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PARALLEL MEASUREMENTS ON FLUORESCENCE LIFE-TIME AND INTENSITY CHANGES FROM LEAVES DURING THE FLUORESCENCE INDUCTION

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INTRODUCTION

Several reports show an almost linear relation between the life-time (τ) and the intensity of fluorescence (F) measured with a constant incident light, as they change with time upon illumination, in intact cells of algae [1—3] and chloroplasts [4]. These data seem to exclude the independent photosynthetic units concept which predicts two exponential components in the fluorescence decay (corresponding to a mixture of units with "open" and "closed" reaction centers, respectively, in which the relative proportions of the two vary as the total fluorescence changes with time). From these results, Tumerman and Sorokin [1] and Sorokin and Tumerman [2] concluded (see discussion in ref. 3) that the concept of the photosynthetic unit has only statistical significance and that each reaction center can be reached, in principle, by excitons created anywhere in the antenna pigments ("lake model" [5]). Various models of the relation between the antenna and the reaction center assume photosynthetic units with limited numbers of reaction centers accessible to them, but still approaching closely the behavior of the "lake" model [6,7]. Moya [8] found small deviations from the above-mentioned linear relation between τ and F and accounted for it by a model of connected units between which exists a certain high frequency of energy exchange; this is still a close approximation of the "lake" model.

On the other hand, there are phenomena which seemingly do not conform to the above concept. For example, flash yield studies in chloroplasts (for flashes in the μs region) show that an exponential relation exists

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between flash intensity and the electron transport yield per flash, indicating very limited amounts of excitation transfer [9]. These results, however, could be explained alternatively by the formation of quenching entities, i.e. the oxidized primary donor P^+, and the triplet state [10] of several μs life-time, which quench any extra excitons around a given reaction center, preventing their spread to neighboring reaction centers. Another example is that the fluorescence induction in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) is sigmoidal in many circumstances and can be satisfactorily explained in terms of energy transfer [11] but tends to an exponential rise when the salt concentration in the medium is low [4]. This again could be explained by an increase in a quenching process, e.g., by energy transfer to the less fluorescent photosystem I (PS-I), which decreases the extent of energy transfer in PS-II (cf. ref. 12), reducing the maximum yield of fluorescence, as well as of photochemistry. Only photons absorbed in the vicinity of an open reaction center have, in this case, a good probability of being captured by the nearby reaction center; the others decay before arrival.

Dark adapted algae and leaves of plants produce complex fluorescence transients when suddenly exposed to continuous bright light [13]. These transients (the Kautsky effect) consist of several phases: The first is a generally rising phase ($O \rightarrow I \rightarrow P$), similar to that of isolated chloroplasts which was interpreted on the basis of PS-II reaction center closure and the accompanying increase of the fluorescence probability. In the second phase ($P \rightarrow S$), the fluorescence decreases from the peak (P) to a quasi steady-state (S). This phase has not been explained satisfactorily and could result from any of several events: opening of the reaction center (oxidation of Q^-) by PS-I (according to this interpretation, PS-I is not active until about a second after illumination); quenching of fluorescence by increased energy transfer from the more fluorescent PS-II to the weakly fluorescent PS-I; or a regulatory mechanism which causes less excitation to be absorbed in PS-II. In the last case, the life-time and the yield of fluorescence will not necessarily be correlated with each other. Data on this problem is scarce; a preliminary demonstration of the linear τ vs. F relation during the $P \rightarrow S$ transition was, however, given by Tumerman and Sorokin [1] for the green alga *Chlorella*. It is clear that more work has to be done to check the correlation of the life-time and the intensity of fluorescence in the $P \rightarrow S$ phase in various situations and species, particularly with higher plants. We provide here the first such measurements on living leaves of several higher plants. The conclusions from our measurements is that the life-time and yield are correlated also during the $P \rightarrow S$ transition, but that this correlation is not necessarily a linear one.

