REVIEW LETTER

ENERGY CONSERVATION IN THE PHOTOCHEMICAL REACTIONS OF PHOTOSYNTHESIS AND ITS RELATION TO DELAYED FLUORESCENCE

A.R. CROFTS and C.A. WRAIGHT

Department of Biochemistry, Medical School, University of Bristol, Bristol, BS8 1TD, England

and

D.E. FLEISCHMANN

Charles F. Kettering Research Laboratory, Yellow Springs, Ohio, USA

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Introduction

During recent years, strong evidence has accumulated from several fields which suggests that the photochemical reactions are intimately involved in the generation of energy which is directly available for ATP synthesis, and also that the oxido-reductive poise of the photochemical reaction centres is directly dependent on the potential of the intermediate energetic state leading to ATP synthesis. The evidence is discussed below under three headings:

1) stoichiometry and available energy;
2) energy dependent pigment spectral shifts of chloroplasts and photosynthetic bacteria; and
3) delayed fluorescence.

Photosynthetic energy conservation has been reviewed more generally elsewhere [1–5], and in addition Cheniae [6] has recently discussed the oxygen-evolving reactions of photosynthesis in some detail. These aspects will not be treated in depth here.

1. Stoichiometry and available energy

1.1. *Photophosphorylation*

There is some direct evidence that electron flow between water and the high potential electron acceptor produced by photosystem II of green plants is linked to phosphorylation [7–9]. Böhme and Trebst [10], in a recent study of ascorbate photo-oxidation by chloroplast, showed that ascorbate was oxidized in a DCMU* sensitive reaction in which ATP was produced with P/e_2^- of 0.5. When water replaced ascorbate as H-donor, the P/e_2^- was 1 under otherwise similar conditions. Both ascorbate and H_2O oxidation were stimulated by uncouplers but, in contrast to the reaction with water, ascorbate oxidation and coupled phosphorylation were insensitive to heat treatment. Böhme and Trebst [10] suggested that a phosphorylation site was located between water and photosystem II, and that ascorbate by-passed this site by donating electrons directly to the photosystem.

Kok et al. [11] suggested that insufficient energy was available during dark electron flow from the acceptor of photosystem II to P700 for the synthesis of ATP with the stoichiometry of 1 ATP/2 electron ($ATP/e_2^- = 1$), and that energy for ATP synthesis may be conserved in parallel with the light reactions. Although Kok's estimates of the oxidation–reduction potentials have since been challenged [12, 13], and the stoichiometry of ATP synthesis is probably greater than he assumed, more recent evaluation of these and other parameters suggests that his conclusions as well as those of Böhme and Trebst, are at least partially correct.

In spinach chloroplasts, the commonly measured stoichiometry of phosphorylation linked to ferri-

* *Abbreviations*:
DCMU: 3-(3,4-dichlorophenyl)-1,1-dimethylurea
DMO : 5,5-dimethyl-2,4-oxazolidinedione

cyanide or NADP reduction is between 1 and 1.6 ATP/e_2^- [2, 5, 14–16].

When the rate of electron flow in the absence of phosphate acceptor is subtracted from the rate of electron flow in the presence of acceptor, the stoichiometry under a variety of conditions is close to 2 ATP/e_2^-*, where e^-* is the electron flow stimulated by phosphorylation. It has been suggested that these stoichiometries reflect the presence of two 'sites' for ATP synthesis in electron flow from water to ferricyanide or NADP [14].

Kraayenhof, Groot and Van Dam [17] and Slater [18] have recently estimated the energetic potential at which the phosphorylation reaction is maintained by chloroplasts. Class I and class II chloroplasts were able to maintain a phosphorylation potential of 15.5 and 14.5 kcal/mole, respectively. When these values are revised to take account of the recent re-evaluation of the $\Delta G'_0$ for ATP hydrolysis [19, 20] the potentials become approximately 17 kcal/mole. To maintain this chemical potential, assuming that two electrons are required for each ATP synthesised, each electron traversing a phosphorylation site must lose at least 0.37 V of electrical potential. Electrons traversing two sites between H_2O and ferricyanide or P700 would be expected to drop through at least 0.74 V of potential.

In photosynthetic bacteria, values for the stoichiometry of phosphorylation with respect to electron flow are harder to evaluate because of the cyclic nature of the pathway [21, 22]. However, Nishimura [23] has estimated from flashing light experiments that the ATP/e_2^- ratio is probably 2 in *Rhodospirillum rubrum* and that there are two distinct 'sites' for phosphorylation in cyclic electron flow. Crofts and Jackson [24] have measured the phosphorylation potential maintained by *Rhodopseudomonas spheroides* chromatophores to be 13.3 kcal/mole, which may be revised [19, 20] to about 15.5 kcal/mole, a value which is close to that reported by Keister [25] for the phosphorylation potential maintained by *R. rubrum* chromatophores. These values, together with values for electron work required to maintain these potentials, are summarised in table 1.

