Derivation of an Electron-Transport Rate Equation, Energy-Conservation Equations and a Luminescence-Flux Equation of Algal and Plant Photosynthesis

By YUNG-SING LI

Institute of Botany, Academia Sinica Nankang, Taipei, Taiwan, Republic of China 115

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On the assumptions that the photosynthetic electron-transport rate is sometimes limited on the water-splitting side of Q (the oxidized primary electron acceptor), and that Q reduction, as well as primary charge recombination, is not kinetically a monomolecular process, a rate equation, a luminescence-flux equation and several versions of energy-conservation equations are derived. The energy-conservation equations explain most, if not all, observed relationships between rate and fluorescence. In particular, by assuming that the limiting site on the water-splitting side of Q is uncoupler-sensitive, these equations explain the uncoupler-induced simultaneous stimulations of rate and fluorescence as well as inhibition of luminescence without additional assumptions *ad hoc* for each individual phenomenon. A newly introduced parameter central to the derivation of these equations is the specific affinity between two electron carriers.

The steady-state rate of photosynthetic electron transport is sometimes assumed to be determined by the concentration of the oxidized primary electron acceptor (Q) of Photosystem II, for the limiting steps of electron transport are generally believed to be located between Q and the site of the terminal reduction (designated as limiting Site I). The rate equation based on this assumption and on the concept of fixed intermediates of electron transport is:

$$R = \lambda q \quad (1)$$

(Malkin & Kok, 1966; Forbush & Kok, 1968), where R is rate, q is the effective concentration of Q and λ includes light intensity, absorbances and quantum yield for the photochemical conversion:

The real value of q (qr) depends on the model of photosynthetic unit. {For the relationships between q and qr , see Joliot & Joliot, 1964; Wraight, 1972; Otten, 1974. For instance, in the Joliot & Joliot (1964) model,

$$q = qr/[1 - p(1 - qr)]$$

where p is the probability of interunit transfer. Unless otherwise defined, the lower-case letters stand for effective concentrations of the corresponding electron carriers throughout this paper.}

In any case, the rate which is proportional to q , and the fluorescence (variable) which is proportional to q^- should show complementary linear changes according to eqn. (1), for $q + q^- = 1$ for all models of photosynthetic unit. Although a linear relation

between rate and fluorescence has been observed (Joliot, 1965), it is by no means universal; non-linear changes between the two have also been observed, for example, in the case of partial inhibition of rate by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (Joliot, 1966), and with the use of hydroxylamine oxidation to monitor the rate of Q reduction in the presence of a saturating amount of 3-(3,4-dichlorophenyl)-1,1-dimethylurea [a minute derivation from linearity in Fig. 3 of Bennoun & Li (1973)]. In both cases variable fluorescence and rate show rectangular-hyperbola relationships. Even parallel changes of rate and fluorescence have been found in well coupled chloroplasts on uncoupler addition [Li (1973), but note, not the photochemistry and fluorescence potential increase on salt addition as reported for example by Li (1975)] or in transient states in algae (Bannister & Rice, 1968). Various treatments that inactivate the water-splitting system produce chloroplasts with low rates of electron transport, in spite of the fact that in these chloroplasts the concentration of Q is high as indicated by the low intensity of fluorescence and the stimulation of fluorescence by Photosystem-II donors and inhibitors (Yamashita & Butler, 1969).

A photophosphorylation coupling site located around Photosystem-II (Böhme & Trebst, 1969; Izawa *et al.*, 1973) and photosynthetic control has been found (Reeves & Hall, 1973; Trebst & Reimer, 1973; Heathcote & Hall, 1974). From fluorescence studies Gimmmler (1973) & Li (1973, 1978) have concluded that there is an uncoupler-sensitive rate-limiting site on the water-splitting side of Q (limiting Site II). Eqn. (1) is therefore not a general

equation for describing the rate of photosynthetic electron transport, even in normal chloroplasts or algae, for it is based on the assumption that electron transport is limited solely by steps located between Q and the site of the terminal reduction, and this assumption limits its ability to describe all the experimental observations.