MATERIALS AND METHODS

Leaves were picked from the following plants: spinach (*Spinacia oleracea*), cucumber (*Cucumis sativus*), mustard (*Brassica* sp.), pigweed (*Amaranthus*

sp.) and umbrella tree (*Schefflera* sp., also known as *Brassica actinophylla*), and pea (*Pisum sativum*) and used fresh.

Fluorescence life-time measurements were made with a phase-delay fluorimeter similar to the one described previously [14]. A continuous train of pulses (modulation frequency, 75 MHz) from a mode-locked He-Ne laser (632.8 nm) was used as the excitation source. Both the phase-delay and the intensity of the modulated fluorescence were recorded simultaneously as function of the time of illumination. The time constant of the recording system was set at 50 ms. All measurements were made at 20°C. The life-time (τ) of fluorescence was calculated from the phase-delay as $\tau = \tan \Delta\phi/2\pi\nu$ where $\Delta\phi$ = phase shift and ν = frequency of modulation.

The relative fluorescence intensity was measured by the same system as the amplitude of the 75 MHz modulated component. To calculate the relevant d.c. component the results had to be multiplied by the correction factor $\sqrt{(1 + 4\pi^2\nu^2\tau^2)}$ which depends on τ. This correction factor applies only to the case of the 1-component fluorescence decay; when two components of decay exist, the amplitude of the a.c fluorescence component is related to the apparent life-time through the following relations:

$$\overline{\tau} = (\gamma_1\tau_1^2 + \gamma_2\tau_2^2 + 4\pi^2\nu^2\tau_1^2\tau_2^2)/(\gamma_1\tau_1 + \gamma_2\tau_2 + 4\pi^2\nu^2[\gamma_1\tau_1\tau_2^2 + \gamma_2\tau_1^2\tau_2^2]) \quad (1)$$

$$\overline{F}_{a.c.} = \sqrt{\frac{\gamma_1^2\tau_1^2}{1 + 4\pi^2\nu^2\tau_1^2} + \frac{\gamma_2^2\tau_2^2}{1 + 4\pi^2\nu^2\tau_2^2} + \frac{2\gamma_1\gamma_2(1 + 4\pi^2\nu^2\tau_1\tau_2)\tau_1\tau_2}{(1 + 4\pi^2\nu^2\tau_1^2)(1 + 4\pi^2\nu^2\tau_2^2)}} \quad (2)$$

where $\overline{\tau}$ is the measured life-time (an "average" from contributions of the two components having life-times τ_1 and τ_2), γ_1 and γ_2 are the relative amplitudes of the τ_1 and τ_2 modes ($\gamma_1 + \gamma_2 = 1$) and τ_1 and τ_2 are the measured life-times corresponding to $F = F_o$ and $F = F_{max}$, respectively. From a theoretical plot of $\overline{\tau}$ vs. γ_1 according to Eq. (1) one finds the value of γ_1 (and γ_2) corresponding to each $\overline{\tau}$ and hence calculates the relative values of $F_{a.c.}$ as expressed by Eq (2).

Fluorescence kinetics from leaves are usually complicated, as the exciting light intensity is not uniform along the optical path, due to its absorption and scattering. This leads to an asynchronous contribution by different cross-sections of the leaf, along the excitation pathway, to the "total fluorescence" transient. It has been shown [15], however, that this complexity can be largely avoided if the ratio of the extinction coefficient for the actinic light to that of the fluorescence is small compared to unity. The best situation is achieved [15,16] when the extinction coefficient is minimum at the actinic wavelength (i.e., at ~550 nm) and as high as possible at the fluorescence wavelength (i.e., at ~680 nm). For the wavelengths indicates the above ratio is about 0.1, meaning that the average spread of intensity of the actinic beam is of the order of 10%. In our less optimal case (excitation wavelength, 632.8 nm, and emission wavelength, 685 nm), the above ratio is close to 0.25, which roughly means a spread of intensities around 25%. Assuming an inverse relation between time and intensity for the kinetics of the fluores-

cence induction, it is estimated for our experimental conditions that, if the original relation between τ and the fluorescence yield is linear for the fluorescence contribution from each cross section along the actinic path, the relation between τ and total F will remain linear with an error of less than 1%. Therefore, it seems that the choice of actinic wavelength is still appropriate.

