THE INFLUENCE OF TWO NATURALLY OCCURRING FACTORS, FROM THE LEAVES OF *RICINUS COMMUNIS*, ON THE FLUORESCENCE INDUCTION OF HIGHER PLANT CHLOROPLASTS AND INTACT ALGAE

MARCIA BRODY

From the Department of Biological Sciences, Hunter College of the City University of New York, New York 10021

ABSTRACT Changes in fluorescence induction, brought about by incubation of chloroplasts (*Zea mays*) in an aqueous extract of *Ricinus* leaf, have been divided, on the basis of speed of manifestation, into two categories: "fast" changes and "slow" changes (i.e. those observed after 5 min and 1½ hr of incubation, respectively). The former, which include a large increase in the magnitude of the fast component of variable fluorescence and a retardation of decay from maximum to minimum levels of fluorescence, have been ascribed to inhibition of electron transport at a site beyond that of 3-(*p*-chlorophenyl)-1,1-dimethylurea (CMU)—i.e., towards system I; these changes result from the action of a fraction of the extract consisting of molecules of small size. The latter changes, which include a marked attenuation of the variable part of fluorescence induction, have been associated with system II and may arise from inhibition of electron flow between water and Q or from decrease in number of functional reaction centers; these changes result from the activity of a proteinaceous fraction of the extract, that simultaneously converts the low temperature steady-state emission spectrum of the chloroplasts into a one-banded one, with maximum at 698 nm.

INTRODUCTION

Because the 698 nm, low temperature fluorescence band (1–7) is thought to originate from a form of chlorophyll active in photosystem II, perhaps as the energy trap (3–6, 8), it has generated considerable interest. Recently, Brody, Nathanson, and Cohen (9) reported changes in spectra of steady-state fluorescence of chloroplasts (isolated from various organisms) incubated in aqueous leaf extract of castor oil plant (*Ricinus communis*). As revealed by measurements at 77°K, the three-banded emission spectrum, with maxima at about 685, 698, and 735 (or 720) nm, was converted, as a function of time and temperature of incubation, into an essen-

tially one-banded system, with maximum at about 698 nm. In the present work is reported the influence of this extract on the induction of fluorescence in isolated chloroplasts of higher plants and in intact algae. This influence appears to be of dual nature, and may involve two separate components in the extract, acting at different sites and at different rates.

The first action of the extract is very rapid, and results in the following changes in fluorescence induction: (*a*) a small increase in maximum fluorescence yield, primarily in the variable portion, (*b*) a large increase in amplitude of the fast part of the rise of variable fluorescence, leading to more rapid achievement of maximum yield, and (*c*) a progressive decrease in the rate of decay from the fluorescence peak to the stationary level, accompanied by a rise in the stationary level itself.

The second action of the extract is manifested more slowly, and appears to be related to the similarly time-dependent changes in steady-state fluorescence referred to above (9); it results in the following changes in fluorescence induction: (*a*) a marked decline in fluorescence yield, primarily in the variable portion, (*b*) a retardation of the slow part of the rise in variable fluorescence, and (*c*) some loss of the initial increase in amplitude of the fast part of variable fluorescence.

Since preliminary experiments revealed that some of the changes in fluorescence induction brought about by the extract were similar to some produced by CMU, the action of this herbicide was also studied, for purposes of comparison.

In the discussion section of this paper, interpretations of the data are given in terms of possible sites of action of the extract in the electron transport chain of photosynthesis. It is suggested that the faster acting effect of the extract blocks electron transport at a site beyond that where CMU blocks, i.e. towards system I (perhaps *within* system I, per se). It is also suggested that the second or slower effect of the extract on fluorescence induction results from an inhibition of electron transport somewhere between water and Q, or from a decrease in number of functional system II reaction centers.

Preliminary experiments on the molecular size (and nature) of the active components of the extract are described. Of the two types of molecules resulting from Sephadex G-25 separation, the smaller appears to be associated with the fast changes in fluorescence induction, while the larger appears to be associated with the slow changes (and also with the transformation of the steady-state emission spectrum).

MATERIALS AND METHODS

Aqueous extracts of leaves of *Ricinus communis* were prepared as described earlier (9) (by a modified method of McCarty and Jagendorf [10]) from greenhouse-grown plants of variety Baker 296 (seeds courtesy of Dr. R. L. Ory, Seed Protein Laboratory, Department of Agriculture, New Orleans, La.) or variety zanzibariensis (seeds purchased from Burnett Brothers, New York). The medium used for both extraction of leaves and isolation of chloroplasts (to be referred to as E-I medium) was composed of 0.35 M NaCl and 0.04 M Trizma-HCl (source of Trizma: Sigma Chemical Co., St. Louis, Mo.) pH 7.8 or 7.2. Leaves of green-

house-grown dwarf corn (*Zea mays*, hybrid variety INRA 200) were used as the source of chloroplasts (see references 9 and 11 for isolation procedure). Intact *Chorella* cells were centrifuged out of their culture medium (12) and resuspended in a small volume of E-I medium. At the beginning of each experiment, predetermined quantities (less than 2 ml) of chloroplasts or algae, at 5°C, were mixed (in the reservoir of the fluorescence induction apparatus) with 250 ml of suspending medium, also at 5°C. This suspending medium was either E-I medium, or *Ricinus* leaf extract, the latter henceforth to be abbreviated RLE. In any one series of experiments, separate and equal aliquots of the same plant materials were used for treatment and control. Suspensions of algae or chloroplasts were prepared so as to yield final chlorophyll concentrations of 10–50 μg chlorophyll ($a + b$) per ml (as determined by the method of Arnon [13]).

