RESEARCH NOTE
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ESTIMATION OF RELATIVE LIVE FLUORESCENCE QUANTUM YIELD BY THE AREA OVER FLUORESCENCE-RISE CURVE[1]

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Based on the linear relation between the yields of electron transport and fluorescence, the area above the fluorescenece-rise curve has been used as an estimation of the concentration (per chlorophyll) of the electron carriers involved in the process (Malkin and Kok, 1966; Forbush and Kok, 1968; Homann, 1968; Bennoun and Li, 1973). Conversely, by adding known amount of Hill acceptors and observing the fluorescence-rise curve one can estimate the quantum yield of the fluorescence. The essence of the working hypothesis is as follows. Each time a Hill acceptor receives an electron from Q, the primary electron acceptor of system II, a certain amount of fluorescence is quenched. The total amount of fluorescence being quenched $\left[f_{qu}=\int_0^\infty (F_\infty - F)dt,\ F_\infty\text{: steady state level fluorescene; F: fluorescence level at any specific time}\right]$ by Hill acceptors depends on the amount of the Hill acceptors (m) and the fluorescence quantum yield (ϕ_f), that is

$$f_{qu}=m\cdot\phi_f$$

Since m is known and f_{qu} can be measured, one therefore is able to calculate ϕ_f.

In this article, a calcium chloride induced increase in live fluorescence yield is reported.

Oats chlorophasts were prepared and assayed as described in the preceding article in this journal (Li, 1973). Fiqure 1 shows two fluorescence traces observed with chloroplasts incubated with the same amount of ferricyanide, but one of them containing added calcium chloride. Fluorescence emissions are

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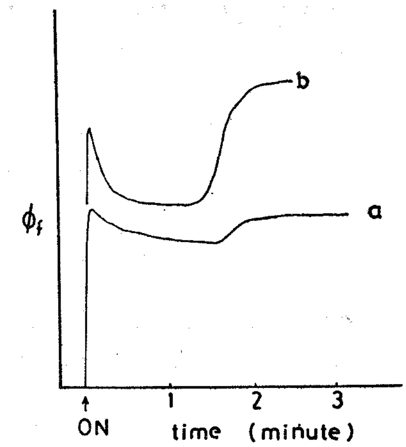

Fig. 1 The effect of calcium chloride on the fluorescence rise curve. The reaction mixture (1 ml) contained chloroplasts 13 μg chl/ml; sorbital, 150 mM, pH 8; ferricyanide 2.8×10^{-5}M. a. no other addition, b. calcium chloride, 5 mM. ϕf: relative fluorescence intensity. Arrow indicates light on.

kept low in all cases during the Hill reaction, and then rise to a steady level at the end of the Hill reaction. The fluorescence quenched (f_{qu}), represented by the area above the fluorescence rise curve, is a linear function of ferricyanide concentration (table 1). However, calcium chloride increases the value of f_{qu} for a five fold at all concentrations of ferricyanide tested. The constancy of

Table 1. *Ferricyanide concentrations and the area over the fluorescence rise curve (fqu).*

The reaction mixture (1 ml) contained chloroplasts, 13 σg chl/ml; sorbitol, 150 mM; tricine-NaOH 50 mM, pH 8; ferricyanide concentration as indicated.

Ferricyanide	$M\times10^{-6}$	5.6	11.2	28
fqu	no addition	2.6	4.9	10.9
fqu	$CaCl_2$	14.3	26.2	59.7
Ratio	Ca/control	5.46	5.35	5.44

this ratio with varied ferricyanide concentrations indicates that the endogenous oxidant pool does not contribute much to this ratio. A five fold increase of f_{qu} in the presence of calcium chloride over that of control seems to indicate that calcium chloride induces an increase in fluorescence yield. The 27% inhibition of Hill reaction by calcium chloride (table 2) can not account for the five fold increase of f_{qu} (seven fold increase of f_{qu}, if we take consideration of the fact that less ferricyanide was reduced in the presence of calcium chloride, later this report).

In the presence of calcium chloride, there is an unexpected feature, that is, the Hill reaction finishes sooner than when there is no calcium chloride (table 3), although calcium chloride slightly inhibits the rate. Table 3 shows that both the half time, t$\frac{1}{2}$ for fluorescence rise and t$\frac{1}{2}$ for ferricyanide

Table 2. *The effect of calcium chloride on the rate of ferricyanide Hill reaction.*

The reaction mixture (3 ml) contained chloroplasts 6 μg chl/ml; sorbitol, 150 mM; tricine-NaOH 50 mM, pH 8; ferricyanide 0.50 mM.

Addition	Ferricyanide reduced, μ moles/mg chl. hr
none	252
$CaCl_2$, 5 mM	185

Table 3. *Comparison of Hill reaction rates and fluorescence rise times.*

The reaction mixture contained chloroplasts, 13 μg chl/ml. sorbital, 150 mM; trichine-NaOH 50 mM, pH 8; ferricyanice 2.8×10^{-5}M.

	Control	$CaCl_2$	Remarks
Hill reaction rate, $\frac{\mu \text{ moles}}{\text{mg chl} \cdot \text{hr}}$	180	159	Broad-band interference filter (centered at 620 um)
Time for 50% completion of the Hill reaction (sec), measured	11.5	9	
Calculated		9.6	
Time for 50% rise of fluorescence induction, measured	43.5	36	blue filters
calculated		35.9	

reaction are reduced by calcium chloride. By examing the 420 nm absorption change. we found that only 58% of the added 28 n mole ferricyanide were reduced in the presence of calcium chloride, and 79% in control. This could be the reason for a shortened t½. Based on this assumption, using the following formula (1 and 2) it is found that the calculated and the measured t½ fit excellenty. This testifies strongly the soundness of our assumption that calcium chloride increases the fluorescence quantum yield.

$t\frac{1}{2}$ (ferricyanide reduction, calculated, $CaCl_2$)

$$= \frac{\text{The control Hill reaction rate}}{\text{Calcium Hill reaction rate}} \times t\tfrac{1}{2} \text{ (measured control)}$$

$$= 9.6 \text{ sec (measured value, 9 sec)} \qquad (1)$$

$t\frac{1}{2}$ (flluorescence, calculated, calcium)

$$= t\tfrac{1}{2} \text{ (fluorescence, measured, control)}$$

$$\times \frac{t\frac{1}{2} \text{ (ferricyanide reduction, calculated, calcium)}}{t\frac{1}{2} \text{ (ferricyanide reduction, measured, control)}}$$

$$= 35.89 \text{ (measured value, 36 sec)} \qquad (2)$$

The much larger change in F_∞ than that in F can be understood if we assume the rate constant of chemistry (k_c) of the trap is much larger than the rate constants of fluorescence (k_f) and heat (k_h). An increase in k_f will change the ratio of $k_f/(k_f+k_h)$ more pronouncedly than the ratio of $k_f/(k_f+k_h+k_c)$. The later is the fluorescence yield when the Hill reaction is in effect.

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由螢光誘導曲線上方的面積來估計葉綠體活螢光的量子產值

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1. 螢光誘導曲線(fluorescence induction curve) 上方的面積與加入葉綠體懸浮液中的 Hill 氧化物的量成正比。這一性質可用來估計活螢光的量子產值。

2. 氯化鈣增强葉綠體的螢光强度，原因可能是它增加螢光的量子產值。