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FLUORESCENCE QUENCHING IN PHOTOSYSTEM II OF CHLOROPLASTS

W. L. BUTLER and M. KITAJIMA*

Department of Biology, University of California, San Diego, P.O. Box 109, La Jolla, Calif. 92037 (U.S.A.)

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SUMMARY

A simple photochemical model for the photosynthetic units of Photosystem II based on first-order rate constants for de-excitation of excited chlorophyll molecules is presented in the form of equations which predict the yields of fluorescence (i.e. at the initial F_0 level, at the maximal F_M level and the fluorescence of variable yield, $F_V = F_M - F_0$). Two types of quenching mechanisms are recognized: (1) increasing nonradiative decay processes in the bulk chlorophyll by creating quenching centers which complete with the reaction centers for the excitation energy (this mechanism quenches both F_0 and F_V) and (2) increasing nonradiative decay of the excited reaction center chlorophyll (this mechanism quenches F_V but not F_0). Quenching in the bulk chlorophyll preserves the relationship that F_V/F_M is equal to the maximum yield of photochemistry; quenching at the reaction center chlorophyll decreases F_V/F_M substantially (since F_V is quenched specifically) but may have very little effect on the yield of photochemistry. Estimates are made of the relative magnitudes of the rate constants for de-excitation of the excited reaction center chlorophyll by photochemistry, k_p, by nonradiative decay processes, k_d, and by energy transfer back to the bulk chlorophyll, k_t. Fluorescence is assumed to emanate only from the bulk chlorophyll. Energy transfer from Photosystem II to Photosystem I may occur from either the excited bulk chlorophyll or from the excited reaction center chlorophyll. The model is valid for any degree of energy transfer between Photosystem II units.

The results of the accompanying paper [1] on the quenching of fluorescence and photochemistry by dibromothymoquinone (DBMIB) in chloroplasts at -196 °C showed that both the initial, F_0, and the final, F_M, levels of fluorescence were quenched in a manner which indicated that a major part of the fluorescence at F_0 emanated from Photosystem II and was the same type of fluorescence as the fluorescence of variable yield ($F_V = F_M - F_0$). In these experiments the ratio F_V/F_M appeared to be a reliable index of the maximum yield of photochemistry of Photosystem II, φ_{P_0}. However, not all types of fluorescence quenching experiments yield

* On leave from the Fuji Photo Film Co., Ltd., Tokyo, Japan

similar results: in fact, some fluorescence quenching treatments quench F_V specifically with little influence on F_0. For instance, irradiation of chloroplasts with ultraviolet can practically eliminate F_V but have very little effect on F_0 [2, 3]. In such experiments, electron transport through Photosystem II may be partially restored by artificial electron donor compounds but with little or no restoration of F_V [3]. Irradation of Tris-washed chloroplasts with visible light in the absence of an electron donor (which results in partial photooxidation of the chloroplasts) has the same effect as ultraviolet irradiation in that F_V is quenched specifically and almost totally and even though electron transport through Photosystem II can be restored by artificial electron donors, that restoration of photochemical activity is not accompanied by a restoration of F_V [4]. It has also been shown that addition of ferricyanide to chloroplasts prior to freezing quenches F_V at low temperatures [5, 6, 7] but such addition has little influence on F_0. Fig. 1 shows the redox titration curve for the oxidizing effect of ferricyanide on the F_0 and F_M levels of fluorescence at −196 °C. It is apparent that F_M is quenched specifically while F_0 is unaffected. Even though F_V is quenched approximately 70 % by the oxidizing conditions at low temperature, the primary photochemistry is affected very little: the initial rate of photoreduction of C-550 at −196 °C in the presence of ferricyanide is 80–90 % of the rate in the absence of ferricyanide (data not shown).