Derivation of a Rate Equation

The rate of the reaction between any electron donor and acceptor pair is sufficient to express the steady-state rate of photosynthetic electron transport. However, the reaction between the Photosystem II primary donor and its acceptor is of particular interest, for a rate equation written in terms of them can correlate the fluorescence parameter with the rate parameter because of the conservation of light energy at the Photosystem II reaction centre. A rate equation may therefore be derived, on the basis of the following sequential reactions of Photosystem II:

D^+ and D are the oxidized and reduced primary electron donor of Photosystem II respectively; k is the composite rate constant of reactions (uncoupler-sensitive) that regenerate D ; Q and λ have been defined, and λ_- is the rate constant of the back reaction between D^+ and Q^- .

From eqn. (4) we write:

$$R_2 = \lambda(d, q) - \lambda_-(d^+, q^-) \quad (5)$$

R_2 is the rate of the overall reaction of eqn. (4); (d, q) is the effective percentage concentration of DChlQ, which is the photochemically active reaction centre, and (d^+, q^-) is that of D^+ChlQ^- , which is photochemically inactive. The question now is how to write (d, q) and (d^+, q^-) in terms of concentrations of D and Q .

The concept of fixed intermediates requires, in theory, that the maximal value of (d, q) is equal to the concentration of either D or Q , whichever is smaller. $(d, q) = d$, when $d < q$, or $(d, q) = q$, when $q < d$. In reality the concentration must be smaller than that, for the fates of Q^- and D^+ are largely independent. And eqn. (5) can then take the form:

$$R_2 = \lambda qx - \lambda_-(1-q)x_- \quad (6)$$

where x and x_- are smaller than or equal to unity, x is unity whenever d equals unity, for other values of x , let:

$$x = ds \quad (7)$$

$$x_- = d^+s_- \quad (8)$$

s is defined as specific affinity between Q and D , which is normally equal to or greater than unity, i.e. $qx = qds \geq qd$, for the reaction taking place between fixed intermediates, and s_- is the specific affinity between Q^- and D^+ . From eqn. (3), we obtain:

$$R_d = kd^+ \quad (9)$$

where R_d is the rate of D regeneration, and:

$$d^+ = R_d/k \quad (10)$$

$$d = 1 - R_d/k \quad (11)$$

Substituting eqns. (7), (8), (10) and (11) into (6), we obtain:

$$R_2 = \lambda qs(1 - R_d/k) - \lambda_-(1 - q)s_-R_d/k \quad (12)$$

In steady state, $R_2 = R_d = R$, where R is the rate of the overall electron transport. In transient states, owing to the fact that the substrate of reaction (3) is regenerated by reaction (4), therefore $R_2 \approx R_d$ in any finite time interval. (Transient states can be considered as many short-lived steady states.) On these assumptions we obtain by re-arrangement of eqn. (12):

$$R = \lambda skq/(k + \lambda qs + \lambda_-s_- + \lambda_-s_-q) \quad (13)$$

if λ_-s_- is small (high photochemistry quantum yield), and λ_- and λ_-q can be neglected, then:

$$R = \lambda skq/(k + \lambda sq) \quad (14)$$

here, q is also a function of the activity of Photosystem I (see the Appendix).

Discussion of the Parameter s

To express correctly rate in terms of q and d , one has to take both the concept of fixity of intermediates (first order in space domain) and that of the independent fluctuations of the states of D and Q (second order in time domain) into consideration. For non-fixity intermediates reaction, $R = \lambda dq$, but for centre-fixity reaction, $R = \lambda sdq$, $s \geq 1$ normally. In what follows, s is defined in terms of both concepts.

In continuous light and at any particular moment we may group all the Photosystem-II reaction centres into two categories. In the first category, all the reaction centres have recently received one or more photons, which have initiated charge separations and produced D^+ and Q^- in some of these centres. At this moment the photochemically active reactants (D and Q) are being regenerated in these centres.

