Photosynthetic chromatic transitions and Emerson enhancement effects in intact leaves studied by photoacoustics

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1. INTRODUCTION

The decrease of the quantum yield of photosynthesis in long wavelength light ($\lambda > 690$ nm; 'light 1'), and its enhancement by addition of short wavelength light, and vice versa (Emerson enhancement [1,2]), were among the most decisive experimental results that led to the concept of the two photosystems in photosynthesis. These phenomena are due to an initial, uneven light distribution between the two photosystems, and can be used to estimate such excitation imbalance quantitatively. It was suggested [3–5] that there exists a mechanism to reduce the initial imbalance of light distribution by allowing excitation energy transfer from photosystem (PS) II to PS I. This mechanism, known as 'spill-over' [3], operates under short wavelength illumination ('light 2', $\lambda < 690$ nm), when initially there is excess excitation of PS II. The extent to which this 'spill-over' mechanism is operating depends on illumination conditions, with slow adaptations occurring to two physiological states; viz. state 1 with no or minimal energy transfer, obtained after adaptation to long wavelength illumination and state 2 with much more effective energy transfer obtained during exposure to short wavelength light. State 2 is characterized by low fluorescence and low Emerson enhancement relative to state 1 [5,6]. The biochemical basis of such transitions between the two states was partly elucidated [7–11].

Abbreviations: PS, photosystem; PA, photoacoustics

Most of the previous experiments were performed with suspensions of algae due to inherent difficulties of measuring leaf photosynthesis rapidly enough and in a non-destructive way. State 1-to-2 transitions in leaves were mainly inferred from fluorescence measurements [9]. Here, we demonstrate Emerson enhancement and state 1-to-2 transitions in intact leaves using a novel technique, photoacoustics (PA), for measuring photosynthetic activities [12–14]. By this method, we also identified new physiological states and transitions between them. One of these states is characterized by an imbalance between the photosystems due to inefficient energy transfer between the antenna pigments of PS I and their reaction centers.

2. MATERIALS AND METHODS

The apparatus was a combined PA and fluorimeter set-up. A circular leaf disc was placed in a hermetically closed cavity which communicated to a microphone. A triple-branched light guide delivered both modulated light and (non-modulated) saturating light to the leaf and directed part of the emitted fluorescence through a 730 nm interference filter to a photovoltaic detector. The modulated PA and fluorescence signals were processed separately by two lock-in amplifiers and displayed on a recorder. For more details see [14]. Tobacco leaves (*Nicotiana tobacum* ev. Xanthi) were used. Dark adaptation was 8 min. Light 2 was provided by either a broad band (400–600 nm), or a 640 nm interference filter.

Light 1 was provided by 700 or 710 nm interference filters. The PA signal at low chopping frequencies (e.g., ~20 Hz) has a major contribution from modulated photosynthetic oxygen evolution [14]. The relative quantum yield of O_2 can be obtained from the change (decrease) in the PA signal upon addition of non-modulated background light of saturating intensity [14]. This contribution is damped out at higher (200 Hz) chopping frequencies and then the relative change (increase) in PA signal upon addition of the background light reflects directly the photochemical energy storage (appropriate to the particular chopping frequency, cf. [12]) and is related to the average photochemical efficiency of both PS I and II.

3. RESULTS AND DISCUSSION

In general a leaf exhibits a very complex pattern of transients depending on its previous illumination regime. We searched for effects related to the light distribution and interactions between the two photosystems and therefore did basically two kinds of experiments:

(1) Irradiation with modulated light-2 (short wavelength), looking at the influence of additional non-modulated light-1 ($\geqslant 700$ nm).

(2) Irradiation with modulated light-1 and addition of non-modulated light-2.

Sometimes non-modulated white light of saturating intensities was introduced, instead of the monochromatic continuous light, in order to obtain a PA signal from a reference state in which the photosynthetic efficiency approaches zero (cf. [13,14]).

Illumination of a dark-adapted leaf with 640 nm light, modulated at low frequency (22 Hz) and with an intensity in the limiting light range of photosynthesis (30 W/m²) yielded a relatively small initial PA signal, which increased gradually (10–60 s), sometimes in a complex way involving several waves, until a steady state was reached, similar to that in [14,15]. The reference level with white saturating light was usually quite close to the initial level. Fluorescence also changed during this transition period. After a steady state was reached the addition of non-modulated 710 nm light caused both fluorescence quenching and enhancement of the PA signal. Fig. 1 demonstrates this enhancement of O_2 evolution in an intact leaf by light ab-
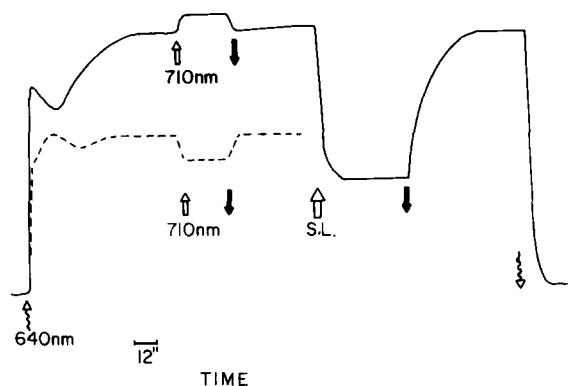