Although fluorescence induction measurements were begun immediately after mixing, the data presented here are for suspensions which had reached 25°C (see footnote 2); this warming-up to constant temperature took less than 5 min. The stop-flow apparatus used for the fluorescence induction studies has been described previously (14). Excitation was with a band of blue light isolated from a high pressure 900 w xenon arc lamp by means of a transmission grating monochromator set at 480 nm. A half-band width of 10 nm was utilized. In the experiments reported here, the light was attenuated by a neutral density filter of metalized glass, which transmits 44.8% of the available light; under these conditions, the exciting intensity was about 9×10^3 ergs/cm^2 per sec, and may be considered to be "moderate" compared to the designation "low" or "high" used by Lavorel (14). In the results section of this paper, the data given in the figures are taken directly from recorder tracings, therefore the values of O fluorescence levels (see below) are apparent ones;[1] actual O levels are given in the corresponding tables. (Corrections were based on measurements of fluorescence as a function of the intensity of the exciting light.) The wavelength at which fluorescence induction was monitored was 685 nm; a red cut-off filter (Corning glass 2-58, Corning Glass Works, Corning, N. Y.), complementary to the exciting light, was placed before the analyzing Bausch & Lomb monochromator (Bausch & Lomb Optical Products, Bausch & Lomb, Inc., Rochester, N. Y.), the slits of which were set to pass light of half-band width of $\simeq$10 nm. The speed of recorder chart paper was generally 120 mm/min; however, rise and decay times were also recorded at speeds of 300 and 30 mm/min, respectively.

For experiments involving CMU, aliquots of 0.1 ml of stock solution (1×10^{-2} M in absolute ethanol) were delivered to the 250 ml suspension in the reservoir, to yield concentrations up to 8×10^{-5} M CMU.

During the course of the fluorescence induction measurements, small samples (about 2 ml) were periodically removed from the reservoir of the apparatus, so that concurrent determinations of steady-state emission could be made. Excitation of steady-state fluorescence was accomplished by an optical system which passed blue light (the 436 nm line) isolated from a 100 w mercury arc lamp by Corning glass filters 3-73 and 5-58 (Corning Glass Works). The sample vessel was lowered into a flat bottom Dewar flask filled with liquid nitrogen, and fluorescence was both excited and detected from the same (bottom) surface of the sample. Fluorescence spectra were measured with the aid of a Bausch & Lomb monochromator (Bausch & Lomb Inc.) (half-band width 6.6 nm) in combination with a red cut-off filter, Corning glass 2-58. A Dumont 6911 multiplier phototube (Dumont Laboratories, Fairchild Camera and Instrument Corp., Clifton, N. J.) was used as detector, and the resulting signal was measured with a sensitive galvanometer.

[1] In the range of exciting light intensity used, the level in O state is invariably overestimated with the flow method (14), the more so as the OP photochemical process (see below) increases in activity; see also J. Lavorel (15), where this relationship is considered in the case of *Chlorella* treated with CMU.

The Sephadex G-25 (coarse grade), used for chromatography of crude RLE was obtained from Pharmacia Fine Chemicals Inc., Uppsala, Sweden. Before introduction into the column, the Sephadex was washed and swollen, and then equilibrated with E-I medium.

As an aid to understanding the results presented below, there are given in Figs. 1 *A* and *B* schematic diagrams of the types of fluorescence induction curves obtained with control chloroplasts and control algal cells, respectively. The various parameters to which reference will be made are labeled either in accordance with earlier usage (16), or are newly designated. Their meaning is as follows: *d* indicates zero fluorescence (dark level); *c*, in relative fluorescence units (mm intervals on the chart paper) represents the constant portion of fluorescence, obtained when the suspension is flowing past the exciting beam; *v*, obtained when the suspension is stopped in the light beam, represents the contribution of the variable portion to total fluorescence, $c + v$; *v* is composed of a fast part, beginning at the *m* or *O* level and extending to the break or inflection point, *I*, and a slow part, beginning at *I* and rising to the *P* level, which represents maximum or peak fluorescence; 25%, 75%, and 100% rise time represent the time (in seconds) for these respective percentages of the *OP* rise to be realized; in chloroplasts, 10% "*PO*" decay time is used here to refer to the time required for the variable portion of fluorescence to fall 10% of its value from the *P* level (measured from the time at which *P* is initially attained) to the previously determined *O* level (the reader should note that this time interval does *not* correspond to that required for 10% of the *PO* transition, which occurs when the suspension is allowed to flow again); in intact algae, *S* represents that stationary state to which the yield drops in the stopped system; the *S* level is monitored by the magnitude of the *PS* transition; 20%, 40%, and 100% *PS* decay times refer to the intervals required to achieve these percentages of the *PS* decay. The following example is given to demonstrate the manner in which rates of transition (Tables I and II) are calculated: the rate of the 75% *OP* rise is obtained by dividing the time it takes to achieve

