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ENERGY TRANSFER BETWEEN PHOTOSYSTEM II AND PHOTOSYSTEM I IN CHLOROPLASTS

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SUMMARY

A model for the photochemical apparatus of photosynthesis is presented which accounts for the fluorescence properties of Photosystem II and Photosystem I as well as energy transfer between the two photosystems. The model was tested by measuring at −196 °C fluorescence induction curves at 690 and 730 nm in the absence and presence of 5 mM $MgCl_2$ which presumably changes the distribution of excitation energy between the two photosystems. The equations describing the fluorescence properties involve terms for the distribution of absorbed quanta, α, being the fraction distributed to Photosystem I, and β, the fraction to Photosystem II, and a term for the rate constant for energy transfer from Photosystem II to Photosystem I, $k_{T(II\rightarrow I)}$. The data, analyzed within the context of the model, permit a direct comparison of α and $k_{T(II\rightarrow I)}$ in the absence (−) and presence (+) of Mg^{2+} : $\alpha^-/\alpha^+ = 1.2$ and $k^-_{T(II\rightarrow I)}/k^+_{T(II\rightarrow I)} = 1.9$. If the criterion that $\alpha + \beta = 1$ is applied absolute values can be calculated: in the presence of Mg^{2+}, $\alpha^+ = 0.27$ and the yield of energy transfer, $\varphi^+_{T(II\rightarrow I)}$ varied from 0.065 when the Photosystem II reaction centers were all open to 0.23 when they were closed. In the absence of Mg^{2+}, $\alpha^- = 0.32$ and $\varphi_{T(II\rightarrow I)}$ varied from 0.12 to 0.28.

The data were also analyzed assuming that two types of energy transfer could be distinguished; a transfer from the light-harvesting chlorophyll of Photosystem II to Photosystem I, $k_{T(II\rightarrow I)}$, and a transfer from the reaction centers of Photosystem II to Photosystem I, $k_{t(II\rightarrow I)}$. In that case $\alpha^-/\alpha^+ = 1.3$, $k^-_{T(II\rightarrow I)}/k^+_{T(II\rightarrow I)} = 1.3$ and $k^-_{t(II\rightarrow I)}/k^+_{(II\rightarrow I)} = 3.0$. It was concluded, however, that both of these types of energy transfer are different manifestations of a single energy transfer process.

INTRODUCTION

Our previous investigations [1, 2] of the quenching of fluorescence and of primary photochemistry in chloroplasts at low temperature led us to a simple model for the photochemical apparatus of Photosystem II. In that model excitation energy trapped by a closed Photosystem II reaction center can be transferred back to the

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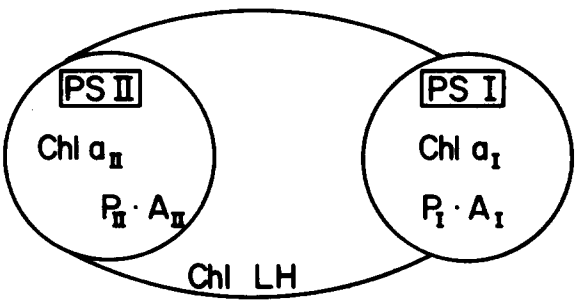
antenna chlorophyll where fluorescence can again occur. The minimum level of fluorescence, F_0, at 690 nm is due to emission from the antenna chlorophyll of Photosystem II which occurs before the excitation energy is trapped by the reaction centers; the fluorescence of variable yield, F_V, is due to excitation energy which has been returned to the antenna chlorophyll from the closed reaction centers.

The model was then expanded to consider both Photosystem I and Photosystem II in the context of a model which assumed three major types of chlorophyll complexes [3] (Scheme 1).