It is generally assumed that F_0 and F_V are of different origin (i.e. from Photosystem I and Photosystem II, respectively) and the results of treatments which specifically quench F_V without affecting F_0 appear to be consistent with that assumption. Furthermore, the absence of correlation between the ratio F_V/F_M and the yield of photochemistry with such quenching treatments raises doubts of the validity of the simple theory of fluorescence quenching used in the previous paper [1]. The purpose of the present paper is to reconcile the different types of fluorescence quenching, exemplified by the results obtained with DBMIB and ferricyanide at low temperature, within the same theoretical framework. It will be shown that both types of quenchers are consistent with a simple model in which F_0 and F_V have the same origin.

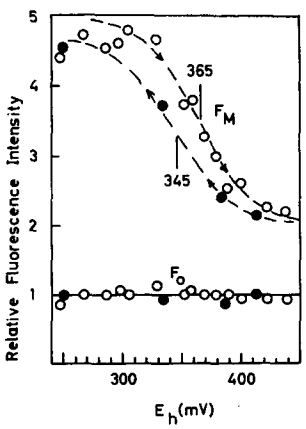
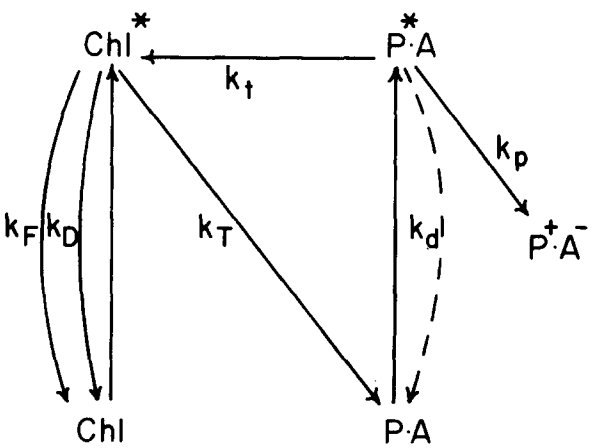


Fig. 1. Redox titration curves of F_0 and F_M measured at −196 °C as a function of the redox potential of the chloroplast medium before freezing. ○, values obtained in an oxidative titration; ●, values obtained in the reverse reduction titration. - - -, plots of one electron Nernst equations with midpoint potentials of 365 and 345 mV (taken from ref. 5).

One reason for assuming that a major part of the fluorescence at F_0 does not emanate from the active photochemical apparatus of Photosystem II is that the ratio F_V/F_M in intact algal cells is generally 0.6–0.7 which seems too low a value to accept for the photochemical efficiency. That dilemma has been resolved by assuming that a major part of F_0 was some other type of fluorescence so that the "true" value of F_V/F_M would be closer to unity. It will be presented in the following discussion, however, that there are two distinct types of fluorescence quenching mechanisms and that one of these does not preserve the direct relationship between the ratio F_V/F_M and the yield of photochemistry. Thus, a value of 0.6 for F_V/F_M in intact algae is not inconsistent with a high yield of photochemistry for Photosystem II or for overall photosynthesis. Furthermore, it will be shown that a part of the excitation energy which might be considered to be lost from Photosystem II through non-radiative de-excitation processes may be transferred to Photosystem I and used in the overall efficiency of photosynthesis.

In the previous paper the analysis of the quenching of fluorescence and photochemistry by DBMIB considered only the bulk chlorophyll. In the present paper the analysis is expanded to include energy transfer to a reaction center complex $P \cdot A$ where the photochemistry occurs. It is assumed that P^+ is rapidly restored to P either by a backreaction with A^- or by a secondary electron donor so that the reaction center chlorophyll, P, is always in an energy-trapping state. Equations will be developed on the basis of the "separate package" model of the photosynthetic apparatus but analogous equations will be presented for the "matrix" model as well (see ref. 1).