1.2. *H^+ uptake*

Estimates of the pH difference maintained by chloroplasts in the light following H^+ uptake have varied between 2.5 and 3 pH units [26–28]. Of these, the value of 2.7–3 pH units measured by Rumberg and Siggel [28] is probably the most reliable. Recently Rottenberg et al. [27] have estimated the pH gradient by using methylamine in a method similar to that using DMO [29], and have shown that at pH 9, the gradient is at least 2.3 pH units. Witt and his collaborators [30, and see below] have estimated from the 515 nm absorbancy change that the electrical potential across the chloroplast membrane may reach a value of 200 mV, dropping to about 100 mV in the steady state. If uptake is the expression of an electrogenic H^+ pump [5], the total proton motive force in the steady state would be at least 0.28 V, and may be as high as 0.38 V.

The stoichiometry of H^+ uptake and electron flow has been estimated variously as between 1 and 5 H^+/e^- [31–38]. When a sufficiently rapid response of recording system has enabled measurements of electron flow and H^+ uptake to be made with respect to the same point in time, values of 2 H^+/e^- have been found, suggesting two sites at which H^+ uptake can occur in electron flow between H_2O and ferricyanide or P700 [34–36]. Similarly, flash yield experiments suggest that 2 H^+ are taken up following a single flash of short duration [37]. This suggests that electrons must transverse two 'sites' for H^+ uptake, with a potential drop of at least 0.28 V at each site.

In chromatophores from both *Rps. spheroides* and *R. rubrum* Jackson and Crofts [24, 39] have estimated that a pH gradient of about 1 unit and a membrane potential of at least 200 mV were maintained in the steady state following H^+ uptake, suggesting a total proton motive force of about 260 mV. During the initial phases of illumination the potential may have reached more than 400 mV. Estimates of the stoichiometry of H^+ uptake both by flash yield experiments [40–42] and by direct measurement [43] have suggested that, at least in the presence of valinomycin, 2 H^+ are taken up for each electron flowing through the cyclic system.

The values for stoichiometry, potential and electron work for H^+ uptake in chloroplasts and chromatophores are also summarised in table 1.

1.3. *Energy available from light, and from dark electron flow*

The efficiency of conversion of light to chemical energy has been treated theoretically by Duysens [44]

Table 1
Values for the probable stoichiometry and potential of phosphorylation and H^+ uptake in chloroplasts and chromatophores.

Reaction	Probable stoichiometry	Potential	Free energy/ electron* (eV)	References
		Chloroplasts		
phosphorylation	2 ATP/e_2^-	17 kcal/mole	0.74	2, 5, 10, 14–18
H^+ uptake	4 H^+/e_2^-	0.28–0.38 V	0.56–0.76	1–5, 26–38
		Chromatophores		
phosphorylation	2 ATP/e_2^-	15 kcal/mole	0.61	21–25
H^+ uptake	4 H^+/e_2^-	0.25–0.4 V	0.5–0.8	24, 39–43

* Minimal free energy loss per electron on passing through all the 'sites' indicated by the stoichiometry.

and more recently by Ross and Calvin [45] and by Knox [46]. These authors conclude that the maximal theoretical efficiency of conversion is in the region of 70% [44] and that photosynthesis would occur most readily with an efficiency of conversion of about 60% [45, 46].

In Duysen's original treatment [44] the photosynthetic trapping mechanism was regarded as a heat engine operating in a cycle in which work was performed between a pigment system in thermal equilibrium with the exciting light at temperature T_L, and a trapping system in thermal equilibrium with the environment at temperature T_E. From the general equation for maximal efficiency of such a heat engine, and by estimating an appropriate value for T_L (1100°K) and the environment (300°K), Duysens showed that the maximal efficiency of photosynthesis was

$$\frac{W}{E} = \left(1 - \frac{T_E}{T_L}\right) = 73\% \qquad (1)$$

where W is the maximal work obtainable from the system, and E is the energy of the absorbed light. For light at 680 nm, the maximal free energy that could be sustained by the trapping system would be 1.33 eV.

Ross and Calvin [45] approached the problem from a consideration of the rates at which energy transfer between the ground and excited states of chlorophyll, and between the excited chlorophyll and the trapping system might occur. At thermal equilibrium, the rate of transitions between the ground and excited states of chlorophyll (Chl–Chl* transitions) must be equal in the forward and reverse direction. The radiative rate could be calculated directly from the product of the electronic absorption spectrum of chlorophyll with the black body radiation curve at the temperature of the equilibrated system. Chl–Chl* transitions would also occur by non-radiative pathways, so that the total rate in the forward direction would be

$$R_{Tot} = R_R + R_{NR}$$

where R_{Tot} is the total rate, R_R and R_{NR} are the rates by radiative and non-radiative pathways.