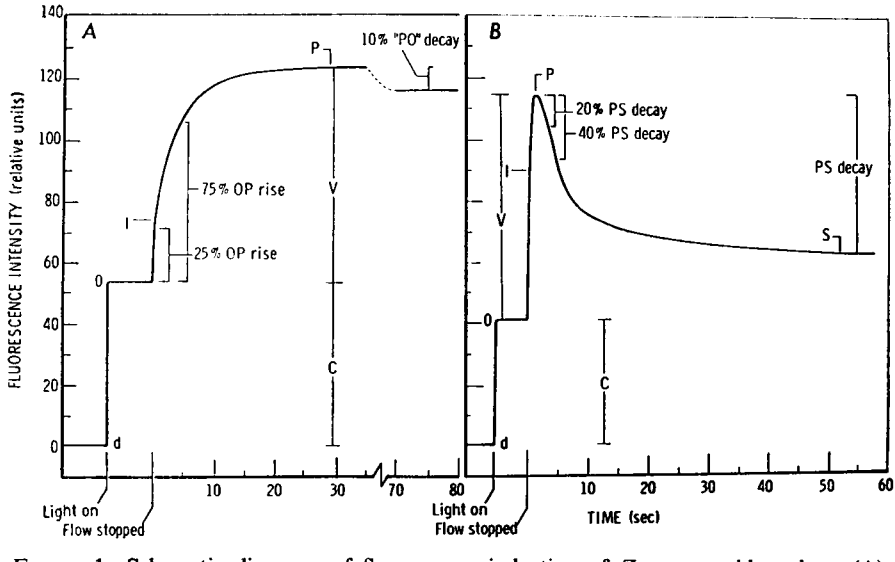


FIGURE 1 Schematic diagrams of fluorescence induction of *Zea mays* chloroplasts (A) and intact cells of *Chorella* (B). Zero fluorescence is given by *d* (dark level); for definition of labeled parameters, refer to body of paper (section on Materials and Methods). Note break in continuity of abscissa (time scale) in Fig. 1 A.

75% of the *OP* rise by the magnitude of this portion of the rise (i.e. 75% *OP* = 75% *v*) in relative fluorescence units (mm); rates are given, therefore, in terms of sec/fluorescence unit. One would like to measure, with the stopped suspension of chloroplasts, the *PS* transition (the "*PO*" measurement being a rather less desirable substitute) but such a measurement is practical only in the case of intact algae; it is impractical in the case of isolated chloroplasts, in which the *PS* decay is extremely slow.

RESULTS

A Comparison of the Influence of RLE and CMU on Steady-State Fluorescence and Fluorescence Induction in Chloroplasts

The Influence of RLE. Data of the type presented in Table I were obtained in the following way. Chloroplasts prepared on any one day were divided into two aliquots. Fluorescence induction was monitored for the control aliquot (chloroplasts in E-I medium) during a period of 1½ hr. Since very little change with time (generally a decrease of ≈5%) was observed in the control, data are given only for the measurement made 5 min after mixing.[2] Fluorescence induction was also monitored for the second aliquot (chloroplasts in RLE) during a period of 1½ hr; in this case, data are given both for measurements made 5 min and 1½ hr after mixing.

Although the data shown here are representative of the kinds of changes brought about by RLE, the degree of change was found to vary somewhat from day to day, and will be commented upon where appropriate. While a part of these variations reflect, no doubt, dissimilarities in the chloroplast preparations per se (i.e. in the controls), the remainder may be attributed to differences in concentration of active material in the RLE's which were freshly prepared each day. To facilitate comparison, the data have additionally been expressed in terms of ratios (experimental-to-control).

The reader may wish to refer to Fig. 2, in conjunction with Table I, for in this figure may be found recorder tracings of these fluorescence induction curves.

Although the data given in the present paper are for plant materials suspended in E-I media (or RLE) of pH 7.8, essentially similiar results were found at pH 7.2.

Concurrent steady-state fluorescence emission measurements, made at 77°K, revealed that in the case of chloroplasts suspended in RLE, the 685 nm band was disappearing as a discrete entity (or had largely "disappeared"), 5 min after mixing, while at 1½ hr after mixing, the 698 nm band was the major one which could be detected. No such changes in steady-state fluorescence emission were observed in the controls.

We will now turn our attention back to Table I (and Fig. 2), to consider, in

[2] Temperature, in common with several other factors (see reference 15) has a strong influence on the relative rates of the light-dependent and light-independent reactions associated with fluorescence induction; at 5°C, almost all of the rise in variable fluorescence is of the fast type, i.e., there is a similarity of effects of low temperature and RLE on the fast component of variable fluorescence.

TABLE I

A COMPARISON OF THE EFFECTS OF RLE AND CMU TREATMENT ON FLUORESCENCE INDUCTION OF *ZEA MAYS* CHLOROPLASTS

Fluorescence induction parameters	Control *C*	Period of incubation in RLE				CMU (added to control) 8×10^{-5} M	
		5 min		1½ hr			
		Experimental *E*	$\frac{E}{C}$	Experimental *E*	$\frac{E}{C}$	Experimental *E*	$\frac{E}{C}$
1. *v* (mm)	70.0	86.0	1.23	41.0	0.585	52.4	0.748
2. *c* (mm)	61.5	69.0	1.12	56.0	0.911	64.6	1.05
3. *c* + *v* (mm)	131.5	155.0	1.18	97.0	0.737	117.0	0.89
4. *OI* (mm)	14.0	60.0	4.28	16.0	1.14	45.9	3.28
5. *OI*/*v*	20.0%	69.8%	3.49	39.0%	1.95	87.7%	4.39
6. *OI*/*c* + *v*	10.6%	38.7%	3.72	16.5%	1.56	39.2%	3.70
OP rise							
7. 25% *OP*							
*a**	0.500	0.250	0.500	0.500	1.00	0.150	0.300
*b**	0.029	0.012	0.400	0.049	1.69	0.012	0.397
8. 75% *OP*							
a	7.50	1.25	0.167	2.50	0.333	0.500	0.067
b	0.143	0.019	0.136	0.081	0.563	0.013	0.089
9. 100% *OP*							
a	25.0	7.50	0.300	18.0	0.72	2.00	0.080
b	0.357	0.087	0.244	0.438	1.23	0.038	0.107
"*PO*" decay							
10. 10% "*PO*"							
a	45.0	56.0	1.24	85.0	1.89	90.0	2.00
b	6.43	6.50	1.01	20.7	3.22	17.2	2.68