Suppose the regenerations of D and Q are totally random, and let the concentrations of regenerated D and Q be d_1 and q_1 respectively in the first category and at this particular moment, then the concentration of Q being re-reduced ($q_{\bar{r}1}$) at this particular moment and in the first category is:

$$q_{\bar{r}1} = id_1q_1$$

here i is the number of new photons just received by all reaction centres in the sample at this moment. If, however, D and Q in some reaction centres are regenerated more or less synchronized, while in others they are not, then

$$q_{rr1} = id_{1n}q_{1n} + iq_{1s}$$

the term iq_{1s} , disregard i , is first order for all Q_{1s} have a D_{1s} (concept of fixed intermediates).

In the second category, the reaction centres have been idling for such a long time that all Q_2 centres are in DChlQ state [all D_2 centres may not be in DChlQ state, however, because (1) that as a whole, Q regeneration is slower, and (2) that there is a Q reduction by the electron acceptor of Q, A, in the dark and the rate of this back-reaction is appreciable (Joliot *et al.*, 1968; Forbush & Kok, 1968)], therefore:

$$q_{rr2} = iq_2$$

(If, however, dark oxidation of D occurs, the expression will be different.) The total Q re-reduced (q_{rr}) is then

$$q_{rr} = i(d_{1n}q_{1n} + q_{1s} + q_2) = isdq$$

and

$$s = (d_{1n}q_{1n} + q_{1s} + q_2)/dq$$

in which the terms q_{1s} and q_2 reflect the property of centre fixity, q_{1s} indicates further the degree of synchronization (if it exists) of D^+ reduction and Q^- oxidation, whereas the term $d_{1n}q_{1n}$ reflects the independent fluctuations of states of D and Q.

With our present knowledge of photosynthesis it is not even possible to identify D, not to say the values of d_{1n} , q_{1n} . . . etc. However, one may estimate the s value by the following procedure: (1) simultaneous measurements of rate and fluorescence better if variable fluorescence is estimated, or still better simultaneous measurements of rate and the concentration of x-320, the latter is assumed to be the oxidized Photosystem-II primary acceptor (Stiehl & Witt, 1969); (2) λ and k may be estimated from rate-light-intensity measurement, on the assumption that k and λ do not change as light intensity varies; Wang & Myers (1974), however, provide evidence that a factor included in our λ values does vary with intensity, but the variation seems to be small (less than 10%); (3) calculate s from eqn. (14).

Properties of the Rate Equation

Fig. 1 shows the theoretical relationships between sq and rate calculated according to eqn. (14). Their relationships depend on the values of k . When k is much greater than λ , a D is ready most of the time for a Q (for example, when $k = 10^4$ and $\lambda = 100$, D regeneration will be much faster than the rate of

Fig. 1. Theoretical relationships between rate and sq as a function of the value of k

Rates are calculated according to $R = \lambda skq/(k + \lambda sq)$; $\lambda = 100$ for all cases. ●, $k = 10^4$; □, $k = 10^2$; ○, $k = 10$.

photon absorption, i.e. $d \approx 1$, $s \approx 1$ and $R = \lambda qx \approx \lambda q$) as a result rate and fluorescence are expected to be linear. With a smaller value of k , non-linearity between rate and sq becomes apparent.

Without a knowledge of s , there are always uncertainties when a set of experimental rate and fluorescence data are compared with a calculated curve, for the latter is calculated with sq , whereas the experimental observed fluorescence is assumed to be proportional to $(1-q)$. However, so far as we are interested only in showing that, contrary to eqn. (1), eqn. (14) predicts non-linearity of rate and fluorescence, we can ignore the differences between sq and q for the following reasons: we have assumed that s is normally equal to or greater than unity, i.e. $sq \geq q$, therefore the substitution of q for sq will move the middle portions of all curves in Fig. 1 closer to the ordinate while the end points, i.e. $sq = q = 1$ and $sq = q = 0$, are fixed; in other words, if rate and sq are non-linear, rate and fluorescence are more so. The non-linearity of rate and fluorescence observed by Joliot (1966) and Bennoun & Li (1973) may therefore be explained by eqn. (14). I emphasize again that eqn. (1) does not predict non-linearity between the two, assuming whatever model of photosynthetic units appeared in the literature.