Fig. 1. PA signal (———) and fluorescence (---), from a dark-adapted tobacco leaf, with excitation by modulated (22 Hz) light 2 (on ⬆, off ⬇, 640 nm, 11 W/m²). Induction transients were reported in [14,15]. O_2 evolution is proportional to the difference in PA signals with and without the addition of non-modulated saturating white light (SL 350 W/m²) relative to the level obtained with the white light. Emerson enhancement of O_2 evolution and fluorescence quenching are demonstrated by adding non-modulated light 1 (710 nm, 6.8 W/m²; ⬆, on; ⬇, off).

sorbed primarily by PS I and shows the correlation with fluorescence quenching. Both phenomena had very short rise and decay kinetics and presumably resulted from the oxidation of PS II reaction-centers by light 1. Prolonged illumination (~7 min) with 710 nm background light (in this case still in the presence of 640 nm modulated light) brought the leaf to state 1, characterized by a low level of O_2 evolution and a high fluorescence yield upon removal of light 1 background (fig. 2). The Emerson enhancement ratio (ratio of O_2 yields with and without background light 1) in state 1 was 1.25. Illumination with 640 nm modulated light only induced a transition to light 2 state, characterized by a slow increase in O_2 evolution and a slow decrease in fluorescence. In state 2, the degree of Emerson enhancement was smaller than before and equal to 1.13. The results of fig. 2 show that the imbalance of photon delivery to the two photosystems due to excess PS II light (in state 1) decreased during the transition to state 2 giving rise to a change from a maximum to a minimum enhancement.

When the intensity of the 640 nm light was gradually reduced below a certain optimal intensity, steady states were achieved in which unex-
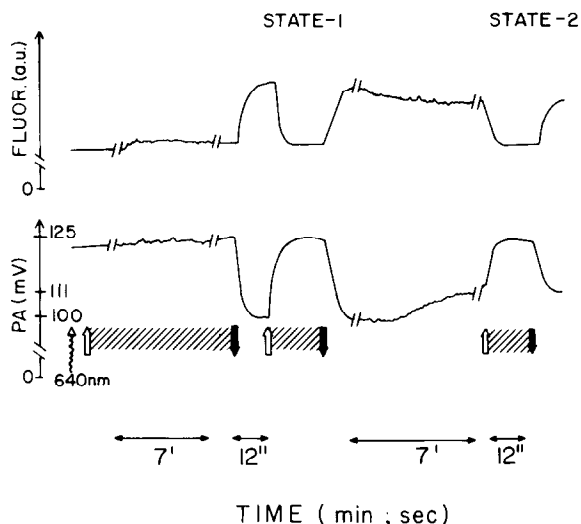
Fig. 2. State 1 $\rightleftarrows$ state 2 transition: $\dagger$ -modulated light (640 nm, 13 W/m^2) on; $\Uparrow$ $\Downarrow$ 710 nm non-modulated background light (2.4 W/m^2) on and off; other details as in fig. 1.

Fig. 4. Transients measured with modulated light 2 at low intensity (640 nm, 1.5 W/m^2). Effects of: Saturating non-modulated light SL (white, 350 W/m^2); non-modulated far-red light (710 nm, 2.4 W/m^2); non-modulated 640 nm light (1.4 W/m^2); frequency, 15 Hz.
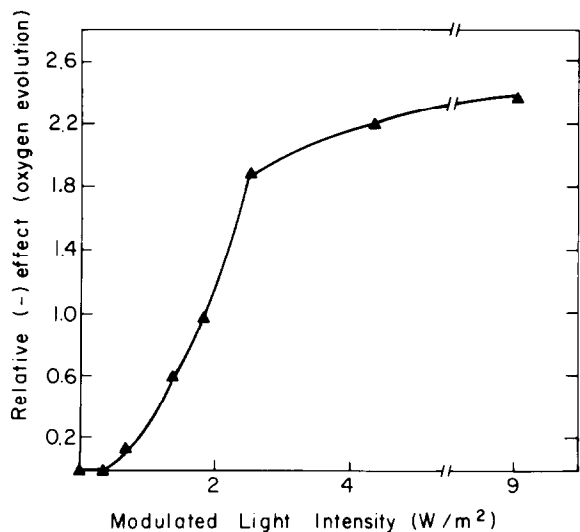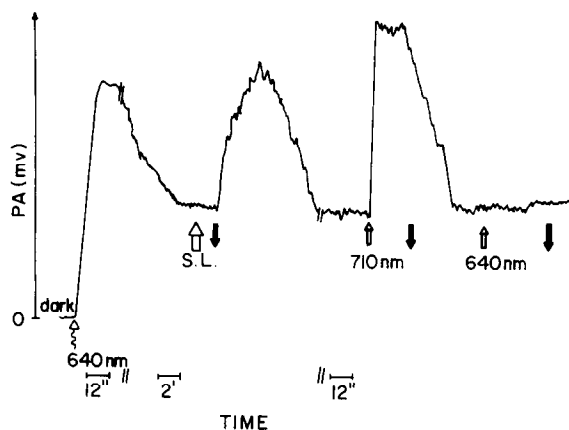

Fig. 3. Dependence of the relative quantum yield of steady-state O$_2$-evolution on the intensity of modulated 640 nm light. Modulation frequency 15 Hz. Other details as in fig. 1.

pectedly the oxygen evolution quantum yield became smaller, gradually approaching zero. The steady-state yield of O$_2$ evolution as a function of the modulated light intensity is shown in fig. 3.