* *a*, time (sec); *b*, rate (sec/mm).

relation to the controls, the "fast" and "slow" changes in fluorescence induction brought about by RLE. These represent, respectively, changes which are detected 5 min and 1½ hr after mixing.

In respect to total fluorescence, the following (see Table I, item 3) may be observed: there is a small increase in magnitude of *c* + *v* when chloroplasts are first mixed with RLE; however, after 1½ hr this maximum yield has fallen to about three-quarters of the control value. From items 1 and 2, it may be observed that the initial increase in *c* + *v* results, in greater part, from increase in variable fluorescence; the subsequent fall in total fluorescence may also be predominantly attributed to attenuation of the variable portion. In some preparations, *v* was found to fall to about 40% of its control value after 1½ hr in RLE.

A very striking change, seen upon mixture of chloroplasts and RLE, is marked enhancement of the amplitude of the fast part, *OI*, of the rise in variable fluorescence (see items 4–6). (In the control, *OI* generally constitutes about ⅕ to ¼ of *v*.) This increase in magnitude (usually two- to fourfold) of the *OI* phase results in accelerated achievement of the *OP* transition, which may be seen at both the 75%

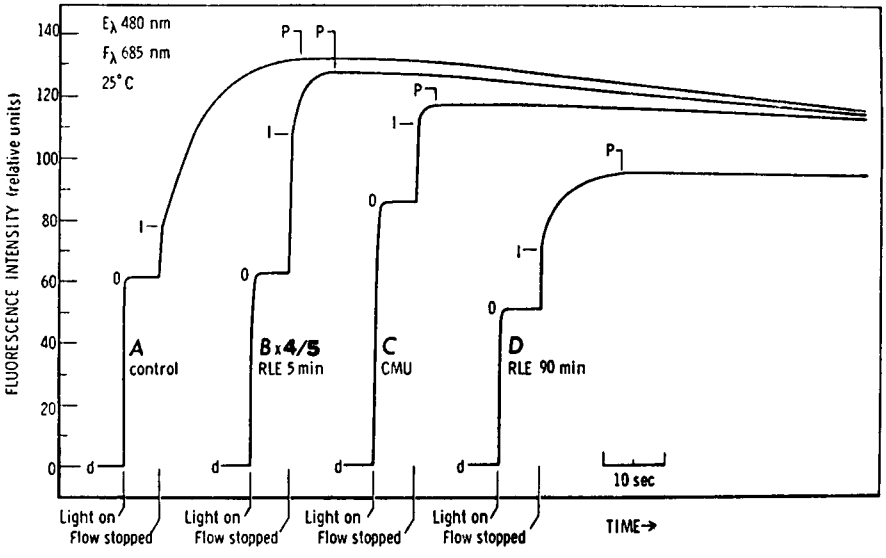


FIGURE 2 Fluorescence induction of *Zea mays* chloroplasts. Curve A, control; curves B and D, chloroplasts incubated in RLE for 5 min and 1½ hr, respectively; curve C, CMU-treated chloroplasts (8×10^{-5} M). Curves A–D are tracings of recordings; curve B has been multiplied by ⅘ for ease of comparison with other curves. Fluorescence excited at 480 nm and monitored at 685 nm. Recorder chart speed was 120 mm/min; abscissa time units indicated by scale of 10 sec.

and peak levels (items 8 and 9); an additional factor in the accelerated rise to maximum fluorescence is the increase in slope of the fast component, reflected in the reduction in time to achieve the 25% level of the *OP* rise (item 7). With increasing time in RLE, the contribution of the *OI* phase diminishes, but generally remains higher than in the control (see items 4–6). Note the influence of prolonged exposure to RLE on 100% *OP* rise times (items 7–9); although the 75% *OP* rise time remains short (because of the increased amplitude of the *OI* transition) the *IP* rise gets slower and slower. It was noted in some preparations that because of this phenomenon, the 100% *OP* rise time was considerably longer—and the rate of rise considerably slower (sometimes two to three times slower)—in chloroplasts exposed to RLE for extended periods of time than in control chloroplasts.

The already slow decay from the fluorescence peak *P*, in control chloroplasts is further arrested by suspension of the chloroplasts in RLE; the time required to reach the 10% "*PO*" decay level increases with time in extract (see item 10). This retardation by RLE can be observed more readily in the case of intact *Chlorella* cells (to be described in detail, below), in which the decay to the actual *S* level is very rapid in the control situation. (See Fig. 3 and Table II.)

The Influence of CMU. Aliquots of CMU were added to the control, subsequent to the fluorescence induction measurement made 1½ hr after mixing

(see Table I); these data have also been expressed in terms of ratios (experimental-to-control).