As stated before, the mutual dependence of d^+ and q in the primary light reaction of Photosystem II makes eqn. (14) valid for the transient-state rate description as well, and it is not limited to the normal transient state. The transient state of Photosystem-II primary reactions of chloroplasts incubated with 3-(3,4-dichlorophenyl)-1,1-dimethylurea and hydroxylamine (Bennoun & Li, 1973) are still going on according to eqns. (3) and (4), even though the electron transfer between Q^- and its electron acceptor, A, is inhibited. The reaction $D+Q \longrightarrow D^++Q^-$ must be followed up by $D^+ \xrightarrow{k} D$, even in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea and hydroxylamine to stabilize the primary charge separation. (Hydroxylamine provides the electron to D^+ , therefore the k contains the hydroxylamine concentration term in this case.) Recombination of D^+ChlQ^- in the presence of these two agents is suggested by submillisecond luminescence (Lavorel, 1973).

In general, Fig. 1 indicates that, depending on the relative values of k and λ and on whether k is constant, rate and fluorescence can be complementally linear, non-linear or even non-complemental. Li (1973) has explained his observation of non-complemental changes of rate and fluorescence in terms of changes in k values.

Table 1 shows the relationship between the normalized concentration of the reduced primary electron donor, d , and sq , when the values of k changed. The fact that d increases when q decreases is not physically surprising; it happens under various conditions. For example, with 3-(3,4-dichlorophenyl)-1,1-dimethylurea-inhibited chloroplasts or dithionite-incubated chloroplasts, q is small in the light, and the conversion of D into D^+ is slowed down for lack of Q, which results in a high value of d . One may look into the matter in a different situation: under constant high-light conditions, the faster is D regenerated from D^+ (i.e. the larger the k is), the faster the reaction $Q+D \longrightarrow Q^-+D^+$ goes, and the more Q^- there is. Here, the more D there is, the less the Q; clearly a high steady-state concentration of Q^- does not

necessarily lead to a low rate of photosynthesis under all conditions. Experimentally, this is illustrated again by the parallel enhancements of rate and fluorescence in well coupled chloroplasts on uncoupler addition, in this particular case fluorescence is enhanceable only when photochemistry is going on and the concentration of Q is kept low (Li, 1973).

In the study of the photochemistry rate, it is necessary to establish the relation between reaction rate and light intensity. Lumry & Spikes (1957) observed light curves that closely approximate to rectangular hyperbolas, and Rieske (1956) has obtained the following rate law:

$$R = k_L k_D I / (k_L I + k_D) \quad (15)$$

where k_L is the rate constant for the limiting light step, k_D is the composite rate constant at infinite light intensity, and I is the constant intensity within the reaction layer of infinitesimal depth. By assigning λsq to $k_L I$ and k to k_D , eqn. (14) gives, or rather confirms, a mechanistic interpretation of eqn. (15).

Lumry & Spikes (1957) have found that both k_L and k_D are complex functions of Hill-oxidant concentrations and pH of the reaction medium, and are inhibited by 2H_2O . As the comparison of eqns. (14) and (15) shows, k_L , which corresponds to $\lambda sq/I$, is not a pure limiting light step, because the q is controlled by the Photosystem-II light reaction, dark limiting steps between the two photosystems as well as the light reaction of Photosystem I (see the Appendix), while s , which is also included in k_L , is a function of the property of the water-splitting system and that of Q. Perhaps it is the s factor that makes k_L sensitive to 2H_2O .

Energy Conservation Equation

The 'fundamental alternative' law expressed by:

$$\Phi_{ps} + \Phi_F / \alpha = 1 \quad (16)$$

is not satisfactory, as it is defined by Lavorel & Joliot (1972), to describe many observations on chloroplast fluorescence. This equation shows a

Table 1. Normalized concentration (d) of reduced primary donor (D) as a function of sq , k and λ , according to the relationship $d = k/(\lambda sq + k)$

For all cases $\lambda = 100$. See the text for definitions.

sq	Normalized concentration of D		
	$k = 10000$	$k = 100$	$k = 1$
1	0.99	0.5	0.0099
0.8	0.992	0.555	0.0123
0.5	0.995	0.666	0.0196
0.3	0.997	0.769	0.0323
0.1	0.999	0.909	0.0909
0	1	1	1

linear variation of the photochemical yield Φ_{ps} with the fluorescence yield Φ_f . Fluorescence is assumed to be a constant proportion (α) of all non-photochemical losses. However, there are cases where photochemistry and fluorescence are not linear. A new energy-conservation equation that describes non-linearity as well as linearity of rate and fluorescence is derived as follows.

