PRELIMINARY NOTE

BBA 41121

Fluorescence excitation of chlorophyll in green plants by β particles

We are investigating the use of β particles to excite the fluorescence of chlorophyll *in vivo*. The ultimate aim of this study is to observe the subnanosecond decay profile of impulsively excited fluorescence without making any *a priori* assumptions about that profile. Preliminary steps in the study are reported in this paper. The yield of the β-induced luminescence* of green leaves is experimentally estimated and the luminescence spectrum tentatively identified, both without time resolution.

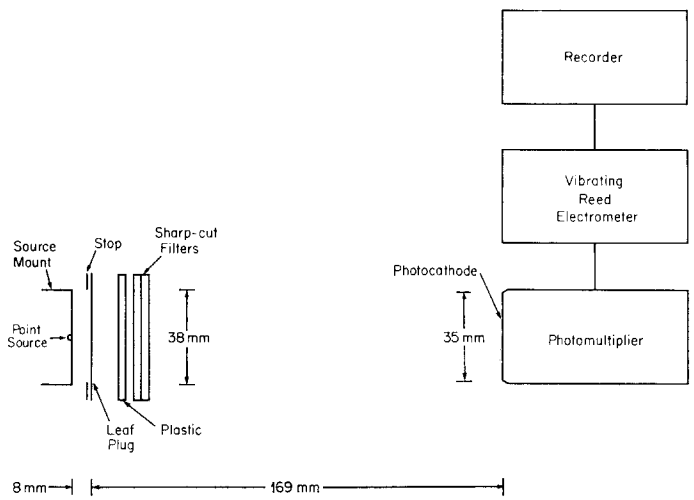

Fig. 1. Experimental set-up.

The experimental arrangement is shown schematically in Fig. 1. β particles from a 35-mC encapsulated source of ^{147}Pm (a pure β particle emitter with maximum β energy of 220 keV) impinge on the underside of a two-inch-square leaf plug of wild kudzu (*Pueraria lobata*). A photomultiplier (RCA 7102) cooled by liquid nitrogen observes the resulting luminescence from the top side of the leaf. A piece of clear plastic, to absorb the more penetrating radiation, and one or two Corning sharp-cut filters are placed between the leaf and the phototube. In the control experiment a dim light replaces the β source. This light is provided by a tungsten lamp operated well below rated voltage, and is filtered through 22 cm of saturated $CuSO_4$ solution

* In this paper, the term "luminescence" is used generally to refer to any externally excited emission of light from a leaf. The term "chlorophyll fluorescence" is reserved for luminescence (at least provisionally) identified as originating from lowest-excited-singlet to ground-state transitions of chlorophyll *a*.

(and thus has wavelengths close to 500 nm). Experimental geometry is otherwise unchanged. The light-tight housing incorporating the sample and filter chambers and several shutters is not shown in Fig. 1.

The present experimental arrangement limits the reliability of the spectral identification, since all luminescence is filtered through the leaf plug itself. (Future experiments, involving time resolution, ultimately must deal with optically thin, low scattering samples, such as chloroplast layers, which will allow more reliable spectral determinations.) Nevertheless, as Table I shows, the integral spectrum of the β-induced luminescence closely resembles the light-induced spectrum. Results in the β-induced column represent averages from measurements on 2 leaf plugs and those in the light-induced column are from measurements on 1 plug. (Each measurement with a given plug and filter is repeated at least 3 times.) Results from β irradiation are corrected for background due primarily to scintillation by the plastic absorber. Results reproduce to no better than $\pm 10\%$, which probably accounts for apparent anomalies in Table I.

TABLE I

LUMINESCENCE OF LEAF PLUGS THROUGH SHARP-CUT GLASS FILTERS

Corning Filter No.	*λ at 50% transmission* (nm)*	*Emission (%)*	
		*β-induced***	*Light-induced****
3384 (CS3-70)	595	100	—
2412 (CS2-61)	632	98.8	100
2030 (CS2-64)	672	106	91.2
5850 (CS7-59) + 3480 (CS3-66)	714	95.5	91.6
5031 (CS5-56) + 2408 (CS2-60)	740	60.0	48.6

* Filter curves corrected for photomultiplier response (S-1) and taken to be 100% at 800 nm.
** Relative to β-induced emission through Filter No. 3384.
*** Relative to light-induced emission through Filter No. 2412.

Even though the light-induced emission spectrum is, not surprisingly, distorted due to self-absorption, it must be that of chlorophyll fluorescence[1]. The correspondence of β-induced and light-induced spectra therefore justifies *tentative* identification of the former also as that of chlorophyll fluorescence.