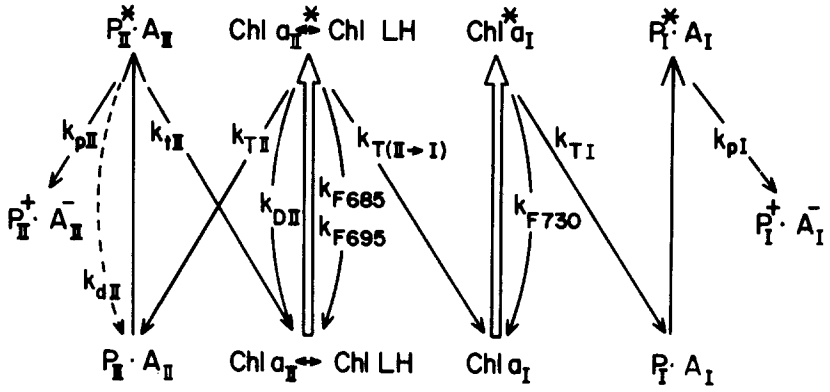


Scheme 1. Chla_I, antenna chlorophyll a_I; Chla_{II}, antenna chlorophyll a_{II}; CHl LH, light–harvesting chlorophyll complex.

The Photosystem I and Photosystem II complexes each contain appreciable amounts of antenna chlorophyll, chlorophylls a_I or a_{II}, and their respective reaction center couples, $P_I \cdot A_I$ or $P_{II} \cdot A_{II}$. The two photosystem complexes are separated by a light-harvesting chlorophyll complex, which can transfer excitation energy to either of the two photosystem complexes. The light-harvesting chlorophyll complex is the chlorophyll *a/b* protein complex isolated by Thornber and coworkers [4, 5]. Wessels and Borchert [6] recently described the separation of chloroplasts into three chlorophyll containing fractions, F_I, F_{II} and F_{III}, which appear to be analogous to the Photosystem I, Photosystem II and light-harvesting chlorophyll complexes.

The three chlorophyll emission bands at 730, 695 and 685 nm that are observed in the fluorescence spectrum of chloroplasts at –196 °C were ascribed to the three types of antenna chlorophylls, chlorophyll a_I, chlorophyll a_{II} and the light-harvesting complex, respectively [3]. We see no evidence for fluorescence emission from the reaction center chlorophylls. The model for the photosynthetic apparatus was described diagramatically (Scheme 2) in terms of the rate constants to depopulate the excited states of chloorphyll in the different types of complexes. It was assumed that excitation energy can be transferred readily between the light-harvesting complex and chlorophyll a_{II} in either direction [3].

It was also necessary to specify how the absorbed quanta are partitioned initially between Photosystem I and Photosystem II. α specifies the fraction of the quanta which excites chlorophyll a_I by direct absorption or by energy transfer from light-harvesting complex; β is the fraction which either excites antenna chlorophyll a_{II} or is dissipated by fluorescence or nonradiative decay in the light-harvesting complex ($\alpha+\beta = 1$). Quanta in the β fraction may also excite antenna chlorophyll a_I by energy transfer from antenna chlorophyll a_{II}.



Scheme 2. See Scheme 1 for abbreviations.

The mathematical description of the model was presented [3] in terms of three equations which describe the fluorescence yield at 690 and 730nm and the yield of energy transfer between Photosystem II and Photosystem I as a function of the fraction of the primary electron acceptor molecules of Photosystem II present in the oxidized state, A_{II}.

$$\varphi_{F690} = \frac{\beta k_{F690}}{k_{F690} + k_{DII} + k_{T(II \to I)} + k_{TII}} \left(A_{II} + \frac{1 - A_{II}}{1 - \varphi_{TII} \varphi_{tII}} \right) \tag{1}$$

$$\varphi_{F730} = (\beta \varphi_{T(II \to I)} + \alpha) \frac{k_{F730}}{k_{F730} + k_{TI}} \tag{2}$$

$$\varphi_{T(II \to I)} = \frac{k_{T(II \to I)}}{k_{F690} + k_{DII} + k_{T(II \to I)} + k_{TII}} \left(A_{II} + \frac{1 - A_{II}}{1 - \varphi_{TII} \varphi_{tII}} \right) \tag{3}$$

The primary photochemical activity of Photosystem I does not result in any fluorescence yield changes of Photosystem I, e.g. careful irradiation of chloroplasts at −196 °C with far-red light will completely oxidize P_{700} without any change of fluorescence yield. (It was proposed [3] that $P^{+}{}_{700}$ is also a trap for excitation energy so that the efficiency of trapping in Photosystem I does not change as P_{700} is oxidized.) Irradiation with red light, however, does cause fluorescence yield changes at 730 nm which are related to Photosystem II activity; the fluorescence of variable yield at 730 nm is ascribed to energy transfer from Photosystem II to Photosystem I, the $\varphi_{T(II \to I)}$ term in Eqn 2. It is apparent from a comparison of Eqns 1 and 3 that $\varphi_{T(II \to I)}$ should have the same dependence on the Photosystem II reaction centers as φ_{F690} and, as such, should have a constant and a variable part that are in the same ratio as the constant and variable parts of φ_{F690}.

