THE RATIO BETWEEN DELAYED LIGHT AND FLUORESCENCE EMITTED BY CHLOROPLASTS

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ABSTRACT Electric fields of a few hundred volts per centimeter greatly stimulate the emission of delayed light from "broken" chloroplasts. At low intensities of exciting light the fluorescence of these chloroplasts is also stimulated by the electric field, but to a lesser extent. Assuming that the electric field has no effect on prompt fluorescence, and has the same effect on the delayed light emission during illumination as in the dark, we can determine the ratio of delayed light to fluorescence under steady-state illumination.

INTRODUCTION

It has long been known that a small part of the light energy absorbed by a green plant is reemitted in the red region of the spectrum. In addition to this fluorescence, sometime ago it was found that green plants emit a delayed light (delayed fluorescence) long after they are illuminated (Strehler and Arnold, 1951). This delayed light is emitted at times far too long to be directly caused by an excited state of chlorophyll. It must represent the storage of light energy and then the reexcitation of chlorophyll. Since there is no reason to believe that the mechanisms making delayed light do not operate during the time the plant is being illuminated, we must believe that the experimentally observed fluorescence of a green plant represents a mixture of delayed light and prompt fluorescence.

A question of some interest is, under continuous illumination, what fraction of the experimentally observed fluorescence is delayed light. Müller and Lumry (1965) found that with modulated light, the delayed light amounts to some 8% of the total fluorescence. We present here a method of making this measurement for continuous illumination. This method is limited to chloroplasts and does not measure all the components of delayed light.

At the International Conference on the Photosynthetic Unit, held in Gatlinburg, Tenn., in 1970, we reported that an electric field across a suspension of broken chloroplasts stimulates the delayed light (Arnold and Azzi, 1971). This stimulation of delayed light is surprisingly large, up to a 50-fold increase in intensity. In that paper we argued that there are four different mechanisms producing delayed light: (*a*) recombination of electrons and holes, (*b*) untrapping of electrons, (*c*) untrap-

ping of holes, and (*d*) a process involving oxygen. Experiments since that conference indicate that the electric fields stimulate mechanisms *b* and *c*. Thus our measurement really gives only that fraction of delayed light made by these two mechanisms.

MATERIALS AND METHODS

Chloroplasts were prepared from *Chenopodium bonus-henricus* (Good King Henry goosefoot), by the method of Bertsch et al. (1969). A volume of 0.05 ml of "whole" chloroplasts was diluted in 35 ml of 10^{-4} M Tris-HCl and $MgCl_2$, pH 7.4. Under these conditions the chloroplasts form spheres 10–20 μ in diameter that we have referred to as broken chloroplasts. The suspension is contained in a 3 cm diameter plastic cuvette. The exciting light from an incandescent lamp was filtered through 10 cm of saturated $CuSO_2$. The fluorescence was measured with a 9558 B photomultiplier with a Corning No. 2403 filter (Corning Glass Works, Corning, N. Y.) to remove the exciting light. The photomultiplier was operated at 1000 v applied through the appropriate resistance chain. The signal was passed through an electrometer and on to a Brown recorder. Flat platinum electrodes were on each side of the light beam so that the chloroplast suspension could be subjected to an electric field.

RESULTS

The effect of an electric field on both delayed light and fluorescence is shown in Table I. The intensity of the highest exciting light used was approximately 5×10^{12}

TABLE I

THE EFFECT OF AN ELECTRIC FIELD ON BOTH DELAYED LIGHT AND FLUORESCENCE*

(*a*) Delayed Light 30 sec after Flash

S Delayed light intensity; No field	ΔS Increase in delayed light; Field on	$\frac{\Delta S}{S} = \theta$
2.2×10^{-10}	2.0×10^{-9}	9.1

(*b*) Fluorescence Steady State

X Fluorescence intensity; no field	ΔX Increase in fluorescence; field on	$\frac{\Delta X}{X}$	$\frac{\frac{\Delta X}{\theta X}}{\text{Delayed light / fluorescence}}$
6.05×10^{-7}	3.5×10^{-8}	0.058	0.0064
5.25×10^{-7}	3.5×10^{-8}	0.067	0.0074
5.90×10^{-8}	3.5×10^{-9}	0.059	0.0065
7.15×10^{-9}	5.0×10^{-10}	0.070	0.0077
5.60×10^{-10}	3.5×10^{-11}	0.063	0.0069
			0.00698

* 314 v/cm, 60 cycle AC was used. For the fluorescence measurements, the exciting light was varied over a range of more than 10^3. No Hill reagent added. The light intensities are in amperes from the photomultiplier.

quanta/cm² per sec. A photosynthetic unit (500 chlorophyll molecules) was absorbing a quantum about every 10 sec. At higher exciting light intensities, where an appreciable fraction of the reaction centers become filled, the story becomes more complex and will not be discussed here.

The results in Table I show that an electric field of 314 v/cm makes the delayed light 10 times brighter. This same field only increases the observed fluorescence by 6.3%. We obtain the same relative increase over a range of exciting light intensities of more than 1000 times.

DISCUSSION

If we let S be the intensity of the delayed light and ΔS the increase when the field is applied, then we can define θ by the relation

$$\Delta S = \theta S.$$

Similarly, let X be the observed intensity of fluorescence and ΔX the increase when the field is applied. We let F be the intensity of prompt fluorescence plus that part of the delayed light that is not changed by the field. We believe that the prompt fluorescence is not affected by the field, since the electric field has no effect on the fluorescence of chloroplasts that have been heated to 50°C for 5 min, a treatment that destroys the delayed light.

With these definitions, we have with no electric field

$$X = F + S.$$

With the field on we have

$$X + \Delta X = F + (\theta + 1)S.$$

The difference of the two equations gives

$$\Delta X = \theta S,$$

or

$$\frac{S}{X} = \frac{\Delta X}{\theta X}.$$

Using the data from Table I we see that the delayed light is 0.70% of the observed fluorescence. The value of 0.7% is for broken chloroplasts, in 10^{-4} M buffer and $MgCl_2$ with no added Hill reagent, and for low intensities of exciting light, and represents only that part of the delayed light that we believe is due to electron and hole untrapping.

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