to intact leaves for the study of in vivo photosynthesis.

Transients were observed in the photoacoustic signal from dark-adapted *Artemisia lactiflora* leaf samples measured with light modulated at 8.3 Hz [6]. They were partly attributed to changes in thermal parameters of the leaf, induced as secondary effects of photochemistry. We show that the direction (i.e., increase or decrease of the signal) of the transients from dark-adapted leaves, as well as the effect of background light application depends on the modulation frequency. At low modulation frequencies (<200 Hz), additional illumination with background light changed the signal in a direction opposite to that expected for an energy storing system (i.e., the signal decreased if photochemistry was saturated). Only at higher modulation frequencies (>200 Hz) the normal 'photochemical loss' [3] was observed, i.e., the signal increased when background light was applied. During the first few minutes of low frequency modulated illumination, the signal increased (as in [6]), whereas at high frequencies a decreasing transient was observed.

We interpret the photochemical loss at high frequencies as a direct expression of the yield of photochemistry [3–5]. The photoacoustic signal at low frequencies apparently contains a considerable contribution from modulated oxygen evolution. The transient behaviour of both signals probably reflects the induction period of photosynthesis.

2. Experimental

Leaf strips of ~2 X 3 cm were cut from mature tobacco plants (*Nicotiana tabacum* L. cv. Xanthi) and placed in a home-built air/microphone photoacoustic cell [7]. When desired, leaf strips were vacuum infiltrated with water or inhibitor solutions.

Mechanically chopped light from a 450 W Xe-lamp was passed through water and interference filters (Schott). A bifurcated randomized light guide (Schott) was used to direct both modulated (680 nm, 14 W/m^2) and saturating DC background light (400–720 nm, 340 W/m^2) onto the sample. The microphone signal was fed into a lock-in amplifier (Ortholoc 9502, Brookdeal) and recorded. For more details see [4].

3. Results and discussion

Fig.1A shows the photoacoustic signal measured at 35 Hz for a dark-adapted leaf. During an induction of ~2 min there is an increase in signal with biphasic kinetics, tending to a steady state level. This transient is qualitatively similar to that reported at 8.3 Hz in [6].

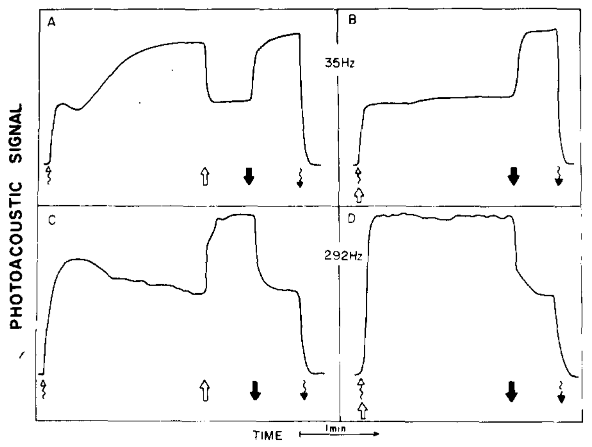

Fig.1. Photoacoustic signals from dark-adapted tobacco leaves. (↑) modulated light on; (↓) modulated light off; (⇧) saturating background light on; (⬇) background light off; (A,B) modulation frequency 35 Hz; (C,D) modulation frequency 292 Hz. Note that the changes due to simultaneous DC background illumination are rapidly reversible (faster than the time resolution of the lock-in amplifier). They can be repeated at least 25 times. For further details, see section 2.

Background light application lowers the signal to its initial level. The photoacoustic signal measured at 292 Hz (fig.1C) shows, on the other hand, a transient decrease during the same period. This behaviour during the induction period is the reverse of that observed at lower frequency. Saturating background light results in a 50% increase in signal, appreciably higher than in isolated broken chloroplasts [4].

Transients are not observed if saturating background light is applied from the start of illumination of dark-adapted leaves (fig.1B,D). This strongly suggests that the transients are somehow associated with photochemistry. However it is extremely unlikely that they involve changes in the thermal parameters as suggested in [6]. Such changes should persist also in the presence of background light, which obviously is not the case. Moreover, one would expect the changes at low and high modulation frequencies to be in the same direction, again in contrast with the experimental results (fig.1A,C).

An alternative suggestion, supported by [8], is that the low frequency signal is composed of two mechanistically independent contributions:

(i) Due to the usual conversion of modulated heat to modulated pressure;

(ii) The other is due to oxygen evolution, which is partly modulated, and hence results directly in pressure modulation.

Using a fast-responding oxygen electrode, modulated oxygen evolution from chloroplasts and algae was detected at 200 Hz, the signal being strongly damped as the frequency increased [9,10]. Oxygen uptake by the photooxidation of rubrene or photocatalytic decomposition of acetic acid have been followed by photoacoustic spectroscopy [11]. Preliminary calculations were made [8] of the possible contribution of modulated oxygen evolution to the signal, using independently determined [7] values for the sensitivity of the microphone/cell combination. They are in agreement with the observed decrease in the low frequency photoacoustic signal upon saturation with background light (fig.1A). According to these calculations, the oxygen evolution component becomes too small to be detected at >200 Hz. We expect this component to be progressively damped with increasing frequency because of the time required for oxygen diffusion from the chloroplasts to the gas phase (see below). Thus at high frequency only the photochemical loss persists, and the transient decrease in this case (fig.1C) reflects the gradual onset of photochemistry, lowering the measured heat release upon light absorption during photosynthetic induction. It is very unlikely that modulated CO_2 uptake significantly contributes to the signal, being strongly damped due to successive intermediary reactions, involving relatively slow rate constants and large pool sizes, which separate between the photoact and CO_2 uptake.

Additional support for the above interpretation comes from the fact that leaves, vacuum infiltrated with DCMU (3-(3, 4-dichlorophenyl)-1, 1-dimethyl urea) solutions (50 μM) do not show any transients or effect of background light at any frequency. Water-infiltrated leaves, however, show a ~15% photochemical loss from 10–300 Hz. The oxygen evolution component in the photoacoustic signal was lost (damped) in this case, as the diffusion path of oxygen increased [from ~1 μm (the average chloroplast to cell wall distance) to an average of ~30 μm (the distance to the leaf surface in the case where water fills the intercellular spaces)]. The thermal component was also damped but not as much. A further indication that the transient reflects photosynthetic oxygen evolution is its similarity to the reported induction pattern of oxygen evolution in leaves [12].

Attempts to separate the signal into gas evolution and thermal components were unsuccessful: coating the leaf on both surfaces with silicon grease attenuated the total signal, but did not significantly change the relative decrease with background light, in agreement with the normal transient from a leaf coated with lanolin [6]. However, since the source of both the thermal and gas evolution components of the signal must be inside the leaf, in the intercellular air space, the combined pressure changes due to heat and/or gas exchange will reach the leaf surface as acoustic waves. These can be transmitted through mechanical barriers, especially in our photoacoustic cell [7].

If oxygen evolution is indeed measured photoacoustically at low frequency, while high frequency experiments show only the conversion to heat (and hence the extent of chemical energy storage), it may be possible to follow easily the energy balance and the rate of photosynthesis both during the induction period and in the steady state. The method may become suitable for field studies, especially if combined with fluorescence detection.

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