The nature of the energy trapping may be somewhat different for the reaction centers of Photosystem I and Photosystem II. We have assumed that there is very little energy barrier for the transfer of excitation energy out of the Photosystem II reaction centers, that excitons can flow readily into and out of P_{680}. Energy trapping in Photosystem II depends on the state of the primary electron acceptor and the statement

that $k_p \gg k_t$. Essentially A_{II} is the energy trap for Photosystem II. In Photosystem I, however, the situation is somewhat different. P_{700} represents an energy trap of some depth. Once the exciton is trapped in P_{700} the chance of back transfer may be small because of that energy barrier. In the case of the presumed trapping by $P^+{}_{700}$ we must assume that the probability for non-radiative decay is much greater than that for a back transfer of energy.

The distribution of the various forms of chlorophyll between the two photosystems would appear to favor (or even direct) a flow of excitation energy from Photosystem II to Photosystem I. Of the eight different absorbing forms of chlorophyll resolved by low temperature absorption spectroscopy, the four longest wavelength forms are all associated with Photosystem I [7].

The primary purpose of the present work was to examine and interpret energy transfer between Photosystem II and Photosystem I. Murata [8] proposed that such energy transfer could be regulated by divalent cations. It is reasonable to expect that energy transfer from one of the photosystems to the other should be reflected in fluorescence emission bands characteristic of the two photosystems. Murata obtained such evidence in fluorescence emission spectra at –196 °C by showing that, in the absence of divalent cations, the Photosystem II emission bands at 685 and 695 nm were diminished while the Photosystem I emission band at 730 nm was increased. We have sought evidence in fluorescence induction curves at 690 and 730 nm measured at –196 °C in the absence and presence of Mg^{2+}. The results obtained in the absence of divalent cations show a marked quenching of the fluorescence of variable yield, F_v, at 690 nm and a pronounced increase of the initial level of fluorescence, F_o, at 730 nm, in accordance with predictions made from the tripartite model [3]. The mathematical expression of the model (Eqns 1, 2 and 3) permit a detailed analysis of the data including quantitative estimates of the effect of Mg^{2+} on the distribution of excitation energy between the two photosystems.

METHODS

Chloroplasts prepared from spinach leaves by methods described previously [9] were suspended at a concentration of approximately 3 mg chlorophyll per ml in a medium containing 0.4 M sucrose, 10 mM NaCl and 5 mM Tris · HCl, pH 7.8, and kept near 0°C. Prior to an experiment, 0.2 ml of this concentrated chloroplast suspension was diluted with a reaction medium (20 mM NaCl and 15 mM Tris · HCl, pH 7.8) to give a final concentration of 10 μg chlorophyll per ml.

Samples (0.3 ml) of the diluted chloroplast suspension were frozen to liquid nitrogen temperature in a vertical cuvette and Dewar system [10]. The frozen samples were about 2 mm thick. For measurements in the presence of Mg^{2+}, 15 μl of 0.1 M $MgCl_2$ was added to the sample (to give a concentration of 5 mM Mg^{2+}) prior to freezing. The measurements were made on chloroplasts frozen to –196 °C in the absence of glycerol. Mg^{2+} had very little effect on the fluorescence of chloroplasts which were frozen in 60 % glycerol media to give a clear glass.

Fluorescence emission spectra were measured at –196 °C with a grating monochromator and a gallium arsenide phototube (Hamamatsu R666S) which gave a spectral sensitivity curve nearly independent of wavelength over the spectral region of the scan ($\pm$ 10 % from 675 to 750 nm). The samples were irradiated with 633 nm light

from a small Ne-He laser through a fiber optics light pipe in contact with the top surface of the frozen sample. Another coincident light pipe transmitted the fluorescence from the illuminated surface to the monochromator. The motor-driven monochromator scanned the fluorescence spectrum while the output of the phototube was recorded on a wavelength-coupled X-Y recorder.