RESULTS AND DISCUSSION

Fig. 1 shows the simultaneous tracings of intensity (F) and phase-delay (labelled as "shift") of the fluorescence from a spinach (*Spinacia oleracea*) leaf, for both the rising (O → P) (left set of curves) and declining (P → S) (right set of curves) phases of the transient. Qualitatively, at least, both fluorescence intensity and life-time show parallel changes.

Fig. 2 shows plots of the life-time vs. the relative d.c. fluorescence intensity (as corrected according to the single component recipe (cf. Materials and Methods) for several plant species: the above is the first demonstration of the relationship between τ and F for the P → S phase (Fig. 2F) of leaves of higher plants. Roughly, τ versus F curves appear linear, but in each case (although to a different degree), there is a convex curvature. This suggests (cf. refs. 1, 2, 4, 8) that there are, at least, two components and, thus, the "lake" model with one fluorescence component is questionable in leaves. This kind of result is quite universal and holds for both the fluorescence rise and decay during the induction (cf. Fig. 2F and 2E). The absence of extrapolation to the origin is just what would be expected from a two component system in the fluorescence. This is however not in agreement with the observation of Briantais et al. [3] who studied the O → P phase in *Chlorella*.

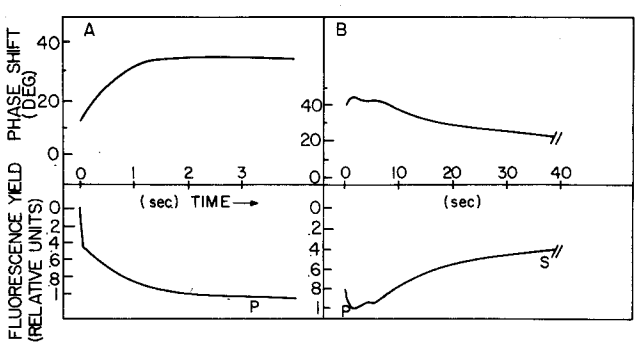

Fig. 1. Simultaneous recording to transient changes in the fluorescence intensity (F), related to the fluorescence yield (ϕ_F), and phase-delay (Shift), related to the life-time (τ) of the chlorophyll a fluorescence in vivo excited by a He-Ne laser (632.8 nm) modulated at 75 MHz. The exciting light intensity was constant, approx. 4 mW · cm^{-2}. Separate spinach leaves, dark adapted for about 10 min, were used for each measurement. A) Faster time scale; O → P transition. B) Slower time scale; P → S transition.