It will be assumed initially for purposes of simplification that $k_p \gg k_t$ or k_d so that $\varphi_p = k_p/(k_p+k_t+k_d) \simeq 1$ for reaction centers in the "open" $P \cdot A$ state and zero for those in the "closed" $P \cdot A^-$ state where $k_p = 0$. Excitation energy trapped by closed reaction centers may be transferred back to the bulk chlorophyll, k_t, where fluorescence may occur (fluorescence from the reaction-center chlorophyll is not considered) or may be dissipated in non-radiative decay processes, k_d, from the excited reaction center chlorophyll. Fluorescence from the photosynthetic apparatus will be the sum of the fluorescence from the independent units:



$$\varphi_F = \frac{k_F(A)}{k_F+k_D+k_T} + \frac{k_F(1-A)}{k_F+k_D+k_T}\left[1+\varphi_T\varphi_t+(\varphi_T\varphi_t)^2+\ldots\right] \quad (1)$$

where the first term represents the contribution to fluorescence from units with open reaction centers and the second term the contribution from units with closed reaction centers. The infinite series $\sum_{n=0}^{\infty}(\varphi_T\varphi_t)^n$ in the second term derives from the nature of the model for units with closed reaction centers. The first term in the series, unity, represents the fluorescence emanating from the unit before the exciton is trapped. The second term, $\varphi_T\varphi_t$, is the probability of the exciton being trapped by the reaction center and transferred back to the bulk chlorophyll where fluorescence has a second opportunity to occur; and the subsequent terms in the series to further recycling of excitation energy into and out of the closed reaction center. The infinite series converges to a simple expression $1/(1-\varphi_T\varphi_t)$ so that:

$$\varphi_F = \frac{k_F}{k_F+k_D+k_T}\left[A+\frac{(1-A)}{(1-\varphi_T\varphi_t)}\right] \quad (2)$$

This expression for φ_F is identical to the analogous expression derived for the "separate package" model in the previous paper [1] if we make the assumption that normally $k_t \gg k_d$ so that for closed reaction centers $\varphi_t = k_t/(k_t+k_d) \simeq 1$. Then the denominator in the second term of Eqn 2 becomes:

$$1-\varphi_T = \varphi_F+\varphi_D = \frac{k_F+k_D}{k_F+k_D+k_T}$$

so that:

$$\varphi_F = \frac{k_F(A)}{k_F+k_D+k_T} + \frac{k_F(1-A)}{k_F+k_D}$$

which is the same expression as that derived previously except that k_T is used in place of k_P. The present model thus predicts the quenching observed with DBMIB if an additional rate constant for quenching, k_Q, proportional to the concentration of DBMIB, is introduced in the first denominator of Eqn 2. The introduction of such a quenching process to the bulk chlorophyll quenches both F_0 and F_M.

F_0 ($A = 1$) represents the fluorescence emitted from the bulk chlorophyll before the excitation energy is trapped by the reaction centers. F_M ($A = 0$) represents, in addition, the fluorescence which results from the excitation energy being transferred back from the closed reaction centers to the bulk chlorophyll. Any conditions which decrease the probability of the back transfer of energy from the closed reaction centers (e.g. an increase of k_d at the reaction center chlorophyll) should decrease F_M (or Fv) without influencing F_0. It is apparent from Eqn 2 that:

$$\frac{F_M}{F_0} = \frac{1}{1-\varphi_T\varphi_t}$$

That ratio can change markedly if k_d increases so that φ_t (for the closed reaction centers) decreases. The action of ferricyanide on the fluorescence at low temperatures can be attributed to an increase of k_d at the reaction center chlorophyll. Assuming

that $\varphi_T = 0.8$, the ratio F_M/F_0 would decrease from 5.0 to 2.0 if φ_t decreased from 1.0 to 0.6 (i.e. if k_d increased from 0 to 0.66 k_t). And since k_p at the open reaction centers should still be large compared to k_t or k_d, the yield of photochemistry ($\varphi_{P_0} = \varphi_T \varphi_p$) will be essentially unchanged. The action of ultraviolet radiation, which, in previous work [3], decreased the ratio F_M/F_0 from 4.0 to 1.1, indicates an even stronger quenching, k_d, of excitation energy in the closed reaction centers to the point where φ_t approaches zero. But even though $k_d \gg k_t$ at the closed reaction centers, photochemistry will persist to the extent that $k_p > k_d$ or k_t at the open reaction centers. Thus, the relationship between F_V/F_M and φ_{P_0} does not hold if quenching occurs at the reaction center chlorophyll: appreciable electron transport may occur through Photosystem II even though F_V approaches zero (e.g. ultraviolet-irradiated chloroplasts).