By assuming that at photosynthetic intensities, the forward rate of Chl–Chl* transitions by non-radiative pathways was negligible, Ross and Calvin were able to conclude that the ratio (Q) of the populations of excited chlorophyll in the light and in the dark was given by the ratio of rates of Chl–Chl* transitions in the light and dark as

$$Q = \frac{R_R^L}{R_R^D + R_{NR}^D} = \frac{R_R^L}{R_R^D}\,\phi_{lum} \qquad (2)$$

where superscripts L and D refer to rates in the light and dark, and ϕ_{lum} is the quantum yield of luminescence. They then assumed that at the light intensities involved, the population of the ground state was not seriously depleted, so that the partial molar free energy difference between Chl* and Chl in the light was

$$\Delta\mu_{max} = kT\ln Q = kT\ln \frac{R_R^L}{R_R^D}\phi_{lum} \quad (3)$$

Since it was possible to compute appropriate rates for the radiative transitions R_R^L and R_R^D, the maximal free energy difference between ground and excited state could be readily calculated by taking values of ϕ_{lum}. When ϕ_{lum} was assumed to be unity (that is, negligible non-radiative transitions), $\Delta\mu_{max}$ was calculated to be 1.36 eV for system II and 1.32 for system I of green plants.

Ross and Calvin showed from kinetic arguments how the potential might vary as non-radiative pathways (including those leading to energy storage) became available, leading to a fall in ϕ_{lum}.

The conditions for maximum power storage were obtained by maximizing the product of the quantum yield for storage with the partial molar free energy difference ($\phi_{st} \times \Delta\mu$). Maximum power storage occurred when the potentials at the traps were 1.16–1.19 eV for system I and 1.23 eV for system II, and when the fraction of quanta lost was about 2%. However, over a range of about 0.12 eV about the optimal potential, the power stored remained greater than 95% of the maximum.

The treatment of Ross and Calvin [45] had many advantages over that of Duysen [44] since it was able to accomodate a broad spectrum of light, and included a consideration of the lowering of the potential of the trap arising from the degree of irreversibility necessary to cause a flow of energy through the system. Ross and Calvin also considered the effects on luminescence of this partial thermodynamic reversibility. They showed that the quantum yield for luminescence included a term dependent on the potential of the trap, and suggested that chemiluminescence (delayed fluorescence) was due to the partial reversibility of the energy storage process.

More recently, Knox [46] has shown that the approaches of Duysens [44] and of Ross and Calvin [45] are equivalent. Knox showed from the black body radiation formula and by integrating over the absorption spectrum, that the rates of radiative transitions of Chl–Chl* calculated by Ross and Calvin are related to the temperature of the light source (T_L) and of the environment (T_E) as follows

$$\frac{R_R^L}{R_R^D} = \frac{e^{-E/kT_L}}{e^{-E/kT_E}} \quad (4)$$

where E is the energy of the lowest excited state of chlorophyll, k is the Boltzmann constant and the other terms have the meanings previously assigned. Knox defined the populations of the ground and excited states of chlorophyll as N_0 and N_i and the thermally excited population as N_i^0. Then following Ross and Calvin

$$\Delta\mu_{max} = kT\ln(N_i/N_i^0) \quad (5)$$

and also

$$\frac{N_i^0}{N_0} = e^{-E/kT_E}.$$

Knox introduced a third temperature (T_C), the 'absorber temperature', which is the temperature to which the chlorophyll would have to be raised to maintain the excited chlorophyll population at the level created by illumination.

$$N_i/N_0 = e^{-E/kT_C}.$$

From kinetic agruments similar to those of Ross and Calvin, Knox was able to show that

$$\Delta\mu_{max} = E\left(1 - \frac{T_E}{T_C}\right) = E\left(1 - \frac{T_E}{T_L}\right) - kT_E\ln\left(\frac{1}{\phi_F}\right). \quad (6)$$

By appropriate substitution it becomes apparent that

$$e^{-E/kT_C} = \phi_F e^{-E/kT_L} \quad (7)$$

and that

$$N_i/N_i^0 = \left(\frac{e^{-E/kT_L}}{e^{-E/kT_E}}\right)\phi_F \quad (8)$$

Comparison between equations (1) and (6) shows that $\Delta\mu_{max}(W)$ as calculated by Duysens is equivalent to $\Delta\mu_{max}$ of Knox when the quantum yield for fluorescence (ϕ_F) is unity. This is the condition when chlorophyll is in thermal equilibrium with the exciting source. When (as in real life) ϕ_F is less then unity, the maximal free energy available at the trap is less than the Duysens maximum by an amount given by the second of the right hand terms in equation (6).