Study of relevant experimental parameters gives a rough estimate of the luminescence yield (the ratio of fluorescence photons emitted to β particles incident on the sample). In addition to the geometrical parameters indicated in Fig. 1, other relevant parameters are the observed photomultiplier anode current for the integrated β-induced spectrum, $3 \cdot 10^{-12}$ A; the photomultiplier gain, $3 \cdot 10^4$ (at 1000 V); and the photocathode quantum efficiency of about 0.4%. The encapsulating materials reduce the measured β emission rate to about $2.2 \cdot 10^8$ sec^{-1} into the forward hemisphere; source–sample geometry further reduces the rate to about $1.3 \cdot 10^8$ sec^{-1} into the sample. The resulting luminescence, from the anode current and gain just given, induces a current of 630 electrons/sec at the photocathode. With the sample–photocathode geometry of Fig. 1 the geometric efficiency of the photomultiplier is 0.3% which, together with the given quantum efficiency, indicates a luminescence rate of $5.2 \cdot 10^7$ sec^{-1}. The luminescence yield for photons escaping the sample is therefore 0.4.

As already noted, the data in the last column of Table I show a strong self-absorption of the emitted light. Comparison of this through-the-leaf integral fluorescence spectrum with front-surface fluorescence spectra of leaves[1] suggests that the actual luminescence yield may be as much as an order of magnitude larger than the value 0.4, just calculated. If, in a fast-timing experiment, the photomultiplier efficiency is increased by a factor of 30 (by using an S-20 photocathode and improved geometry) over the present efficiency, photoelectrons may be produced at the rate of 1 per 1200 β particles. With a counting rate of $6 \cdot 10^4$ β particles/sec, which is readily obtainable, 10^5 β-photoelectron coincidence events may be recorded in little more than 30 min. This coincidence rate is more than ample for the contemplated fast-timing work.

So far the results considered relate only to the specific samples used. A quantity of more general interest is the average energy (lost by a β particle), W^*, required to excite a chlorophyll molecule *in vivo* to its fluorescent state (lowest excited singlet). An estimate of W^* may be made from the present data, although even less precisely than that for the luminescence yield. By approximating the β energy spectrum as a linearly decreasing function, the average energy lost by a β particle in a leaf plug of thickness 13 mg/cm^2 may be estimated as 40 keV. If β-excited chlorophyll has the same chance to fluoresce *in vivo* as does chlorophyll excited by light, nearly 40 chlorophylls must be excited to produce one quantum of fluorescence[2]. Finally, if one accepts 4 as the actual luminescence yield (corrected for self-absorption), one β particle excites some 160 chlorophylls, $W^* = 250$ eV, and the efficiency of β-excitation of chlorophyll is only 0.8 %. However, since chlorophyll constitutes only 0.25 % of the weight of fresh leaf[3], its lowest excited singlet state is clearly an efficient collector of energy deposited in leaves by β particles.

One assumption made in the estimate of W^* which deserves close scrutiny is that on the β-excited chlorophyll fluorescence yield. Implicit in the assumption, and essential to the success of future fast-timing experiments, is the validity of the supposition that those photosynthetic units in which chlorophyll is excited are almost always distinct from those units in which reaction centers are damaged. Data of ARNOLD[4] indicate that the deposition of about 60 eV by ionizing radiation in one photosynthetic unit is sufficient to inactivate that unit (assumed to weigh 10^{-18} g). Thus, since a photosynthetic unit is about 10 % chlorophyll by weight[5], some 17 units are inactivated per β particle in this experiment. Then, for example, if a single chlorophyll molecule in each such unit is concomitantly excited by the inactivating β particle and fluoresces with the 10 times higher chance typical of chlorophyll in nonpolar solvents[6], the actual luminescence yield is again 4. (The probability of chlorophyll excitation in an inactivated unit by a subsequently arriving β particle is negligibly small under the experimental conditions.) Although concomitant excitation and inactivation of the same unit appears unlikely, clear resolution of this question awaits the results of future experiments.

In summary, green leaves luminesce under excitation by β particles. The luminescence yield is at least 0.4 photon per incident β particle from a ^{147}Pm source. The integral luminescence spectrum excited by the β particles closely resembles the integral chlorophyll fluorescence spectrum excited by blue light. The luminescence yield of 0.4 together with reasonable assumptions imply that the passage of one β particle through a leaf excites some 160 molecules of chlorophyll *a*.

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