For kinetic measurements of the induction curves at –196 °C, fluorescence was excited by broad band blue light (defined by Corning 9782 and 9788 glass filters, a Calflex C heat reflecting filter and 2 cm of 3 % $CuSO_4$) at an intensity of 0.2 mW/cm^2 at the top of the sample. Fluorescence was measured from the bottom of the sample through blocking filters (a Corning 9830 plus a Toshiba VR65) and either a pair of 690 nm interference filters (the combination giving a peak transmission at 692 nm with a 7 nm halfwidth) or a pair of 730 nm interference filters (9 nm halfwidth). The blocking filters were chosen for their low fluorescence as well as their spectral characteristics. The Corning 9830 filter has a very sharp cut-off at wavelengths less than 690 nm which shifts the peak transmission of the 690 nm filter combination to 692 nm. The photocurrent from an EMI 9558C was measured and stored as a function of time in a Fabri-Tek 1072 computer. Measurements of F_M/F_o were also made with single interference filters blocked with four VR65 filters.

EXPERIMENTAL RESULTS

Our expression for the yield of fluorescence at 730 nm (Eqn 2) assumes that the emission at 730 nm originates entirely from Photosystem I and is not contaminated appreciably by the long wavelength tail of the Photosystem II emission (fluorescence from both the chlorophyll a_{II} and light-harvesting chlorophyll complex are treated as Photosystem II emission). Fig. 1 compares the emission spectrum of the light-harvesting complex at –196 °C (i.e. a purified sample of the chlorophyll *a/b* protein [5]) with emission spectra of two preparations of chloroplasts, one of which was prepared from summer spinach (s), the other from winter spinach (w).

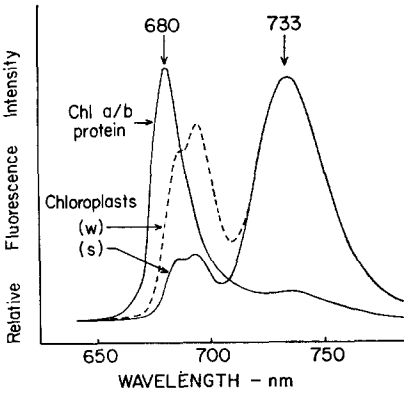


Fig. 1. Fluorescence emission spectra of chloroplasts (10 μg chlorophyll/ml) prepared from spinach grown in the summer (s) and in the winter (w) and the chlorophyll *a/b* protein complex (11 μg chlorophyll/ml) at –196 °C. The chlorophyll *a/b* protein, prepared by methods described in ref. 4, was provided by Drs J. P. Thornber and R. S. Alberte.

The contribution of the light-harvesting complex to the 730 nm emission band of the chloroplasts is considered to be negligible. We did not have a sample of chlorophyll a_{II} to measure but the emission spectrum of the F_{II} particles at −196 °C reported by Wessels et al. [11] shows a single sharp emission peak at 685 nm which is 10 times the intensity of the emission at 730 nm. (The emission spectra of both light-harvesting complex and the chlorophyll a_{II} appear to be shifted 5 nm to shorter wavelength when isolated from the chloroplasts.) Thus, the assumption that the fluorescence at 730 nm is due solely to Photosystem I is reasonably valid, especially for chloroplasts which show a high predominance of the 730 nm band such as the summer chloroplasts in Fig. 1. With chloroplasts which show a less dominant 730 nm band it would be safer to measure Photosystem I fluorescence in the 750 nm region.

We assumed previously [3] from the similarity of the ratios of F_M/F_o measured at 685 and 695 nm at −196 °C that there was a tight coupling for energy transfer between the light-harvesting complex and chlorophyll a_{II}. (Murata [12] drew essentially that same conclusion earlier.) We examined that assumption more closely by measuring ratios of F_M/F_o at different wavelengths and with interference filter combinations of different pass bands (Table I). The chloroplasts used for these measurements had an emission spectrum similar to that of the chloroplasts prepared from winter chloroplasts in Fig. 1. If the three emission bands have different ratios of F_M/F_o, as might be expected, the value of the ratio measured will depend on the degree of overlap between bands in the spectral region measured by the interference filter combination. We can assume that the overlap is negligible at wavelengths shorter than 680 nm or longer than 730 nm. Thus, we take the value of F_M/F_o measured at 679 nm to be representative of the 685 nm band and that measured at 752 nm to be representative of the 730 nm band. It is apparent from the values in Table I that the ratio of F_M/F_o is greater for the 695 nm band than for the other two bands but that the value measured in the 690 - 700 nm region may be less than the inherent value because of the influence of the adjacent overlapping bands. With chloroplasts from either winter or summer spinach we obtain a maximum value of the ratio with our 690 nm filter combination (7 nm halfwidth, 692 nm peak). Using a filter combi-