Table 2
Potentials of some electron flow reactions of photosynthesis.

Reaction	Standard potential (V) E'_0 (pH 7)	Probable working Potential (V) E' (assuming pH 7)	References
		Chloroplasts	
$2H_2O/O_2.4H^+.4e^-$	0.816	0.806*	92 (*air saturated water).
Primary acceptor	−0.035	−0.10 (no acceptor) ∿0 (with ferricyanide) <−0.10 (with DCMU)	13
cytochrome *f*	0.365	variable, probably more oxidised	12, 93
P700	0.43	variable, probably more oxidised	94
Ferredoxin	−0.42	∿−0.50 (no NADP)	95, 96
Ferri/ferrocyanide	0.42	variable	97
		Chromatophores	
high potential *c*-type cytochromes	0.32	∿0.4	see 93 for refs.
mid-potential cytochromes (mainly *b*-type)	0.05	∿0	see 93 for refs.
Primary acceptor, X	−0.15–0	≲−0.1	98–100
P890	0.45	∿0.5	98, 99
	Maximal potential (V)	Optimal potential (V)	Physiological redox span ($\Delta E'$).(V) (assuming pH 7)
Photosystem II	1.34	1.23	−0.82
Photosystem I	1.32	1.19	−1.0
Q to P700	–	–	0.4–0.5
bacteria reaction centre (*Rps. spheroides*)	0.9	0.79	−0.65 to −0.55
X to P890	–	–	+0.55 to +0.65

Comparison between equations 2, 4 and 8 shows the equivalence between Q and ϕ_{lum} of Ross and Calvin, and N_i/N_i^0 and ϕ_F of Knox.

The values of maximal and nominal energy available at the efficiency of conversion indicated by these calculations are listed for various photosynthetic systems in table 2. Also listed in table 2 are values for the energy available from dark electron flow between oxidation-reduction couples spanning a variety of photosynthetic electron flow pathways, assuming either that these couples are poised at their mid-point potentials at physiological pH, or that they have the potential indicated by experiment.

1.4. *'Sites' for energy conservation in photosynthetic electron flow*

It is clear from a consideration of the electron work required to maintain phosphorylation or H^+ uptake in

chloroplasts at the stoichiometries and potentials shown, that sufficient energy is available from dark electron flow when the terminal couples are at either their mid point potentials or apparent physiological potentials for energy conservation at only one of the two 'sites' indicated. However, sufficient energy is available from the overall process for either synthesis of ATP or H^+ uptake at the potentials and stoichiometries suggested by experiment. It is also clear that in each case the oxido-reductive span across the photochemical reactions is considerably less than the optimal potential calculated [45] or indicated by experiment [47]. Both these anomalies are resolved if one assumes that in addition to oxido-reductive work the photochemical reactions perform work which contributes more directly to the high energy state of photophosphorylation.

2. Energy dependent spectral shifts of pigments in chloroplasts and photosynthetic bacteria

The nature of the 515 nm change in chloroplasts and intact green plants and its relation to energy coupling have been discussed at length elsewhere. [5, 30, 48]. The rise time of the change following laser excitation has been shown by Witt and his collaborators [30, 48, 49] to be faster than 2×10^{-8} sec. Since this is more rapid than any electron flow event apart from those associated with photochemical reactions, Witt et al. [30] have ascribed the change to events associated with these reactions. The decay of the 515 nm change is very much slower than its rise, and is markedly accelerated under a variety of conditions in which the high energy state would be dissipated. Junge and Witt [50] have concluded from a detailed study of these effects that the 515 nm change is an indicator of an electrical potential difference developed across the chloroplast membrane as a result of charge separation occurring in the primary photochemical reactions, and that the potential energy thus stored plays an important role in chemiosmotic energy coupling as envisaged by Mitchell [51, 52].

In chromatophores from *Rps. spheroides* and *R. rubrum*, spectral changes occur on illumination, or on modification of the high energy state, which have been ascribed to a red shift in the absorption bands of carotenoids and chlorophylls [24, 39, 53–59]. The rise of the carotenoid change is faster than 10^{-7} sec [39, 59, 60], while the decay is very much slower. The decay of the carotenoid change is accelerated under conditions in which energy coupling is inhibited or dissipated [40, 58, 60, 61], and these kinetic characteristics are very similar to those of the 515 nm change. Fleischman and Clayton [58] tentatively suggested among other possibilities that the carotenoid change may be in response to a membrane potential, and Jackson and Crofts [39] have shown that spectral changes in the chromatophore suspension which are similar to those induced by light may be induced when potentials are generated across the chromatophore membrane by ionic gradients operating through ionophorous antibiotics or uncoupling agents. More recently the close relation between the rapid phase of the carotenoid change and the photochemical reactions in *Rps. gelatinosa* [62] and *Rps. spheroides* (J.B.Jackson and L.Dutton, unpublished observations) has been demonstrated by observing the dependence of these reactions on the ambient redox potential. The two reactions show an identical titration for changes in potential over the range –0.15 to +0.48 V.