The titration curves in Fig. 1 suggest that ferricyanide does not quench fluorescence directly but rather that the quenching is mediated by the oxidation of cytochrome b_{559}. We have made similar experiments with Tris-washed chloroplasts in which the cytochrome b_{559} was oxidized without addition of ferricyanide because the treatment changed the cytochrome to a lower potential, autooxidizable form. Irradiation of such chloroplasts frozen to −196 °C shows a relatively small $F_V(F_M \simeq 2F_0)$ which is increased (with no change of F_0) if ascorbate is added to reduce cytochrome b_{559} prior to freezing. It was suggested previously [5] that an unknown alternative secondary electron donor might be oxidized by P_{680}^+ at −196 °C when cytochrome b_{559} was oxidized prior to freezing and that the oxidized alternative donor might quench fluorescence. We would now make the more specific suggestion that this quenching occurs by increasing k_d at the reaction center chlorophyll.

The model was presented in its form by assuming that $\varphi_p \simeq 1$ (i.e. $k_p \gg k_t$ or k_d). The equations, however, can be written without making any assumptions as to the relative magnitudes of k_p, k_t, and k_d. Using subscript modifiers o and c to indicate open and closed reaction centers, respectively, (the difference being that $k_p = 0$ for closed centers) the equations become:

$$\varphi_F = \frac{k_F}{k_F + k_D + k_T}\left[\frac{A}{1-\varphi_T\varphi_{t_0}} + \frac{1-A}{1-\varphi_T\varphi_{tc}}\right] \tag{3}$$

$$\varphi_P = \varphi_T\left[\frac{\varphi_{po}A}{1-\varphi_{t_0}}\right] \tag{4}$$

so that:

$$\frac{F_V}{F_M} = \frac{\varphi_{F_M} - \varphi_{F_0}}{\varphi_{F_M}} = \varphi_T\left[\frac{\varphi_{tc} - \varphi_{t_0}}{1-\varphi_T\varphi_{t_0}}\right] \tag{5}$$

$$\varphi_{P_0}(A=1) = \varphi_T\frac{\varphi_{po}}{1-\varphi_{t_0}} = \varphi_T\frac{\varphi_{po}}{\varphi_{po}+\varphi_{d_0}} = \varphi_T\frac{k_p}{k_p+k_d} \tag{6}$$

$$\frac{F_M}{F_0} = \frac{1-\varphi_T\varphi_{t_0}}{1-\varphi_T\varphi_{tc}} \tag{7}$$

Particular assumptions as to relative magnitudes of k_p, k_t and k_d can be examined with these equations.

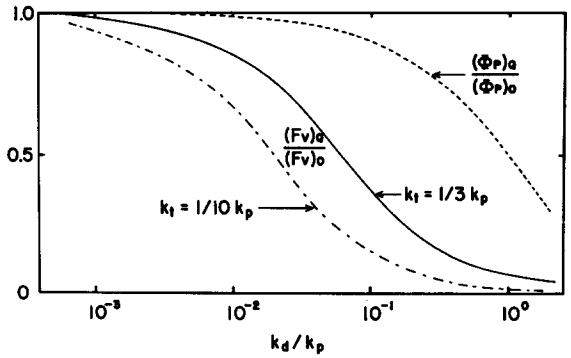


Fig. 2. The quenching effect of increasing k_d relative to k_p on F_V and φ_{P_O}. The ratio $(F_V)_{quenched}/(F_V)_{unquenched}$ is calculated from Eqn 3 and plotted for values of k_t. The ratio $(\varphi_{P_O})_{quenched}/(\varphi_{P_O})_{unquenched}$ is calculated from Eqn 6.