Like eqn. (16), this new energy-conservation equation will be written in terms of photochemistry and fluorescence. Since, at room temperature, fluorescence is basically a Photosystem-II phenomenon, this equation is restricted to Photosystem II. For derivation, two basic postulations are made: (1) open units (with oxidized Q)+closed units (with reduced Q)=total units; (2) equal absorbances of open and closed units. (All the concentration terms are effective ones. The term 'unit' is changed to trap for the matrix model of the photosynthetic unit.) From assumption (1), we have:

$$q + q^- = 1 \quad (17)$$

and from assumption (2), we have:

$$I_a q + I_a q^- = I_a \quad (18)$$

where I_a is the light quanta absorbed.

Since only open units carry out photochemistry, we proceed to express $I_a q$ in terms of R , the photochemistry rate. From eqn. (14), and letting $\lambda = \phi_c I_a$, ϕ_c be the elementary quantum yield of photochemistry of a photoactive unit, we obtain

$$I_a q = R / \phi_c s (1 - R/k) \quad (19)$$

Closed units (Photosystem II) are characterized by high fluorescence emission and photoinactivity. $I_a q^-$ is therefore expressed in term of fluorescence (Malkin & Kok, 1966). Assuming a part (f) of the total fluorescence (F) originates from closed units with an elementary quantum yield of ϕ_f . Then:

$$f = \phi_f I_a q^-$$

or

$$I_a q^- = f / \phi_f \quad (20)$$

Substituting eqns. (19) and (20) into (18), and dividing the resultant equation by I_a , we have:

$$R / I_a \phi_c s (1 - R/k) + f / I_a \phi_f = 1 \quad (21)$$

Putting $R / I_a = \Phi_{ps}$, the 'observed quantum yield of photosynthesis' and $f / I_a = \Phi_f$, the 'observed variable fluorescence quantum yield', we then have:

$$\Phi_{ps} / \phi_c s (1 - R/k) + \Phi_f / \phi_f = 1 \quad (22)$$

or

$$\Phi_{ps} / \beta + \Phi_f / \phi_f = 1 \quad (23)$$

here

$$\beta = \phi_c s (1 - R/k)$$

{Eqn. (23) has been published in an abstract [*Brookhaven Symp. Biol.* (1976) **28**, 364].}

This equation may not be the general energy conservation equation yet; Li (1977) has suggested that there are two fluorescence states of Q^- unit. Immediately after Q reduction, the unit is still in a low fluorescence state (Q_1^-), which is transformed into a high-fluorescence state by state transformations. To derive an equation based on this model, we let:

$$f_1 = \phi_{1f} I_a q_1^- \quad (25)$$

here f_1 is the part of fluorescence emitted by all Q_1^- units (low-fluorescence Q^-) with an elementary quantum yield of ϕ_{1f} . Similarly:

$$f_h = \phi_{hf} I_a q_h^- \quad (26)$$

where h designates high-fluorescence state of Q^- . Eqn. (23) is then changed to:

$$\frac{\Phi_{ps}}{\beta} + \frac{\Phi_{1f}}{\phi_{1f}} + \frac{\Phi_{hf}}{\phi_{hf}} = 1 \quad (27)$$

or

$$\frac{\Phi_{ps}}{\beta} + \frac{\Phi_{fc}}{\alpha_c} = 1 \quad (28)$$

where $\Phi_{fc} = \phi_{hf} \Phi_{1f} + \phi_{1f} \Phi_{hf}$, and $\alpha_c = \phi_{1f} \phi_{hf}$.