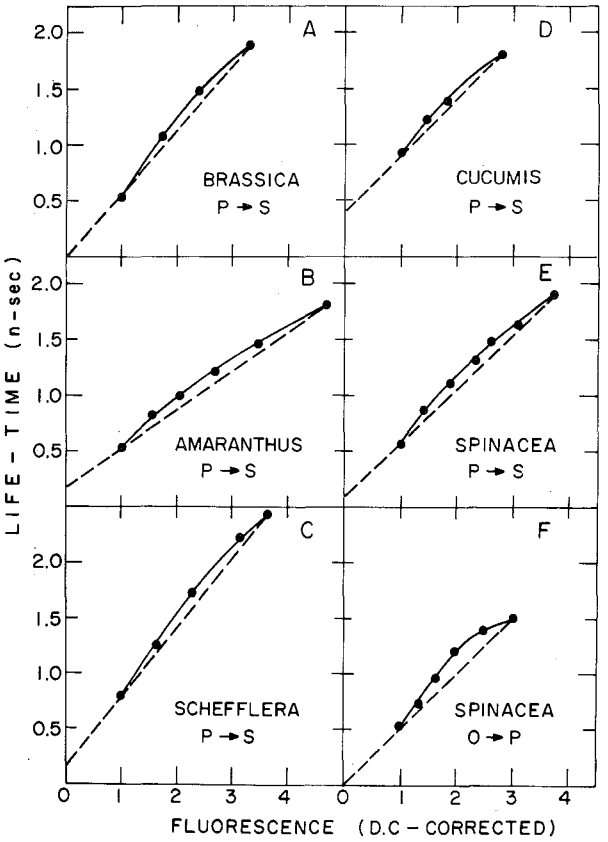

Fig. 2. The reaction between fluorescence life-time (τ) and fluorescence intensity, calculated for chosen time points from curves similar to Fig. 1B. The fluorescence intensity is given in arbitrary units (normalized to 1 for the lower fluorescence). The life-time is calculated from the relation: $\tau = \tan \Delta\phi / 2\pi\nu$, where $\Delta\phi$ is the phase-delay and ν the frequency (75 MHz). The fluorescence is corrected by multiplying each value by $(1 + 4\pi^2\nu^2\tau^2)^{1/2}$ to obtain the d.c. component. The plant species used are indicated in the figure.

The above conclusion is better illustrated by a direct comparison of the plot of the life-time vs. the modulated fluorescence intensity (F_a) with those expected for the cases when the decay of fluorescence consists of one and two components, respectively (Fig. 3). Some experimental plots (solid dots) agree with the curve predicted for a two component system, and some occupy intermediate positions between the predictions for the one and two component systems. It is also possible that an additional emission, not generated in PS-II, contributes to such deviations.

A more careful analysis of the phenomenon investigated here must, however, await a better experimental precision. Nevertheless, it is clear that the relation between fluorescence intensity and life-time is not quite in

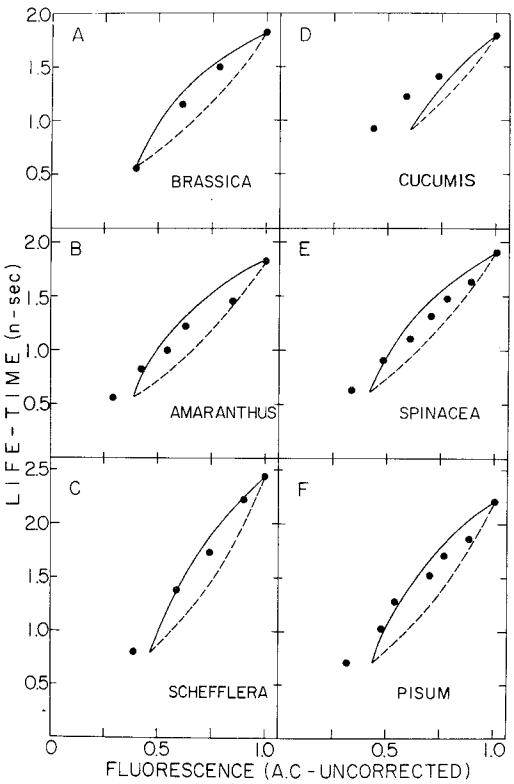

Fig. 3. The relation between fluorescence life-time (τ) and the non-corrected modulated (a.c.) fluorescence intensity. Points indicate chosen experimental results selected from the τ—F experimental curves. Solid line indicates a theoretical τ—F relation according to a fluorescent system with two decaying components (Eqs. 1 and 2); for these calculations, we used the lowest and highest experimental τ values for τ_1 and τ_2 respectively. Broken line indicates a theoretical τ—F relation according to a 1-component system which is linear in τ—$F_{d.c.}$. The calculation is made such that the (F, τ) point for the maximal fluorescence will coincide with the corresponding experimental point and the maximal fluorescence is normalized to 1.

accordance with the concept of the "lake" model; different organizations of the photosynthetic units are observed in the different leaves. The experimental τ—F curves represented in Fig. 3 correspond to interactions between photosynthetic units which are variable with almost no probability of energy transfer at one limit and a significant probability of energy transfer (close to a large "pond") at the other limit. The relations are similar for both the O → P and the P → S transitions, thus excluding the idea that changes in the distribution of light by direct absorption by photosystem II occur during the P → S transition.

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