Low temperature, steady-state fluorescence emission spectra were determined with such CMU-treated chloroplasts and it was found that, even after equally long periods of incubation in CMU, the types of spectral changes which accompany RLE incubation are not observed.

The influence of CMU on fluorescence induction of chloroplasts will now be compared with that of RLE (see Table I and Fig. 2). The presence of 4×10^{-6} M CMU has little effect on total fluorescence. However, with increasing concentration, the apparent level of *O* increases, and the sum of $c + v$ decreases. At 8×10^{-5} M CMU total fluorescence has fallen to about 90% of the control (see Table I, item 3, and Fig. 2), the change here, too, resulting primarily from decrease in variable fluorescence (see items 1 and 2).

In 8×10^{-5} M CMU almost all of the *OP* rise is composed of the fast component *OI* (items 4–6), the slow rise having been practically obliterated. (The magnitude of *OI* is only slightly smaller in 4×10^{-6} M CMU than in 8×10^{-5} M CMU.)

As a consequence of the increase in magnitude of *OI* (and perhaps also increase in its slope—see item 7) the *OP* rise times are shortened (items 7 *a*, 8 *a*, and 9 *a*) and the rates of rise accelerated (see items 7 *b*, 8 *b*, and 9 *b*) as they are with chloroplasts exposed to RLE for 5 min. That the rates of rise in CMU are faster than in RLE (especially to 100% *OP*) is due largely to the contribution of the slow component (*IP*) of variable fluorescence in the latter. Indeed the persistence of the *IP* transition and its marked deceleration with increasing time in RLE constitute two of the major differences in the effects of RLE and CMU. Also, in the case of CMU addition, the time required to reach the 10% "*PO*" decay level is increased, just as it is in the case of chloroplasts suspended in RLE (see item 10).

The Influence of RLE on Steady-State Fluorescence and Fluorescence Induction in Chlorella

Refer to Fig. 3, in which are presented tracings of fluorescence induction curves for *Chlorella* suspended in E-I medium (control), or in RLE for periods of 15 and 45 min; the salient features of these curves have been summarized in Table II. The level of the inflection point, *I*, and, therefore, the exact magnitude of the fast component, *OI*, of the *OP* rise is difficult to determine. In such cells, the rise in variable fluorescence is very fast and there is present little slow component (compare with Fig. 2); however, it may be that here, too, RLE is increasing the amplitude of the fast component (item 4 in Table II) and the proportion which this fast component contributes to variable fluorescence (see items 5 and 6). The situation in regard to the *OP* rise is even less clear (item 7). The most pronounced change brought about by RLE in *Chlorella* cells is in the decay of fluorescence from the peak to the stationary level. Although the actual time it takes to reach the *S* level decreases in the

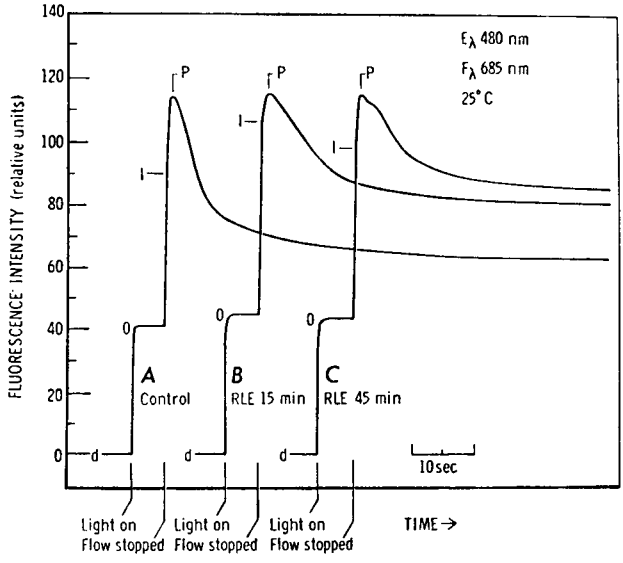


FIGURE 3 Tracings of fluorescence induction recordings of intact *Chlorella* cells. Curve A, control; curves B and C, cells incubated in RLE for 15 and 45 min, respectively. Other conditions as in Fig. 2.

presence of RLE (see item 13 *a*), the level of *S* itself has risen towards *P* (see items 8, 9 and 10). Thus, the rate of decay has actually slowed down (see item 13 *b*). This phenomenon may be additionally observed in the time it takes to decay 20% and 40% from the peak to the *S* level (items 11 *a* and 12 *a*), as well as in the respective rates of decay (items 11 *b* and 12 *b*).

The major difference in the effect of RLE on fluorescence induction in whole *Chlorella* cells and on corn chloroplasts is that in the former, the "slow" changes in fluorescence induction do not seem to occur to any appreciable extent. For example, in intact cells the marked decrease in fluorescence yield, especially in the variable portion, is not observed (see items 1–3).

Steady-state emission spectra (77°K) were determined for intact *Chlorella*, both for the control case, and for RLE-treated cases. It was found that, even after 45 min in RLE at 25°C, few spectral changes had occurred. Although the 685 nm band had "disappeared" the ratio of fluorescence intensities, F 725/F 698, had decreased to only 1.7 in comparison to a ratio of 1.9 in control cells. (It has subsequently been determined with chloroplasts of *Chlorella* that this ratio decreases to 0.6 after 45 min of incubation in RLE at 25°C.)