If the photoreactions are arranged in the thylakoid or chromatophore membranes in such a way that donor and acceptor sites are on opposite sides, as suggested by these experiments, then the consequent generation of a membrane potential would involve the performance of electrical work in addition to the generally accepted oxido-reductive work. In many previous models for the photoreactions, charge separation over a limited distance is explicitly suggested [63–65]. If the oxido-reduction reactions of the donor and acceptor occurred in the same phase, the electrostatic work done in separating charge would be lost. Clearly it may be advantageous for the organism to conserve the electrical energy of charge separation if it can subsequently use the energy in the chemistry of photosynthesis, for example in chemiosmotic coupling [51, 52].

3. Delayed fluorescence in photosynthetic organelles

Photosynthetic organisms, after having been exposed to light, emit a glow which can in some instances be observed as long as an hour after the cessation of illumination. The spectrum of the emitted light is identical to that of the fluorescence of the *in*

vivo chlorophyll which suggests that chlorophyll molecules are somehow being chemically excited to the singlet level.

3.1. *Relation to electron flow*

It is probable that delayed fluorescence results from a direct reversal of the primary photoact, in which electrons return to oxidized reaction centre chlorophyll from acceptor pools via the chlorophyll singlet state [66]. Goedheer found that emission of light with the spectrum of chlorophyll fluorescence occurred when a reductant was added to a solution of chemically oxidized chlorophyll [67]. From studies of the relation between delayed and prompt fluorescence, Lavorel [68, 69 and see below] has concluded that the delayed fluorescence arises from the recombination of electrons from the reduced primary acceptor (Q^-) with the oxidized donor (Z^+) in what is essentially a reversal of the photochemical reaction;

$$Z \cdot Chl \cdot Q \underset{J}{\overset{I}{\rightleftharpoons}} Z^+ \cdot Chl \cdot Q^- \qquad (9)$$

where I (the light intensity) and J are the rates of the forward and reverse reactions. Recently, Bennoun [70] has demonstrated very elegantly the dependence of delayed fluorescence in chloroplasts on a re-oxidation of reduced acceptor (Q^-) through a reversal of the photoreaction. Both the re-oxidation in the presence of DCMU and the emission following a flash were sensitive to very low concentrations of hydroxylamine.

The dependence of delayed fluorescence on the redox state of the donor pool has been shown by observing the emission elicited by flashes of light [71]. The intensity showed a periodicity which was similar to that of oxygen yield [72, 73] except that the maxima were displaced. Barbieri et al. [71] interpret their experiments as indicating a dependence of emission intensity on the redox state of the oxygen evolving mechanisms [72, 73]. Chemiluminescence experiments suggest that similar light emission in bacterial chromatophores can result from the return of electrons to oxidized reaction centre chlorophyll from physiological electron acceptors [74].

3.2. *Relation to prompt fluorescence*

Lavorel [68, 69] and Clayton [75] have investigated the relation between the quantum yield of prompt fluorescence of chloroplasts and the intensity of delayed fluorescence. This has been formulated as follows:

$$F = \phi_1 I \qquad (10)$$

$$L = \phi_1 J \qquad (11)$$

F is the fluorescence intensity, I the actinic intensity, L the intensity of delayed fluorescence and J the rate of regeneration of excitons in the dark. The relationship holds only when ϕ_1 is the quantum yield of the *live* prompt fluorescence [75]. It can be best observed when the actinic intensity is low and when delayed light is observed some time (500 msec) after illumination. Under these conditions, variations in the process J contribute relatively little to the intensity so that the delayed fluorescence depends predominantly on ϕ_1. From the lack of correlation between ϕ_1 and L under a variety of other conditions, Lavorel [69] concluded that the process J must reflect changes in the donor (Z) side of photosystem II, as well as on the acceptor (Q) side. The relation to fluorescence yield has not yet been characterised for delayed fluorescence of bacterial chromatophores.