Fig. 4 shows the slow transition (~5 min) from the maximal level of O$_2$ evolution to the new steady state, which is obtained when a low, rather than a high intensity of modulated light 2 is used. This figure also shows several events induced by various successive treatments. At the new steady-state, when white saturating light was added and then turned off, the 640 nm modulated oxygen yield increased transiently to almost the maximum quantum yield and then decayed again to the steady-state level. The Emerson enhancement in the state of the low yield of oxygen evolution was quite remarkable: Addition of light 1 raised the O$_2$ yield from (almost) zero to the maximal level obtainable. A straight forward interpretation is that the drop of the O$_2$ yield in weak light is due to an extreme imbalance in which PS I receives almost no excitation. The fact that with light 1 immediate full restoration is achieved proves that the electron transfer system was not impaired. A possible explanation is based on the postulated existence, in PS I, of a small antenna of pigments, which is the main site of the far-red absorption, and a large antenna, which is responsible for most of the light absorption. The small antenna is always communicating with reaction center I, while the large one is able to dissociate from PS I as a result of the
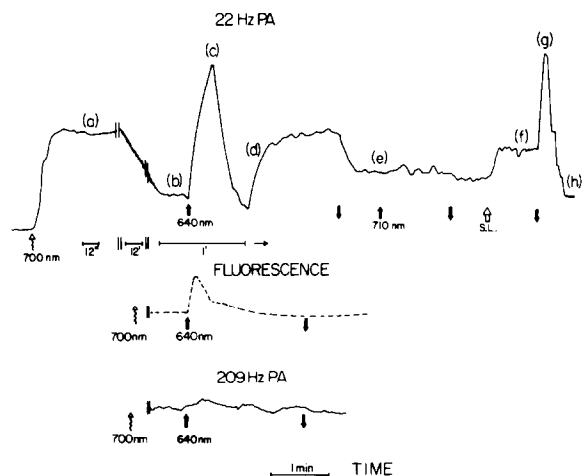
Fig. 5. Transients measured with modulated light 1 (700 nm) at low light intensity (1.6 W/m^2). Effect of frequency (22 and 209 Hz), non-modulated light 2 (640 nm, 4.6 W/m^2) and non-modulated far-red light (710 nm 6.4 W/m^2); for other details, see text.

adaptation to weak light. This hypothesis is consistent with the results of fig. 3,4. The division of the antenna of PS I into small and large separate parts is also suggested in a model derived from development studies [16].

Transients were also observed with modulated light-1 (e.g., 700 nm) (fig. 5). In this case, the initial PA level, immediately after dark adaptation (fig. 5a) was very close to the reference level, obtained with saturating white light (fig. 5f), indicating zero O_2 evolution and zero photochemical activity. After ~1 min the signal decreased, reaching a steady state (b) in ~10–20 min, considerably below the reference level, thus indicating an intensive photochemical energy storage (perhaps cyclic phosporylation), but no O_2 evolution. Addition of 640 nm non-modulated background light, resulted in a dramatic slow transient increase (c) peaking at a considerably higher level than the reference level, ultimately decaying back to the same level, as in (b). A subsequent increase (d), to a level of zero activity (close to the reference level) followed. Upon switching off the 640 nm light, the signal decreased to (e), reflecting photochemical activity somewhat smaller than in level (b). When 710 nm background light of equal absorbed intensity was used, instead of 640 nm light, the above transients did not occur. Irradia-

tion with strong saturating background light caused the signal to increase to the reference level (f). Switching off the saturating light resulted in a transient increase (g) and ultimate decrease of the signal to the steady state (h) similar to (b). The reference level obtained with saturating white light was always the same, regardless of when this light was applied. Clearly, the above phenomena represent quite a complex interplay and superposition of changes in various photochemical activities. Concentrating on the transient obtained by addition of 640 light, it probably reflects transient O_2 evolution, since at high frequency (209 Hz) it disappeared completely (fig. 5, bottom). A parallel fluoresence transient followed indeed the first part of this PA transient (fig. 5, middle). This effect cannot be due to 'opening' of reaction centers in PS II as they are already fully opened with the far-red irradiation and tend rather to be closed by the effect of the 640 nm light. It is possible that the transient modulated O_2 evolution is due to the modulation of the reaction centers of PS II themselves, caused by the modulated oxidizing effect of modulated ligt 1 upon application of non-modulated light 2. These complex phenomena will be explored in more detail in further work.

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