The magnitude of the ratio F_M/F_0 is very sensitive to even small degrees of quenching by k_d. If $k_d = 0.1\ k_t$ (so that $\varphi_{tc} = 0.91$) Eqn 7 predicts that F_M/F_0 (for $\varphi_T = 0.8$) would decrease from 5.0 to 3.7. The additional assumption that $k_t = 0.1\ k_p$ would decrease the ratio further to 3.4. Fig. 2 demonstrates the sensitivity of F_V to small degrees of quenching by k_d. The ratio F_V quenched: F_V unquenched is plotted as a function of the ratio $k_d : k_p$ for two values of k_t assuming $\varphi_T = 0.8$. The marked sensitivity of F_V to k_d justifies the assumption that k_d is very small or negligible when F_V is large (i.e. when F_M/F_0 approaches 5.0). The plot of φ_{P_0} in Fig. 2 is much less sensitive to k_d than F_V and it is apparent from Eqn 6 that φ_{P_0} is independent of k_t. Fig. 2 demonstrates that F_V can be quenched almost completely while a considerable fraction of φ_{P_0} remains. Examination of Eqn 3 shows that F_0 ($A = 1$) should have a very small dependence on k_d being quenched only 4% as k_d/k_p increases from 0 to 1 (assuming $k_p = 10\ k_t$).

The analysis also requires some estimate of the relative magnitudes of k_t and k_p. The results of the previous paper with DBMIB showed good agreement between relative values of F_V/F_M and φ_{P_0}. From Eqns 5 and 6 we can write, assuming $k_d = 0$,

$$\frac{F_V}{F_M} = \frac{\varphi_{po}}{1 - \varphi_T \varphi_{to}} \varphi_{P_0}$$

In the experiment with DBMIB, however, φ_T is a variable depending on DBMIB concentration, so that a direct relationship between F_V/F_M and φ_{P_0} will not be obtained if φ_{to} has an appreciable value. Fig. 3 compares plots of F_V/F_M as a function of DBMIB concentration for various ratios of k_t/k_p. For these calculations φ_T, at various concentrations of DBMIB, was determined from the curve marked zero in Fig. 3 which is the same as the curve of F_V/F_M from Fig. 4 of the previous paper. The agreement between relative values of F_V/F_M and φ_{P_0} determined experimentally in the previous paper [1] is close enough to indicate that k_p is at least three times k_t and may even indicate that $k_p > 10\ k_t$.

We assumed, for estimates of the relative magnitudes of k_d, k_t and k_p, that $\varphi_T = 0.80$. That value was chosen on the basis of the results of the previous

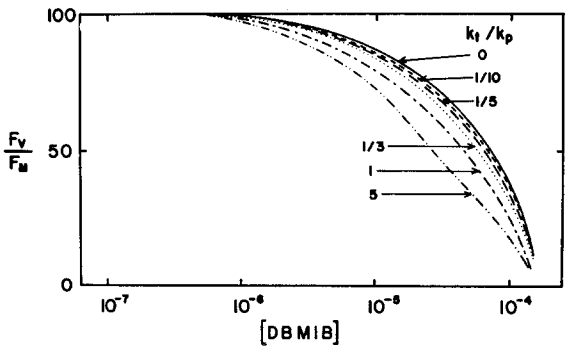


Fig. 3. The influence of k_t relative to k_p (assuming $k_d = 0$) on the curve of F_V/F_M plotted as a function of DBMIB concentration. The curve for $k_t = 0$ is the same as the experimental curve of F_V/F_M obtained from Fig. 4 of the accompanying paper [1]. The curve for $k_t = 0$ is also used to determine φ_T as a function of DBMIB concentration with the assumption that $\varphi_T = 0.8$ in the absence of DBMIB. The equation given in the text is used to plot relative values of F_V/F_M for various values of k_t/k_p assuming $k_d = 0$.