Arnold and Azzi [47] have studied the afterglow emitted on warming chloroplasts previously illuminated at −15 to −150 °C. They assumed that the light emitted was proportional to the rate at which trapped electrons and holes were able to recombine. We may equate this rate with Lavorel's J, so that

$$L = \phi_1 J = -\phi_1 \frac{dN}{dt} = \phi_1 N F e^{-E_{Ac}/kT} \qquad (12)$$

where N is the number of trapped electrons or holes, F is a frequency factor containing rate constant and entropy terms, E_{Ac} the activation energy, and k, T are conventionally assigned. The fluorescence yield (ϕ_1) is seen to be a proportionality constant. Substitution of the rate of heating [47] showed that the peaks of emission corresponded to activation energies of 0.53, 0.60 and 0.63 eV.

3.3. *Delayed fluorescence and the high energy state*

The intensity of delayed fluorescence measured at a fixed time after flashes in a phosphoroscope [76, 77], varies over the duration of illumination. The kinetics depend on the interval between the flash and observation, and on the light intensity. At high light

intensity, and with a short interval (5 msec), the kinetics and response to reagents differ markedly from those of prompt fluorescence [75, 78, 79]. In particular, and in contrast to the effects on prompt fluorescence, inhibition of electron flow, addition of uncoupling agents, or phosphorylation conditions inhibit [78] whereas stimulation of electron flow by addition of acceptor increases the intensity of delayed emission [77]. Delayed emission of chromatophores of *Rps. spheroides* is also sensitive to uncoupling agents and to valinomycin [58]. Of particular interest was the correspondence observed by Fleischmann and Clayton [58] between the kinetics of delayed fluorescence and those of the carotenoid changes.

More recently, Wraight and Crofts [79] have investigated the kinetics of the 1 msec delayed fluorescence in chloroplasts. During the first few seconds of illumination, the emission reached a maximum and subsequently declined. The rise kinetics consisted of an initial rapid phase ($t_{½} \leqslant 100$ msec) followed by a slower phase ($t_{½} \sim 0.3$ sec). From the sensitivities of the two phases to a variety of uncouplers and ionophores, they concluded that the slow phase was dependent on the development of a pH gradient across the chloroplast membrane, whereas most of the rapid phase was dependent on the membrane potential. The dependence of delayed fluorescence on the high energy state could be interpreted as a dependence on the electrochemical H^+ gradient.

3.4. *Induced-delayed light emission and the high energy state*

An alternative approach to the involvement of the high energy state in the delayed fluorescence mechanism has been that of inducing emission from pre-illuminated chloroplasts or chromatophores by chemical addition to the dark suspension [80–84]. The treatments fall into distinct classes.

a) Salt additions [81] in which emission was stimulated when the cation of the added salt permeated the chloroplast membrane more effectively than the anion [82]. The intensity of emission approached proportionality with the cation concentration gradient as the permeability coefficient for the anion became negligible [82, 84]. This condition was most closely approximated when K^+ salts of non-penetrant anions were used in the presence of valinomycin [82, 83, 85].

b) Acid–base and base–acid treatments. On transferring pre-illuminated chloroplasts from an acidic to a basic medium in the dark, an emission of light occurred, the intensity of which depended in a non-linear fashion on the pH difference between the acidic and basic phases [80, 86]. However, the intensity did not depend on the presence of weak acid in the acidic phase to increase the chloroplast buffer capacity [81]. On the transfer from basic to acidic phases, emission also occurred but only when the acidic phase was pH 3.5 or below [81, 84].

c) Addition of DCMU to preilluminated chloroplasts stimulated emission [75]. This, and the emission stimulated by the addition of electron acceptors or donors, have already been mentioned.

While changes in emission elicited by DCMU or redox changes were attributable to changes in ϕ_1 [75], the emissions stimulated by salt gradients or pH changes were not accompanied by any marked change in ϕ_1 [84]. Kraan et al. [84] have concluded from these latter observations that the stimulated emission is not caused by an increase in concentration of reduced electron acceptor (Q).

Mayne [80] suggested that the high energy state of photophosphorylation might be in equilibrium with the photosystem II reaction. However, Miles and Jagendorf [81] suggested that ionic treatment or pH jumps acted as 'barrier-lowering' events to accelerate recombination of electrons and holes. Similarly, Barber and Kraan [82] suggested, among other possibilities that the potential produced by the ionic gradients resulting from salt addition reduced the energy barrier for triggering luminescence.

Crofts (see Fleischman [83]) pointed out that charge separation across the thylakoid membrane in the photoact, would provide a store of energy, the membrane potential, in direct equilibrium with the light reaction. This energy would be available in the dark for luminescence. In order to account for the logarithmic relation between intensity of emission and the membrane potential induced by the K^+ gradient, Fleischman [83] suggested that the membrane potential might act by directly lowering the activation energy for emission. Thus,

$$L = \phi_1 NF e^{-(E_{Ac} - \Delta\psi)/kT} \qquad (13)$$

where $\Delta\psi$ is the membrane potential.