The Size and Nature of the Active Components in RLE

In a preliminary attempt to determine the nature of the active components (i.e., those capable of giving rise to the observed fluorescence induction changes), crude

TABLE II

THE EFFECT OF RLE ON FLUORESCENCE INDUCTION OF *CHLORELLA* CELLS

Fluorescence induction parameters	Control *C*	Period of incubation in RLE			
		15 min		45 min	
		Experimental *E*	$\frac{E}{C}$	Experimental *E*	$\frac{E}{C}$
1. *v* (mm)	73.0	70.0	0.96	71.0	0.973
2. *c* (mm)	42.0	45.8	1.09	43.5	1.03
3. *c* + *v* (mm)	115.0	115.8	1.01	114.5	0.996
4. *OI* (mm)	49.0	59.0	1.21	55.0	1.12
5. *OI/v*	67.0%	84.0%	1.25	77.5%	1.16
6. *OI/c* + *v*	42.6%	51.0%	1.20	48.0%	1.13
7. 100% *OP* rise:					
*a**	1.50	1.80	1.20	2.00	1.33
*b**	0.21	0.026	1.250	0.028	1.375
8. *PS*	52.5	34.0	0.648	30.0	0.57
9. *PS/v*	71.8%	48.5%	0.675	42.3%	0.59
10. *PS/c* + *v*	45.6%	29.4%	0.645	26.2%	0.575
PS decay					
11. 20% decay					
a	1.80	2.80	1.56	2.80	1.56
b	0.171	0.412	2.40	0.467	2.73
12. 40% decay					
a	3.00	5.00	1.67	4.80	1.60
b	0.143	0.368	2.57	0.400	2.80
13. 100% decay					
a	50.00	34.00	0.681	43.00	0.86
b	0.954	1.00	1.05	1.43	1.50

* *a*, time (sec); *b*, rate (sec/mm).

RLE was placed in a cellulose sac and dialyzed against a total of 60 times its own volume of E-I medium (six changes of medium during a 24-hr period) in the cold (5°C). The contents of the sac (predominantly larger molecules), when tested on chloroplasts, gave only very weakly the "fast" changes, but much more strongly the "slow" changes. A second dialysis experiment was made, in which the crude extract, in the sac, was dialyzed against three times its volume of E-I medium for a period of 18 hr. When the nondialyzed portion was diluted to a volume equal to that of the dialyzate, and the two aliquots tested, they gave about equal activity for fluorescence induction changes of the "fast" kind, but changes of the "slow" kind were again only given by the nondialyzed portion.

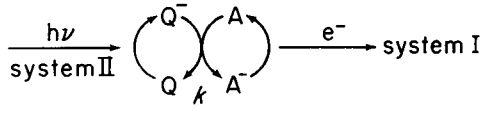
When crude RLE was chromatographed on a Sephadex G-25 column, two distinctly different fractions were collected; these were tested on chloroplasts for fluorescence induction activity of the "fast" type. The first fraction, consisting of large molecules, came quickly through the column. It was colorless and turbid and yielded a broad absorption band (between 260 and 280 nm) in the near ultraviolet.

This fraction gave only slight activity of the "fast" kind; however, it did bring about changes in steady-state emission similar to those produced by the crude extract. The second fraction, consisting of smaller molecules, was removed only very slowly from the column. It was clear, pale lemon-yellow in color, and slightly fluorescent (orange-yellow). It yielded an absorption spectrum consisting of a narrow band with maximum at 270 nm and a secondary (lower and broader) band at 360–380 nm with a long "tail" out to about 440 nm. This fraction, when tested on chloroplasts, showed strong fluorescence induction activity of the "fast" type; low temperature steady-state emission characteristics were not modified in its presence.

DISCUSSION

In the results section of the paper it was seen that two types of changes in fluorescence induction are brought about by RLE. Even though these are probably occurring simultaneously at different rates, "fast" and "slow" changes will be discussed separately.

A frequently used model will be utilized to interpret the data given above:



In the model, Q may be thought of as the quencher of Duysens and Sweers (17), or alternately as Joliot's E (see references 16–18); when Q (the primary oxidant or electron acceptor of system II) is in the Q^- state it cannot quench the excitation of chlorophyll$_{II}$ and therefore, the reduction (by the strong primary reductant) of Q to Q^-, brought about by events following absorption of light by system II, results in the rise of variable fluorescence from the *O* to the *P* level; refer to Fig. 1. The fast component of this rise, *OI*, represents the direct photochemical conversion (19) of Q to Q^- (see, however, references 20 and 21 for the involvement of activation, as well as reduction, in this process), while the slow component of this rise, *IP*, represents the slowing down of the Q to Q^- conversion because of continued restoration of Q by the light-independent A to A^- reduction (A being the large pool of secondary oxidant); hence, this second part of the rise is largely nonphotochemical. The reoxidation of A^- to A (present at about 20 times the concentration of Q) presumably results from electron transfer to system I, to which it is coupled by a chain of intermediates. The decay of fluorescence from the *P* to the *S* level is probably associated with the formation of a quencher distinct from Q; see, for example, the suggestion of Duysens and Sweers (17). (Munday and Govindjee (22) have alternatively suggested that this decline could be associated with induction of the Calvin cycle.)