paper [1] and from our observations that the maximum value of the ratio F_M/F_0 appeared to be about 5.0. However, if k_d were not negligible compared to k_t and k_t were not negligible compared to k_p when F_M/F_0 is 5.0, Eqn 7 indicates that φ_T would be larger than 0.8. For instance, if k_d, k_t and k_p were in the ratio of 1:10:100 when $F_M/F_0 = 5.0$, φ_T would be 0.91 rather than 0.80 and φ_{P_0} would be 0.90 rather than 0.80. While we think that a value of 0.91 may be too high for φ_T the value may be somewhat higher than 0.80 since there are occasional reports in the literature where the ratio F_M/F_0 exceeds 5.0 (a ratio 7.0, for instance, would indicate a minimum value of 0.86 for φ_T). Such considerations, however, set rather minimal upper limits on ratios of k_d/k_t and k_t/k_p under conditions where F_M/F_0 has a high value (the limitation is much more stringent on the ratio k_d/k_t than k_t/k_p).

The model has been presented in rather explicit terms involving a specific quenching at the reaction-center chlorophyll. That could be an oversimplification. It is also possible to present the model in less explicit terms in which the reaction center complex is modified by certain treatments in an undefined manner which gives the same quenching effect as increasing k_d of the reaction-center chlorophyll. The purposes of a model to focus concepts to or gain insights which lead to further exploration are better served, however, by the more explicit description.

The model represented by Eqn 2 may also be useful in interpreting the effects of divalent cations on fluorescence properties of chloroplasts and on energy transfer between Photosystem II and Photosystem I. Murata [8] showed that suspending chloroplasts in the absence of divalent cations caused a marked quenching of F_V (compared to chloroplasts in the presence of divalent cations) with very little quenching of F_0. The low value of F_V in the absence of divalent cations was correlated with a greater energy transfer from Photosystem II to Photosystem I. We would suggest that the absence of divalent cations increases energy transfer from the Photosystem II reaction-center chlorophyll to Photosystem I, a process which, so far as Photosystem II is concerned, is analogous to increasing k_d. It was noted in the previous paper [1] that energy transfer can also occur from the bulk chlorophyll

of Photosystem II to Photosystem I. Thus two types of energy transfer from Photosystem II to Photosystem I can be recognized; one analogous to a k_D process in the bulk chlorophyll, the other analogous to a k_d process at the reaction center chlorophyll of Photosystem II.

The above equations were based on the "separate package" model. Similar equations derived for the "matrix" model (assuming $k_p \gg k_t$ or k_d) are:

$$\varphi_F = \frac{k_F}{k_F + k_D + k'_T}$$

where $k'_T = k_T[A + (1-A)\,\varphi_d]$

$$\varphi_{F_0} = \frac{k_F}{k_F + k_D + k_T}$$

$$\varphi_{F_M} = \frac{k_F}{k_F + k_D + k_T\,\varphi_d}$$

The "matrix" model also predicts that F_V can be quenched from its maximum value when $\varphi_d = 0$ to zero when $\varphi_d = 1$ without any change of F_0. Furthermore the equations:

$$\frac{F_V}{F_M} = \varphi_T(1-\varphi_{dc}) = \varphi_T\,\varphi_{tc}$$

$$\varphi_{P_0} = \varphi_T$$

are the same those as derived for the "separate package" model assuming $k_p \gg k_t$ or k_d.

Although these two models, which represent the extreme cases for energy transfer between Photosystem II units, cannot be distinguished on the basis of the levels of fluorescence and the yield of photochemistry, they should be distinguishable by comparing the kinetics of the fluorescence change with the kinetics of the photoreduction of the primary electron acceptor, A [9]. The "separate package" model predicts a 1 : 1 correspondence between the increase of fluorescence at any time, $F-F_0$, and the amount of the acceptor reduced, $1-A$.

$$\frac{F-F_0}{F_M-F_0} = 1-A$$

However, the "matrix" model predicts a significant lag between the fluorescence and photochemistry which depends solely on the ratio F_M/F_0.