Since $\Delta\psi = \frac{RT}{F} \ln \frac{(K^+)_o}{(K^+)_i}$,

$$L = \phi_1 NF \exp\left[-\left\{E_{Ac} - \frac{RT}{F} \ln \frac{(K^+)_o}{(K^+)_i}\right\} / kT\right]. \quad (13a)$$

Assuming that $(K^+)_i$ is constant, and recognising that $RT/FkT = 1$, equation 13a simplifies to

$$L = NC(K^+)_o;$$

$$\left\{E_{Ac} - \frac{RT}{F} \ln \frac{(K^+)_o}{(K^+)_i}\right\} \geqslant 0 \quad (14)$$

where

$$C = \frac{\phi_1 F}{(K^+)_i} e^{-E_{Ac}/kT}.$$

The recognition of the direct role of the light reaction in generating a membrane potential and the converse effect of membrane potential on the reversal of the photoact leading to emission, provides a ready explanation for the emission induced by salt addition [81–83], and for the sensitivity of a part of the delayed fluorescence to valinomycin [58, 79].

In order to discuss the involvement of the chemical component of the H^+ gradient (ΔpH) in delayed light emission, we must consider a model for electron flow in the region of the photoreactions (fig. 1). This model is a generalized version of similar models previously proposed [24, 50, 51, 79, 84]. It follows from two main postulates.

i) The primary donor (Z/Z^+) and acceptor (Q^-/Q) of the photochemical reaction are situated on opposite sides of the thylakoid membrane.

ii) The primary donor and acceptor are in equilibrium with secondary donor (DH/D) and acceptor (AH/A) pools, which are redox couples of the H carrier type. The pools are in equilibrium with phases on opposite sides of the membrane, and their redox potentials are dependent on the values of pH in these phases.

Kraan et al. [84] have previously suggested an alternative model in which the primary donor and acceptor are themselves H^+ carriers, (QH/Q^-, Z/ZH^+), which are also dissociable weak acids.

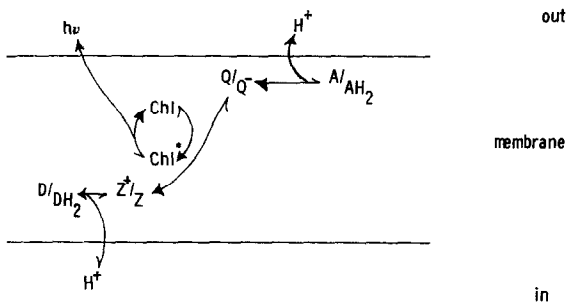

Fig. 1. Hypothetical photochemical reaction centre. Dark arrow heads indicate reactions leading to delayed fluorescence. See text for explanation.

In the case of photosystem II of chloroplasts we envisage Q^-/Q as being on the outside of the grana membrane in equilibrium with a plastoquinone pool [87], and Z/Z^+ on the inside in equilibrium with the water/oxygen couple [88]. The plastoquinone couple would equilibrate with the external pH and the water/oxygen couple with the internal pH.

For the photoreaction operating in reverse (see eq. 9) we may write

$$Q^- + Z^+ \overset{K^*_{eq}}{\rightleftharpoons} Q^*Z \xrightarrow{k_a} Q + Z$$

where

$$K^*_{eq} = \frac{(Q^*Z)}{(Q^-)(Z^+)}; \quad \Delta G^*_0 = -RT \ln K^*_{eq}$$

and k_a is a composite rate constant for de-excitation of the activated state Q^*Z to the ground state Q, Z by various pathways.

The rate of reaction (or the turn-over of the excited state, $(\overrightarrow{Q^*Z})$), is then given by

$$(\overrightarrow{Q^*Z}) = k_a(Q^*Z) = k_a(Q^-)(Z^+)e^{-\Delta G^*_0/RT} \quad (15)$$

Since ΔG^*_0 is the standard free energy of activation for the reaction leading to delayed light emission, equation (15) is equivalent to the formulation of Arnold and Azzi [47] (eq. 12). ΔG^*_0 is the difference between the standard free energy of the overall reaction and that of the de-excitation reaction.

$$\Delta G^*_0 = zF(E^* - \{\Delta E^0_{Z-Q} + \Delta\psi\}) \quad (16)$$

where E^* is the energy difference between ground and excited states (equivalent to the lowest chlorophyll singlet) and the term in curly brackets is the standard free energy change for the overall reaction. It includes a term ($\Delta\psi$) for the difference in electrostatic potential between primary donor and acceptor since redox potentials are defined with respect to zero electro-static potential. Substitution of eq. 16 into eq. 15 makes it equivalent to Fleischman's formulation [83, eq. 13].