Inhibition of system II by CMU is accompanied by augmentation of the *OI* transition, suppression of the *IP* transition, and retardation of the decay from the *P* level—phenomena which have been interpreted (17, 23) in terms of the herbicide

acting between Q^- and A (the break in electron flow to system I presumably keeping fluorescence at its maximal value *P*). Seen among the first effects of RLE is also a more rapid reduction of Q. However, RLE does not suppress the *IP* rise, while CMU does. It is not certain, at the present time, if the fast acting component of RLE behaves as a nonsaturating concentration of CMU—at which the *IP* phase remains always visible but smaller, or if it (more likely) acts in some way or at some site different than CMU, so that *IP* is not suppressed. Decrease in the rate of reaction between Q^- and A, or partial inactivation of A, would result in less slowing of the Q to Q^- conversion (and, therefore, in increased *OI* transition).

Also observed among the first effects of RLE on fluorescence induction is a (small) increase in maximum fluorescence yield, principally in the variable portion. This may reflect a larger concentration of Q^-, perhaps as a result of conversion of some of the quencher distinct from Q, into Q itself, and/or as a result of a "backup" of electrons from a break in the electron transport chain to system I. In regard to the latter, it has been noted in experiments on light-induced changes in absorption of chloroplasts suspended in RLE (24), that there is present a component which affects system I (enhancing the changes at low concentrations and inhibiting them at higher concentrations). Furthermore, studies of oxygen transients (25) reveal an immediate decrease in the photochemical activity of system I upon introduction of RLE to suspensions of chloroplasts.

The slowing up of the fluorescence decay, *PS* or "PO," which results in the yield being kept close to its maximum level, may be interpreted in terms of the disruption of electron transport from system II to system I, as above, or, on the basis of the rise towards *P* of the *S* level itself, especially evident in the case of *Chlorella* (see also reference 26), a diminution in the amount of quencher distinct from Q.

In their 1968 paper on the effect of manganese deficiency on fluorescence properties of chloroplasts, Anderson and Thorne (27) reported an enhancement in the 77°K ratio F 735/F 693 (6.2 in the deficient as compared with 2.3 in the control case), i.e. a change opposite in direction to the one produced by RLE. Additionally, they reported that in the case of chloroplasts in 10^{-5} M 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), the ratio F 735/F 693 also rose (to 2.9). No such change in ratio was observed in the present work, upon addition of CMU to chloroplasts. To determine whether the F 735/F 693 enhancement phenomenon were specific to DCMU, the experiment was repeated with several concentrations of DCMU, up to 10^{-4} M. It was not possible to reproduce the changes reported by Anderson and Thorne, unless the stock solutions of DCMU were prepared so as to yield final suspending media 3–5% ethanolic in water.

It may be recalled that in RLE-treated chloroplasts the "slow" changes in fluorescence induction exhibit a time dependence similar to that of conversion of the three-banded steady-state emission spectrum (77°K) into a (primarily) one-banded spectrum. From this, it seems likely that both these phenomena are brought about by action of the same component of the extract. Among the effects of the slower act-

ing component on fluorescence induction is a marked diminution of the variable portion. Briantais (28), using system II and system I chloroplast fragments, isolated by differential centrifugation of detergent-treated chloroplasts, showed that the variable portion of fluorescence induction is greatly enriched and greatly reduced, respectively, in these fragments, compared to whole chloroplasts.

Since variable fluorescence is closely related to system II, it is not surprising that its marked diminution by the slower acting component of RLE is paralleled by decreases in both Hill activity (9, 29, 30) and system II-associated light-induced changes in absorption (24). Again, it may be worthwhile to compare the action of CMU with that of the slower acting component of RLE. Although CMU also inhibits system II, it does not block electrons from reaching Q. In contrast, one notes that with increasing time of exposure to RLE, less Q^- is produced. Thus, it would seem that the slower acting component of RLE is either blocking electron flow between H_2O and the reaction center of system II or is preventing electrons from getting to Q via the reaction centers. In the latter category are the possibilities of reaction centers being destroyed or electrons leaving the reaction centers but being detoured away from Q. (From oxygen transient studies (25), decrease in number of system II reaction centers was given as one of the effects of RLE on chloroplasts.) On the other hand, the diminution of variable fluorescence may result, not from aberrant photochemistry, but from impairment of energy transfer. Were the transfer of excitation energy to the reaction center chlorophylls diminishing because of disorganization of photosynthetic units, there would obtain a condition tantamount to decrease in number of functional reaction centers; that prolonged incubation of chloroplasts in RLE is accompanied by structural modifications (such as swelling of thylakoids and disruption of normal lamellar arrangement) has already been noted (9, 29, 30).

The observation was also made that prolonged exposure to RLE sometimes results in a strong retardation of *IP*, the slow component of the variable rise. Perhaps this retardation is a secondary effect which comes about because the interaction between Q^- and A has become relatively more efficient. At the present time, no explanation can be offered for the loss of some of the initial RLE-induced increase in amplitude of the fast component of variable fluorescence.

From experiments on the size and nature of the active components of RLE, particularly those with the two different fractions obtained through Sephadex chromatography, the following (see also reference 31) may be derived. The fast changes in fluorescence induction are brought about by the fraction of lower molecular weight, while the slower changes are brought about by the fraction of higher molecular weight; the latter (protein-like fraction) is also responsible for the changes in steady-state emission.[3] The experiments with intact *Chlorella* cells in RLE lend support to

[3] It has been noted that several substances, including long chain unsaturated fatty acids serve as good model systems for the action of the protein fraction of RLE (29, 30); that the changes in steady-state emission brought about by these substances result from a deaggregation of chlorophyll was

these contentions: the changes in fluorescence induction which occurred were almost exclusively of the "fast" type, and additionally, very little modification in steady-state emission (77°K) occurred. Such results could well be interpreted in relation to penetration of a small molecule, and exclusion of a large molecule, by the intact plasma membrane of the *Chlorella* cells.