TABLE I

RATIOS OF F_M/F_o MEASURED AT DIFFERENT WAVELENGTHS OF EMISSION

Filter combinations: a, 4 Toshiba VR65 red cut-off filters and one interference filter; b, one Corning 9830, one Toshiba VR65 and two interference filters. Excitation: Corning blue filters 9788, 9782, 5431, 5433 and 3 % $CuSO_4$ solution, T_{max}, 430 nm; 50 % T_{max}, 400 and 475 nm. Chlorophyll concentration 12 μg/ml in 20 mM NaCl, 5 mM $MgCl_2$, 15 mM Tris · HCl, pH 7.8.

T_{max} (nm)	F_M/F_o	Filter combinations	Width at 50 % T (nm)	Width at 10 % T (nm)	Width at 1 % T (nm)
679	2.8	a	11.5	28	65
683	3.3	a	12	28	75
691	3.8	a	13	21	36
692	4.3	b	7	10	19
696	3.6	a	11.5	29	70
730	1.4	o	9	17	28
752	1.4	a	11	20	37

nation with a broader pass band in this spectral region decreases the value of the ratio because of the greater influence of the overlapping bands. The previous measurements showing approximately equal ratios at 685 and 695 nm are in part fortuitous due to emission band overlaps: the influence of the 730 nm band would be to decrease the ratio measured at 695 nm and the influence of the 695 nm band would be to increase the value measured at 685 nm. We conclude from a number of measurements that the ratio of F_M/F_o for the 695 nm band is at least 50 % greater than the value for the 685 nm band. This conclusion means that the coupling between the light-harvesting complex and chlorophyll a_{II} is not as tight as assumed previously. All of our measurements of Photosystem II fluorescence, however, have been made with the double 690 nm interference filter combination which gives the maximum value of the F_M/F_o ratio.

Fluorescence induction curves at 690 nm and 730 nm measured at -196 °C in the absence and presence of Mg^{2+} are shown in Fig. 2. These measurements were made on chloroplasts prepared from summer spinach which showed a large 730 nm emission band (see Fig. 1). Addition of Mg^{2+} to the reaction medium causes a marked increase of F_V at 690 nm and a small increase of F_o. At 730 nm, the addition of Mg^{2+} causes a marked decrease of F_o and a small increase of F_V. These data will be analyzed within the context of the model represented by Eqns 1, 2 and 3.

The extent of F_V at 690 nm (or the ratio of F_M/F_o) after restoration of Mg^{2+} to the Mg^{2+}-depleted chloroplasts is never as large as it is with the chloroplasts before

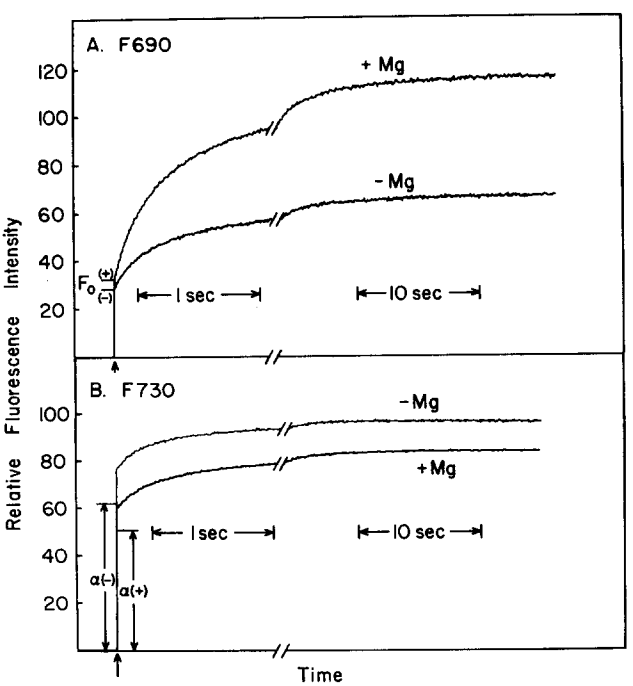


Fig. 2. Fluorescence induction curves of chloroplasts (10 μg chlorophyll/ml) measured at −196 °C in the absence (−) and presence (+) of 5 mM $MgCl_2$. A, at 690 nm; B, at 730 nm. Fluorescence was excited by broad-band blue light at 0.2 mW/cm^2.

depletion. The addition of 2 μM CCCP had no effect on the fluorescence induction curves measured in the presence or absence of Mg^{2+} at room temperature or at −196 °C.

