TRANSIENT VARIATION OF PHOTOACOUSTIC SIGNAL FROM LEAVES ACCOMPANYING PHOTOSYNTHESIS

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1. Introduction

In photosynthesis, the light energy absorbed by photosynthetic pigments is converted to three different energy forms, chemical energy via photochemical reactions, light energy re-emitted as fluorescence and heat. A number of studies have been devoted to the former two conversions but little to the conversion to heat. Photoacoustic spectroscopy (PAS) has been applied to the studies of fluorescence and thermal properties of various samples [1]. In this method, heat pulses generated by light, modulated at an audio frequency, are specifically measured. One can thus obtain information about how much light energy absorbed in the sample is converted to heat. This method has been applied [2] to the photosynthetic system and DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea]-poisoned chloroplasts were shown to generate more heat than normal chloroplasts when illuminated. We report here a new phenomenon, the transient variation of the photoacoustic signal from intact leaves, which was found in an effort to measure the balance of the above three energy forms in photosynthesis. We show that the phenomenon is closely related to photosynthesis and partly interpret it as due to light-induced changes of heat conductivity within the chloroplasts.

2. Experimental

A small leaf disk (1 cm diam., *Artemisia lactiflora*, Wall) was placed in one of the two photoacoustic cells of a CO-gas meter model ZFD (Fuji Electric Co., Tokyo). The two cells, made to hold solid materials on plastic trays, were connected to each other though a micro flow sensor [3], which is a sensitive and stable detector newly devised for the measurement of gas flow or pressure change in place of a microphone. The leaf disk in the cell was illuminated with monochromatic light (478 nm, 3.5 mW/cm^2) from a Xe lamp through heat-absorbing and interference filters. A mechanical chopper in front of the cells modulated the light beam at 8.3 Hz. The fluorescence from the leaf was led to a photomultiplier through a red blocking filter with a light guide. The photoacoustic and fluorescence signals from a single leaf disk were recorded simultaneously.

3. Results and discussion

Trace A in fig.1 shows a transient variation of the photoacoustic signal found on illumination of a dark-adapted leaf. The variation shows two peaks (P1, P2) before the signal reaches the steady high level (S). The P1 peak usually appeared a few seconds after the start of illumination and the P2 peak after around 20 s, and it took ~2 min to reach the S level. The S level was ~50% higher than the initial P1 level. Trace G shows the fluorescence variation, the Kautsky effect [4], measured for the same sample. It is clear from these two traces that the early rapid variation of photoacoustic signal involving P1 and P2 occurred during the course of the Kautsky effect, whereas the slow increase of photoacoustic signal took place when the decrease of fluorescence had almost finished.