Both components of RLE are of interest because they are naturally occurring substances that bring about some effects similar to those of extensively used synthetic compounds. Also, as seen in experiments on steady-state emission, the slower acting component is able to bring about large changes in the pathway of absorbed light energy by acting (directly or indirectly) on the chloroplast lamellae. It is possible that this molecule, or one closely related to it, represents the in vivo monitor for controlling both the partition of energy and electron transfer between system II and system I of photosynthesis.

The author wishes to express her appreciation to Professor Jean Lavorel for so graciously making available his laboratory facilities and for reading the manuscript.
She wishes also to thank Dr. H. de Klerk for his aid in chromatography experiments.

The experimental work reported here was done by the author at the Laboratoire de Photosynthèse, Centre Nationale de la Recherche Scientifique Gif-sur-Yvette, France, during sabbatical leave.

This work was supported, in part, by a CUNY Faculty Research Grant.

Received for publication 8 August 1969 and in revised form 28 August 1970.

REFERENCES

1. LITVIN, F. F., A. A. KRASNOVSKII, and G. T. RIKHIREVA. 1960. *Dokl. Akad. Nauk S.S.S.R.* **135**:1528.
2. BRODY, M., and H. LINSHITZ. 1961. *Science (Washington).* **133**:705, Fig. 1.
3. BERGERON, J. 1963. *Nat. Acad. Sci. Nat. Res. Counc. Publ.* **1145**:527.
4. BRODY, S. S., and M. BRODY. 1963. *Nat. Acad. Sci. Nat. Res. Counc. Publ.* **1145**:455.
5. GOVINDJEE. 1963. *Nat. Acad. Sci. Nat. Res. Counc. Publ.* **1145**:318.
6. KOK, B. 1963. *Nat. Acad. Sci. Nat. Res. Counc. Publ.* **1145**:45.
7. GOEDHEER, J. C. 1964. *Biochim. Biophys. Acta.* **126**:304.
8. BROYDE, S. B., and S. S. BRODY. 1965. *Biochem. Biophys. Res. Commun.* **19**:444.
9. BRODY, M., B. NATHANSON, and W. S. COHEN. 1969. *Biochim. Biophys. Acta.* **172**:340.
10. MCCARTY, R., and A. JAGENDORF. 1965. *Plant Physiol.* **40**:725.
11. JAGENDORF, A., and M. AVRON. 1958. *J. Biol. Chem.* **231**:277.
12. LAVOREL, J., and C. LEMASSON. 1961. *Biochim. Biophys. Acta.* **49**:574.
13. ARNON, D. I. 1949. *Plant Physiol.* **24**:1.
14. LAVOREL, J. 1965. *Photochem. Photobiol.* **4**:819.
15. LAVOREL, J. 1964. *Biochim. Biophys. Acta.* **88**:20.
16. JOLIOT, P., and J. LAVOREL. 1964. *Bull. Soc. Chim. Biol.* **46**:1607.
17. DUYSENS, L., and H. SWEERS. 1963. Studies on microalgae and photosynthetic bacteria. Japan Society of Plant Physiology, University of Tokyo, Tokyo. 353.
18. JOLIOT, P., A. JOLIOT, and B. KOK. 1968. *Biochim. Biophys. Acta.* **153**:635.

proposed by Nathanson and Brody (32). Experiments now in progress indicate that certain polyphenolics (e.g. rutin) induce changes in fluorescence induction similar to those brought about by the fast acting component of RLE.

19. DELOSME, R. 1967. *Biochim. Biophys. Acta.* **143**:108.
20. JOLIOT, P. 1965. *Biochim. Biophys. Acta.* **102**:116.
21. FORBUSH, B., and B. KOK. 1968. *Biochim. Biophys. Acta.* **162**:243.
22. MUNDAY, J., and GOVINDJEE. 1969. *Biophys. J.* **9**:1.
23. MURATA, N., M. NISHIMURA, and T. TAKAMIYA. 1966. *Biochim. Biophys. Acta.* **120**:23.
24. BRODY, M., S. BRODY, and G. DÖRING. 1970. *Z. Naturforsch.* **25b**:862.
25. BRODY, S. 1970. *Z. Naturforsch.* **25b**:855.
26. LAVOREL, J. 1959. *Plant Physiol.* **34**:204.
27. ANDERSON, J., and S. THORNE. 1968. *Biochim. Biophys. Acta.* **153**:819.
28. BRIANTAIS, J. 1966. *Photochem. Photobiol.* **5**:135.
29. BRODY, M. 1969. *Biophys. Soc. Annu. Meet. Abstr.* **9**:A-121.
30. COHEN, W. S., B. NATHANSON, J. E. WHITE, and M. BRODY. 1969. *Arch. Biochem. Biophys.* **135**: 21.
31. BRODY, M. 1969. Abstracts of the 11th International Botany Congress. Seattle. 22.
32. NATHANSON, B., and M. BRODY. 1970. *Photochem. Photobiol.* In press.