DISCUSSION

The purpose of our analysis of energy transfer is to show that, if certain simplifying assumptions are made, parameters such as the yield of energy transfer from Photosystem II to Photosystem I and the fraction of the quanta delivered to Photosystem I can be calculated from measurements of fluorescence. However, the model and the equations derived from the model are not completely rigorous because certain aspects of the energy transfer properties of the photosynthetic apparatus are not definable in terms suitable for our equations. The model presents a useful framework in which to discuss and analyze energy transfer and the distribution of excitation energy but the qualitative aspects should be kept in mind lest the model be taken too literally. In our discussion we will go through the calculations of such parameters as $\varphi_{T(II\to I)}$ and α and then point out how uncertainties such as the spacial distribution of excitons within Photosystem I or Photosystem II or the light-harvesting complex preclude rigorous calculations of these parameters.

$k_{T(II\to I)}$.

The introduction of the energy transfer term $k_{T(II\to I)}$ into the equations for fluorescence yield at 690 and 730 nm [3] suggested that energy transfer between Photosystem II and Photosystem I might be analyzed within the framework of the tripartite model. It is generally assumed that divalent cations can induce conformational changes in the thylakoid membranes and that such changes could affect energy transfer. Within the context of our model we assume that any physical change which brings the Photosystem I and Photosystem II complexes closer together would increase $k_{T(II\to I)}$ but that k_{F690}, k_{DII} and k_{TII} would remain unchanged.

According to Eqns 2 and 3 the fluorescence at 730 nm can be separated into the constant part due to direct excitation of antenna chlorophyll a_I, α, and the constant and variable parts due to energy transfer from Photosystem II to Photosystem I, $\beta\varphi_{T(II\to I)(o)}$ and $\beta\varphi_{T(II\to I)(V)}$. The constant and variable parts of $\beta\varphi_{T(II\to I)}$ should be in the same ratio as the constant and variable parts of φ_{F690}. Thus, the constant part of $\beta\varphi_{T(II\to I)}$ can be calculated from the data of Fig. 2.

$$\beta\varphi_{T(II\to I)(o)} = \frac{\varphi_{F690(o)}}{\varphi_{F690(V)}}\,\beta\varphi_{T(II\to I)(V)}$$

where $\beta\varphi_{T(II\to I)(V)}$ is the extent of F_V at 730 nm measured directly from the curve. Using the relative values of fluorescence measured from the curves, (−) and (+) Mg^{2+}, $\beta^-\varphi^-{}_{T(II\to I)(o)} = (28/39)21 = 15.0$ and $\beta^+\varphi^+{}_{T(II\to I)(o)} = (32/82)23 = 9.0$. Subtraction of the contribution due to $\beta\varphi_{T(II\to I)(o)}$ from $\varphi_{F730\,(o)}$ leaves the contribution due to α. Such calculations are indicated in Fig. 2B. It is apparent that α is somewhat larger in the absence of Mg^{2+}; $\alpha^-/\alpha^+ = 61/51 = 1.20$.

The data in Fig. 2 taken in the context of the model, provide a measure of dependence of $k_{T(II\to I)}$ on Mg^{2+}. The ratio of values, (−) and (+) Mg^{2+}, of that part of F_o at 730 nm due to energy transfer from Photosystem II is:

$$\frac{\beta^- \varphi^-_{T(II\to I)(o)}}{\beta^+ \varphi^+_{T(II\to I)(o)}} = \frac{\beta^- k^-_{T(II\to I)}}{\beta^+ k^+_{T(II\to I)}} \frac{\Sigma k^+}{\Sigma k^-} \quad \text{(a)}$$

where $\Sigma k = k_{F690} + k_{DII} + k_{T(II\to I)} + k_{TII}$. The ratio of F_o at 690 nm, (–) and (+) Mg^{2+}, is:

$$\frac{\varphi^-_{F690(o)}}{\varphi^+_{F690(o)}} = \frac{\beta^- k_{F690}}{\beta^+ k_{F690}} \frac{\Sigma k^+}{\Sigma k^-} = \frac{\beta^- \Sigma k^+}{\beta^+ \Sigma k^-} \quad \text{(b)}$$