$$\frac{F-F_0}{F_M-F_0} = \frac{1-A}{1+\left(\frac{F_M}{F_0}-1\right)A}$$

For instance, the "matrix" model predicts, for a ratio F_M/F_0 of 4.5, that the fluorescence, $F-F_0$, will increase to only 18 % of the maximum change when A is 50 % reduced or that A would have to be 82 % reduced in order for the fluorescence to

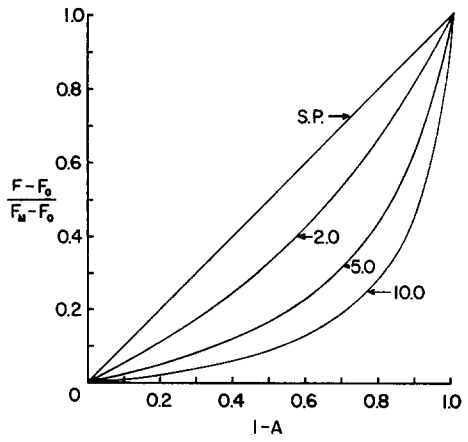


Fig. 4. Theoretical plots of the fractional increase of the fluorescence of variable yield $(F-F_0)/(F_M-F_0)$ as a function of the fraction of the primary electron acceptor reduced, $1-A$. The "separate package" (S.P.) model gives the straight line for all values of F_M/F_0. The "matrix" model gives the curved lines for the values of F_M/F_0 indicated.

increase 50 %. Fig. 4 shows plots of $(F-F_0)/(F_M-F_0)$ as a function of $1-A$ predicted by the two models.

Previous measurements [6] of the kinetics of the fluorescence yield increase and the rate of photoreduction of C-550 indicated a fairly close correspondence between fluorescence and photochemistry* at −100 °C (the half time for the fluorescence increase was about equal to the half time for C-550 reduction) and presumably at higher temparatures. At lower temperatures fluorescence changes lag behind the photochemistry (at −196 °C the half time for the photoreduction of C-550 is 3–4 times faster than the half time for the fluorescence increase) but presumably for reasons related to electron transfer from a secondary electron donor to P_{680}^+ [6] rather than for reasons related to energy transfer between Photosystem II units. One might propose that a phase transition could occur on cooling from −100 to −196 °C which would increase energy transfer between Photosystem II units and thereby favor the "matrix" model. However, the "matrix" model predicts that the kinetics of the fluorescence increase (relative to the photoreduction of A) should be faster in the presence of quenchers, such as ferricyanide, which increase k_d and thereby decrease F_M/F_0 while the "separate package" model predicts that such quenchers should have no effect on the relative kinetics. The available experimental evidence

* Precise correlations between the kinetics of fluorescence and C-550 changes are difficult because both types of measurements must be made under identical conditions and the high chlorophyll concentrations required for the absorbance measurements cause the intensity of exciting light for the fluorescence measurements and the self absorption of fluorescence by the sample to vary markedly with sample depth. Thus, while the absorbance measurements indicate the average kinetic changes occurring throughout the sample, the fluorescence measurements may emphasize kinetics in a particular region of the sample such as at the front surface, if the absorption of the exciting light is greater than the self absorption of the fluorescence, or at the back surface, if the absorption characteristics are reversed. The solution of this problem was discussed previously [6] but even under the best experimental conditions the experimental limits of uncertainty are difficult to ascertain.

[5, 7] indicates that ferricyanide has very little effect, if any, on the kinetics of the fluorescence increase at −196 °C even though the magnitude of F_V is decreased markedly. Also the fluorescence induction curve at −196 °C does not have any of the S-shape character that the "matrix" model predicts. Thus, the "matrix" model does not appear to be valid even at −196 °C where fluorescence changes lag behind the photochemistry. We conclude that the photosynthetic apparatus of Photosystem II is closer to the "separate package" model than to the "matrix" model but, on the basis of the available kinetic data, we cannot predict the degree to which energy transfer may occur between Photosystem II units.

ACKNOWLEDGEMENT

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