Traces B–F show the effect of a dark interval on

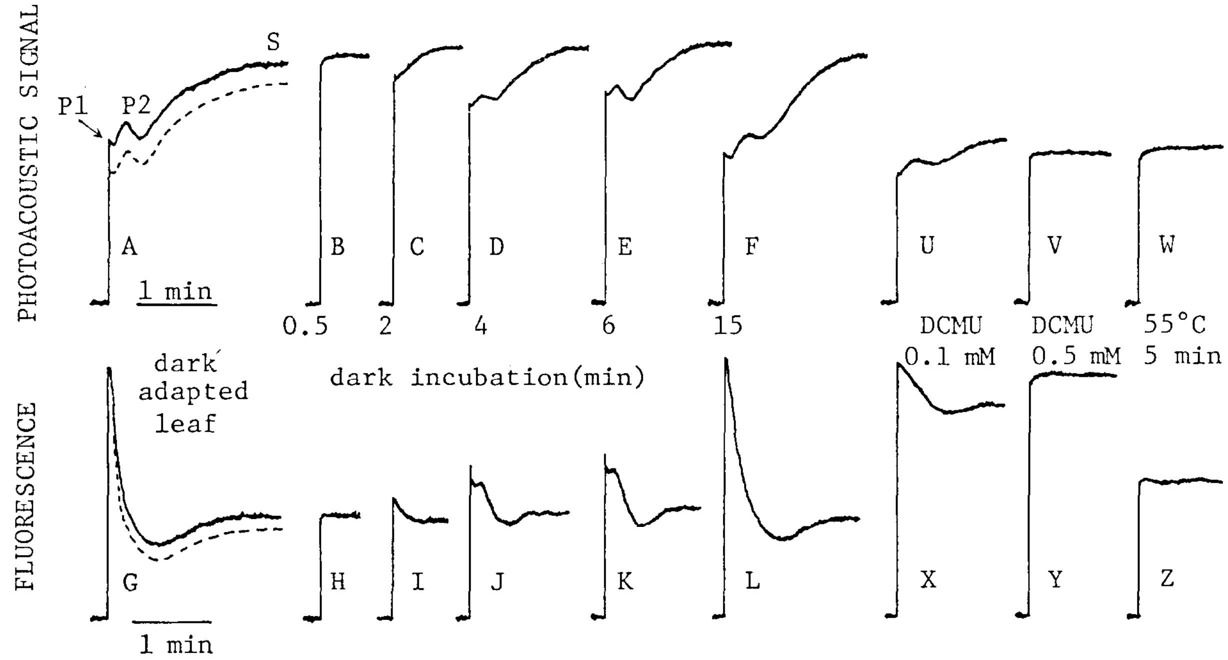

Fig.1. Simultaneous recording of photoacoustic (upper traces) and fluorescence (lower traces) transient variations in intact leaves. Traces A, G are the transient variations measured on the first illumination of a dark-adapted leaf, and traces B–F and traces H–L are those measured again for the same illuminated leaf after dark-incubation of various periods indicated. Broken traces are the variations observed for a leaf covered with lanolin on its surface. Traces U–W and traces X–Z show the effect of photosynthesis-inhibiting treatments. A leaf was kept standing for 24 h in DCMU-containing water in the light to accelerate absorption of the chemical by transpiration, then kept in darkness for 1 h before measurements.

the photoacoustic variation when the same leaf after illumination was kept in darkness for a certain period and then illuminated again for the measurement. Trace B measured after 0.5 min in darkness shows no transient variation. The variation recovered gradually during 15 min dark incubation (traces C–F). Traces H–L show the recovery of the Kautsky effect during dark incubation. The fluorescence variation recovered after 15 min dark incubation, the same period as was required for recovery of the photoacoustic variation.

Traces U–Z show the effect of DCMU or heat treatment, known to specifically inhibit photosynthetic electron transport. Treatment with 0.5 mM DCMU completely suppressed both the photoacoustic and fluorescence variations (traces V, Y); the photoacoustic signal stayed low on the initial P1 level, while the fluorescence remained high on the maximal level. With 0.1 mM DCMU, however, the inhibition was incomplete, and both signals underwent slight but distinct variations (traces U, X). Heating of leaves in water at 55°C for 5 min also suppressed these variations completely (traces W, Z) and both photoacoustic and fluorescence signals were kept at the low levels.

The characteristics of the present phenomenon can be summarized as follows. The photoacoustic transient variation:

(i) Is a phenomenon closely associated with photosynthesis;

(ii) Undergoes light and dark adaptation similar to the Kautsky effect;

(iii) Differs in its transient kinetics from fluorescence variations [4].

In photoacoustic measurements of intact leaves, heat pulses generated in the thylakoid membranes must migrate through the stroma, cytoplasm, cell wall and epidermal cell layer to induce acoustic signals in the surrounding atmosphere. Heat diffusion through these barriers will shift the pulse phase of the acoustic signal, and so the lock-in system measures the heat fraction generated within the thermal diffusion length [5] as in-phase signals, but ignores the heat generated beyond that length as out-of-phase signals. The thermal diffusion length of intact leaves at the modulation frequency of 8.3 Hz can be calculated as 80–120 μm according to the equation in [6], if we assume the thermal constants (thermal conductivity, specific heat and density) of intact leaves to be between those of water and solid sucrose. When the large size of photoacoustic induction (50% increase over the rapid phase of the signal) observed here is considered with respect to this limitation, the slow increase of the signal from P2 to S cannot be due to the increase of the total heat generated, but is rather due to an increase of the in-phase component of the heat signal, which is caused by an increased rate of heat conduction in the leaf tissue. This view is supported by the low level of photoacoustic signal observed for heated or DCMU-poisoned leaf. If the photoacoustic signal would directly represent the total heat generation, as shown [2,7] for isolated chloroplasts or purple membranes having less complicated structure than intact leaves, the signal from the leaves deprived of photosynthetic activity should have appeared higher than that from normal leaves.

The leaf tissue has inner air space open to the atmosphere, so that light-induced opening of stomata might shorten the apparent path for heat. This view is, however, excluded since a leaf covered with waxy lanolin on its surface underwent a beautiful photoacoustic transient variation (broken trace A). It seems also possible that a rise of cytoplasmic temperature might increase the apparent heat conductivity. This view is not likely either because the DCMU-treated leaf did not show any transient variation. Since DCMU is a strong inhibitor, highly specific for photosynthetic electron transport in chloroplasts, the cytoplasm is expected to be preserved in its natural state in the presence of DCMU. Furthermore, the heat conductivity change expected on raising the temperature is not large enough to explain such large induction of the photoacoustic signal.

Judging from the close similarity of the photoacoustic signal to the Kautsky effect both in the response to the inhibition of photosynthesis and in the light and dark adaptation characteristics despite the difference in transient kinetics, the slow induction of the photoacoustic signal found for dark-adapted leaves seems to result from a change, thermal in nature, within the chloroplasts and not in the cytoplasm. Possibly, the large changes of pH and metabolite concentrations in the stroma caused by photosynthetic reactions accelerate the heat conduction from the thylakoids to the cytoplasm, which results in the increase of the photoacoustic signal up to the S level. As to the early rapid variation involving P1 and P2, however, the present data provide no information except the fact that it is related to photosynthesis. Both or either of the changes in heat generation and in heat conduction in chloroplasts are possible causes, which are to be clarified in future studies.

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