Recognising from postulate (ii) that

$$E'_Z = E'_D, \text{ and that } E'_Q = E'_A,$$

where E' is the actual potential of the couple indicated, and by appropriate substitution and cancellation, it can be shown that

$$\overrightarrow{(Q^*Z)} = k_a(Q)(Z)\exp[-(E^* - \{\Delta E'_{D-A} + \Delta\psi\})zF/RT] \quad (17)$$

where the term in curly brackets is the actual free energy difference between the donor and acceptor pools.

E'_D and E'_A are dependent on the values of pH in phase D and A, so that the contribution of pH can usefully be brought out by writing

$$E'_D = E^{\neq}_D - \mathcal{Z}pH_D, \text{ and } E'_A = E^{\neq}_A - \mathcal{Z}\,pH_A$$

where

$$\mathcal{Z} \text{ is } 2.303\,\frac{RT}{F}.$$

The free energy difference between donor and acceptor pools can now be seen to depend on the pH difference and the electrostatic potential difference across the membrane as well as on the poise of the redox couples of the pool.

$$\Delta G'_{D-A} = -zF(\Delta E^{\neq}_{D-A} - \mathcal{Z}\,\Delta pH_{D-A} + \Delta\psi). \quad (18)$$

Recognising that the sum of chemical ($-\mathcal{Z}\Delta$pH) and electrical ($\Delta\psi$) components of the H^+ gradient is the proton motive force (Δp, [51, 52]) and that the proportion of de-excitation reactions going by radiative pathways is given by a fluorescence yield (ϕ_1) we obtain a relation between the intensity of delayed fluorescence and the proton motive force.

$$L = \phi_1 k_a(Q)(Z)\exp[-(E^* - \{\Delta E^{\neq}_{D-A} + \Delta p\})zF/RT] \quad (19)$$

This relation is somewhat artificial since the terms on the right are not independently variable. Thus the fluorescence yield, ϕ_1, contains terms dependent on (Q) and k_a, and would also vary with Δp because of the phenomenon of fluorescence lowering [89, 90]; the interdependence of other terms is obvious from the derivation above.

It is possible to abstract the relation between emission and the pH gradient from this equation.

$$\log_{10}L = -\Delta pH + \log_{10}C'$$

where C' is a constant, such that

$$C' = \phi_1 k_a(Q)(Z)\exp[-(E^* - \{\Delta E_{D-A} + \Delta\psi\})zF/RT]$$

Of course C' may be expected to be constant only under very limited conditions. Wraight and Crofts [79] suggested that the kinetics of the slow component of the rise in delayed fluorescence might reflect the onset of the pH gradient, since replotting with the intensity of emission on a semi-logarithmic scale gave a curve with first order kinetics similar to those of H^+ uptake. This suggests that the terms in C' became approximately constant during the first 0.2 sec of illumination and accounted for the rapid phase of the delayed fluorescence, an observation which fits well with the known rapid rise times of prompt fluorescence, the 515 nm change and plastoquinone reduction.

3.5. *Energy conserved in the photochemical reactions*

In eqs. 17–19 we have recognised that, according to our model, the free energy difference between donor and acceptor pools is contributed by the poise of the pools, and the pH and electrical difference across the coupling membrane. This free energy difference is equivalent to the work done, or the energy conserved, during the photoreaction. It is of interest to see how well the model accounts for the potential of the traps calculated from thermodynamic and kinetic considerations [45, 46]. By referebce to tables 1 and 2 it can be seen that for photosystem II of green plants the poise of the reactants accounts for about 0.82 eV of the energy

conserved. The proton motive force ($-\Delta pH$ and $\Delta\psi$) would contribute a further 0.28–0.38 eV to give a total of 1.1–1.2 eV. This compares with 1.23 eV calculated by Ross and Calvin [45] as the optimal potential. If we assume that the activation energies obtained from the glow curves of Arnold and Azzi [47] (values of $\sim$ 0.6 eV) represent the energy deficit between the conserved energy and the photon energy we get a value of 1.24 eV. Similarly, calculation from the data of Sauer and Park [46, 91] indicate a trap potential of 1.24 eV. It appears therefore that the energy available according to our formulation may not be quite enough to fit the data. However, we feel that the recognition of a contribution of the high energy state (in the form of the H^+ gradient) to the electronic energy available for delayed light emission provides a more realistic basis for a mechanism than do proposals of reactions in which two electronic events directly contribute to the emission of a single quantum, or in which the full oxido-reductive span generated by a two quantum series mechanism is available for the emission of a single quantum of delayed light [47, 65].

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