Discussion of the Energy-Conservation Equations

The equations derived in this paper are examined below with some experimental observations.

From eqns. (18), (19), (20) and (22) we know that Φ_{ps} and Φ_f are not linear, and this is shown experimentally by the observations of Joliot (1966) and Bennoun & Li (1973). However, when $k \gg R$, eqn. (22) becomes

$$\Phi_{ps} = \phi_c s (1 - \Phi_f / \phi_f) = \phi_c (1 - \Phi_f / \phi_f) \quad (29)$$

which shows that the photochemistry and the fluorescence of chloroplasts (Photosystem II) are conservatively linear (Joliot, 1965) (under conditions with $k \gg R$, $d \approx 1$ and $s \approx 1$, Table 1).

Eqn. (13) or a conservation equation derived from it may be used to describe any situation, including perhaps the Tris-treated chloroplasts, where a mini cyclic electron transport around the Photosystem-II reaction centre is suggested to exist (or under some conditions pseudocyclic electron transport), which quenches fluorescence and diverts energy from the linear electron transport.

As stated above, parallel stimulation of rate and fluorescence by uncoupler addition (fluorescence is only enhanced while electron transport is in effect) can be understood if uncoupler increases the value of k (eqns. 14, 19, 20 and 22). Yet, uncouplers increase not only the rate of Q reduction (due to its effect on

Site II), but also that of Q^- oxidation (due to its effect on Site I), consequently uncouplers, depending on conditions, are expected to either stimulate both rate and fluorescence or decrease fluorescence while stimulating rate, or stimulate rate, but have no effect on fluorescence, or enhance fluorescence, but have no effect on rate. Indeed, we have observed that with a Methyl Viologen concentration of $100\mu\text{M}$, gramicidin is found to decrease fluorescence, in contrast with the addition of gramicidin to a sample containing 10nm -Methyl Viologen where fluorescence is increased owing to the addition (Li, 1978).

Parallel stimulation of rate and fluorescence by salt addition to salt-depleted chloroplasts (maximal fluorescence stimulation is observable only when Q is reduced) can also be explained by eqn. (22). In salt-depleted chloroplasts, both rate and fluorescence are low. Fluorescence is low even in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea, or under strong light plus Photosystem-II electron donors. In such a case, the ϕ_c , perhaps, has been lowered by salt depletion, and restored by salt addition (Li, 1975). A small ϕ_c will make the $\Phi_{ps}/\phi_{cs} (1-R/k)$ term in eqn. (22) large even if Φ_{ps} is small. A change in ϕ_c may be an indication of changing properties of the reaction centre. Any change of variable fluorescence in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea may be related to ϕ_r . Under some conditions, changes of ϕ_c and ϕ_r may be 'two faces of a coin'; for instance, a decrease of the rate constant of radiationless loss of energy will increase both ϕ_c and ϕ_r (Li, 1975), whereas under some other conditions, ϕ_c and ϕ_r may not be related, for instance, trypsin treatment lowers the variable fluorescence without changing ϕ_c , at least under light-saturating conditions (Renger *et al.*, 1976). Eqn. (27) may provide an explanation for the last case, i.e. there are two fluorescence states of Q^- unit, and trypsin treatment may change the fluorescence quantum yield of the high-fluorescence state, ϕ_{hr} . The fluorescence yield of photosynthetic units may not be governed by the redox state of Q *per se*, rather a change from low to high fluorescence state may reflect, normally, a state transformation of Q^- unit initiated by the reduction of Q (Li, 1977). Trypsin treatment may interfere with the process of state transformation.

Flux Factor of Chloroplast Luminescence

We have mentioned above that uncoupler stimulates rate and fluorescence simultaneously. Wraight & Crofts (1971) showed that at the same time as the uncoupler enhances fluorescence, it inhibits luminescence. A luminescence-flux equation will be derived in this section, and in the following section an analysis will show that it is possible to explain the uncoupler effects on luminescence in terms of uncoupler-induced increase of the value of k .

A number of pieces of evidence indicate that luminescence is a result of the recombination of electron and hole created by Photosystem-II primary charge separation [for a review, see Laval (1975)]. The luminescence intensity (L) is determined by two factors, a yield and a flux factor. Eqn. (30) is the 'L relation' given by Lavorel (1968):

$$L = (\phi) J \quad (30)$$

it simply says that a flux J of luminescence excitons (ϵ_L) arising from electron-hole recombinations is injected into the photosynthetic unit and is subsequently expressed as a light flux L , with quantum yield (ϕ).

The concept of fixity of centres would suggest that the recombination process was monomolecular (Lavorel, 1975), i.e.

$$L \approx (^+DChlQ^-) \quad (31)$$

However, there is much evidence pointing to the kinetic independence of the two 'sides' of the Photosystem-II centres (the Q side and the D side); the regeneration of D ignores more or less the oxidation of Q^- by A . Furthermore a high rate of back reaction between A^- and Q exists, which also ignores the states of D . Therefore in the time domain the existence of $^+DChlQ^-$ is not a monomolecular phenomenon. The act of recombination itself, so soon as $^+DChlQ^-$ is formed, is monomolecular, and the light reaction which creates the $^+DChlQ^-$ from $DChlQ$ is also monomolecular, but the regeneration of $DChlQ$ and the decay of $^+DChlQ^-$ by way other than recombination are not monomolecular, and it is possible to generate $^+DChlQ^-$ by dark back reactions that are not monomolecular either.

In view of the independent fluctuations of the states of D and Q , the recombination process can be described by the following reaction and rate equations,

and

$$R_b = J = \lambda_- (d^+, q^-) \\ = \lambda_- s_- (1-q) R_d/k \quad (33)$$

for details of the derivation see the section above entitled 'Derivation of a Rate Equation'. In steady state, $R_d = R$, substituting eqn. (14) into (33), we have

$$L = (\phi) \lambda_- s_- \lambda s q^- / (k + \lambda s q) \quad (34)$$

Eqn. (34) is valid only for the steady-state luminescence; it is not applicable to the dark decay of luminescence after either continuous illuminations or flash(es), for then R and R_d may not be equal.

Uncoupler Effect on Luminescence

Fig. 2 shows that an increase of the value of k decreases L . Again s and s_- introduce uncertainties, but Fig. 2 may help to show that it is possible to explain the uncoupler effect on luminescence in terms of changes of k values. This explanation of uncoupler effect on luminescence is named the 'uncoupling hypothesis' to differentiate it from the 'high-energy-state hypothesis' proposed by Wraight & Crofts (1971) to interpret the same experimental observation. The latter hypothesis states that the luminescence flux factor is proportional to the electrochemical proton gradient across the thylakoid membrane; uncouplers dissipate the proton gradient and therefore reduce the luminescence intensity.

The uncoupling hypothesis assumes that there is a coupling site on each side of Q, the reducing and the oxidizing side, and that uncoupler changes the luminescence intensity mainly through its effect on q as a result of uncoupling of both sites. To what extent q is changed on uncoupler addition is not only a function of the properties of the two coupling sites, but also those of the activity of the water-splitting

system, the concentration of the terminal electron acceptor, and the extent of the Mehler (1951) reaction, etc. For these reasons the uncoupler effects on luminescence can be complicated. Furthermore luminescence may be a bimolecular process and the concentrations of both molecules can be affected by uncoupler addition. In general, however, changes of the value of q in either direction due to uncoupler addition may result in an inhibition of luminescence, for if q is decreased due to an increase of k , then d will be increased and the net effect may still be a decrease of luminescence; but, in at least two theoretical cases, luminescence can be stimulated by uncoupler addition: (1) if electron flow through Site I is much more limited than that through Site II (assuming the latter is itself extremely limiting) then addition of uncoupler may bring an $M \rightarrow M'$ transition (Fig. 2) that represents an increase of luminescence due to an increase of the value of q ; (2) if Site II is much more limiting than Site I, an $N \rightarrow N'$ transition may be possible; here a decrease of the value of q enhances luminescence (Fig. 2). There is no report of uncoupler-stimulated luminescence as yet. However, the observation of Wraight & Crofts (1971, Figs. 3 and 4) that a large increment of fluorescence is accompanied with a small decrease of luminescence in one case, whereas a small increment of fluorescence goes together with a large decrease of luminescence in another case on nigericin addition, may be a hint that if conditions are right, it is possible to observe uncoupler-enhanced luminescence. The ways that fluorescence and luminescence respond to nigericin addition and the fact that at about the same fluorescence, luminescence differs vastly (and vice versa) are rather difficult to understand if luminescence is a monomolecular process (Lavorel, 1975) and if high-energy state change is the sole uncoupler effect on luminescence.

The merit of the uncoupling hypothesis is that it explains, without additional assumptions *ad hoc* for each individual phenomenon, the simultaneous increases of rate and fluorescence and decrease of luminescence on uncoupler addition.

Final Remarks

The introduction of the steady-state photosynthetic-electron-transport-rate equation $R = \lambda q$ (eqn. 1) 12 years ago was a natural consequence of the Q hypothesis of Duysens & Sweers (1963) and the observation that fluorescence and rate are complementarily linear. Many fluorescence 'abnormalities' have since been found, and it has become necessary to generate a spectrum of hypotheses to explain each of these 'abnormalities' to keep to the concept that fluorescence and rate are 'normally' complementarily linear. However, the present study shows that the

Fig. 2. Theoretical relationships between luminescence and sq as a function of the value of k

Luminescence is calculated according to eqn. (34); $\lambda = 100$ for all cases. $\square$, $k = 10^2$; $\circ$, $k = 10$; $\triangle$, $k = 1$. For a discussion on $M \rightarrow M'$ and $N \rightarrow N'$ transitions, see the text.

Q hypothesis, together with eqn. (14), is adequate to explain the 'abnormal' non-linear and even the 'abnormal' non-complementary relationships between rate and fluorescence.

Yet to show that the Q hypothesis is adequate to explain these fluorescence phenomena is in no way to assure that Q is the only factor that regulates the fluorescence intensity, for photosynthetic machinery is a complicated system, and fluorescence yield of an emitter is, in general, sensitive to changes in environments of the emitter. Therefore, to establish that the Q hypothesis is a more general hypothesis than previously thought is in no way to disprove the other hypotheses concerning chlorophyll fluorescence, such as the activation hypothesis of Bannister & Rice (1968) to explain the rate-fluorescence parallelism during the induction phase: the State-I-State-II hypothesis of Bonaventura & Myers (1969) to account for the slow fluorescence change in light; the proton-uptake hypothesis of Krause (1974) and of Barber *et al.* (1974) deals with uncoupler stimulation of fluorescence. All these hypotheses, including the Q hypothesis, are not mutually exclusive; several factors may affect fluorescence at the same time. To show to what extent each of these factors influences fluorescence under a particular situation is a difficult task, yet it is one that needs more attention if we are to know more about the photosynthetic primary events.

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APPENDIX

Simplified Reactions and the Rate Equations Derived from them that Relate Photosystem-I Activities with q

$$R_A = k_A(1-q)a - k_{-A}(1-a)q \quad (37)$$

$$R_P = k_P(1-a)p^+ \quad (38)$$

At steady state:

$$q = \frac{k_A a - k_P(1-a)p^+}{k_A a + k_{-A}(1-a)} \quad (39)$$

here a is the normalized concentration of A, p^+ is that of P^+ , the latter is the oxidized pigment P-700,

the Photosystem-I reaction centre; other constants and symbols are defined by eqns. (35) and (36). Substituting eqn. (39) into eqn. (14) we have

$$R = \frac{\lambda s k [k_A - k_P(1-a)p^+]/[k_A a + k_{-A}(1-a)]}{\{k + \lambda s [k_A a - k_P(1-a)p^+]\}/[k_A a + k_{-A}(1-a)]} \quad (40)$$

Comparing eqn. (15) with eqn. (40), we have

$$k_D = k$$

$$k_L I = \lambda s [k_A a - k_P(1-a)p^+]/[k_A a + k_{-A}(1-a)]$$

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