Dividing these two equations gives:

$$\frac{\text{Eqn (a)}}{\text{Eqn (b)}} = \frac{k^-_{T(II\to I)}}{k^+_{T(II\to I)}}$$

The ratios represented by Eqns (a) and (b) can be evaluated from line segment measurements taken directly from Fig. 2. Thus,

$$\frac{k^-_{T(II\to I)}}{k^+_{T(II\to I)}} = \frac{15.0}{9.0} \cdot \frac{32}{28} = 1.9$$

According to the model $k_{T(II\to I)}$ is approximately twice as large in the absence of Mg^{2+}, the condition which favors energy transfer. If we apply the additional criterion that $\alpha + \beta = 1$ (i.e. that $\alpha^+ + \beta^+ = \alpha^- + \beta^- = 1$) we can calculate that:

$\varphi^+_{T(II\to I)(o)} = 0.065 \quad \alpha^+ = 0.27 \quad \beta^+ = 0.73$

$\varphi^-_{T(II\to I)(o)} = 0.116 \quad \alpha^- = 0.32 \quad \beta^- = 0.68$

The internal consistency of these latter values can be verified by comparing the ratios of $\beta^+ \varphi^+{}_{T(II\to I)(o)}/\alpha^+$ and $\beta^- \varphi^-{}_{T(II\to I)(o)}/\alpha^-$ with ratios of the appropriate line segments from Fig. 2B. As the Photosystem II reaction centers close the yield of energy transfer from Photosystem II to Photosystem I, $\varphi_{T(II\to I)}$, increases from an initial value of about 6 % to a final value of 23 % in the presence of Mg^{2+} and from an initial value of about 12 % to a final value of 28 % in the absence of Mg^{2+}. A comparison of results obtained in three separate experiments is presented in Table II.

The determination of the ratio $k^-{}_{T(II\to I)}/k^+{}_{T(II\to I)}$ from the data of Fig. 2 is relatively direct. The calculation of the absolute values of $\varphi_{T(II\to I)}$, α and β requires a greater extrapolation of the model in that these values, (–) and (+) Mg^{2+}, must satisfy

TABLE II

THE EFFECT OF Mg^{2+} ON α AND $k_{T(II\to I)}$

See text for definitions. – and + refer to the absence and presence of 5 mM $MgCl_2$.

Experiment	1	2	3
$\frac{\alpha^-}{\alpha^+}$	1.2	1.2	1.1
$\frac{k^-{}_{T(II\to I)}}{k^+{}_{T(II\to I)}}$	1.9	2.3	2.2

a set of six simultaneous equations; one being the ratio of $k^-_{T(II\to I)}/k^+_{T(II\to I)}$, four being the experimental determinations of α^+, α^- $\beta^+\varphi^+_{T(II\to I)(o)}$ and $\beta^-\varphi^-_{T(II\to I)(o)}$ from the line segment measurements of the data and the sixth being the relationship that $\alpha^+ + \beta^+ = \alpha^- + \beta^- = 1$.

$k_{t(II\to I)}$.

In previous treatments [2, 3] we suggested that two types of energy transfer from Photosystem II to Photosystem I could be distinguished; energy transfer from the antenna chlorophyll of Photosystem II to Photosystem I analogous to a k_{DII} process and energy transfer from the reaction center chlorophyll of Photosystem II to Photosystem I analogous to a k_{dII} process, the latter type of transfer being characterized by a specific quenching of F_V. In the present model we have considered only the first type of transfer, $k_{T(II\to I)}$, analogous to a k_{DII} process. Energy transferred out of the closed Photosystem II reaction centers is assumed to go to chlorophyll a_{II} or to the light-harvesting complex and then, depending on the competition of $k_{T(II\to I)}$ with the other competing rate constants, on to Photosystem I. For reasons of simplicity we have ignored any direct energy transfer from the Photosystem II reaction centers to Photosystem I. In such a treatment, however, the changes of $k_{T(II\to I)}$ account for only a small part of the changes of F_V which are observed at 690 nm. The major part of the change of F_V could be ascribed to the change of an additional non-radiative decay process, k_{dII}, at the reaction center chlorophyll but this latter change would not necessarily be related to energy transfer between the two photosystems. We showed previously [2, 3] (assuming $k_{pII} \gg k_{tII}$ and k_{dII}) that:

$$\left(\frac{F_V}{F_M}\right)_{690} = \varphi_{TII}\varphi_{tII}$$

where $\varphi_{TII} = k_{TII}/\Sigma k$ and $\varphi_{tII} = k_{tII}/(k_{tII}+k_{dII})$. If we made the further tentative assumptions that the maximum value of F_V/F_M was 0.80 and that this value occurred when φ_{tII} was 1.0, we obtained a value of 0.80 for φ_{TII} [2]. We can adopt these assumptions here to estimate the value of φ_{tII} (or $\varphi_{dII} = 1 - \varphi_{tII}$) indicated by the ratios of F_V/F_M at 690 nm shown in Fig. 2A. In the presence of Mg^{2+}, where F_V/F_M was 0.72, φ_{tII} would be 0.90 or φ_{dII}, 0.10. In the absence of Mg^{2+}, φ_{TII} should decrease from 0.80 to 0.77 because Σk increased 4 % due to the increase of $k_{T(II\to I)}$. Thus, the ratio of F_V/F_M of 0.58 obtained in the absence of Mg^{2+} would indicate a φ_{tII} of 0.75 or a ϕ_{dII} of 0.25. On the basis of these assumptions the data indicate that φ_{dII} is 0.25 in the absence of Mg^{2+} and 0.10 in the presence of Mg^{2+}.

However, a part of k_{dII} could be due to a direct transfer of energy from the reaction center chlorophyll of Photosystem II to Photosystem I, a $k_{t(II\to I)}$ process. Such a rate constant would compete with k_{tII} and k_{dII} in the closed reaction centers (we would still assume that $k_{pII} \gg k_{t(II\to I)}$, k_{tII} or k_{dII}) and as such would decrease the value of φ_{tII} and, therefore, the ratio of F_V/F_M at 690 nm. Such energy transfer could be included in the equation for the fluorescence yield at 730 nm:

$$\varphi_{F730} = (\alpha + \beta\varphi_{T(II\to I)} + \beta\varphi_{TII}\varphi_{t(II\to I)}(1-A))\frac{k_{F730}}{k_{F730}+k_{TI}} \qquad (4)$$

The fluorescence of variable yield at 730 nm would then consist of two parts as indicated in Fig. 3.

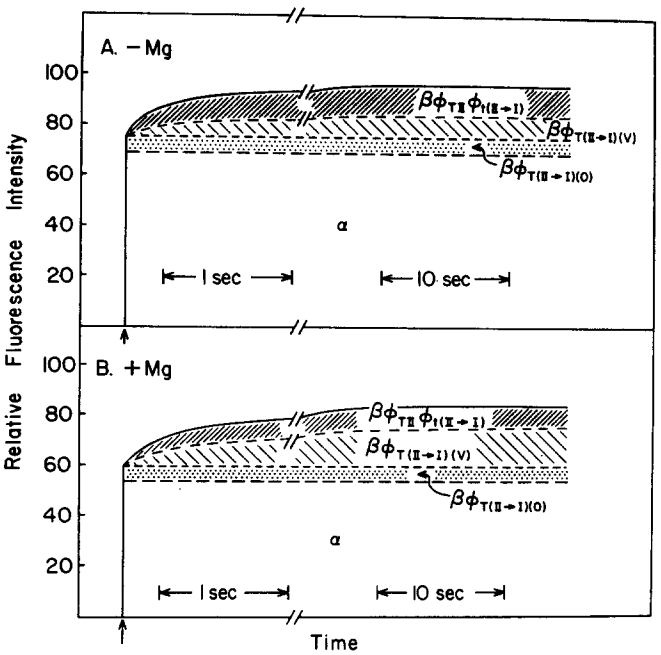


Fig. 3. Fluorescence induction curves at 730 nm measured at −196 °C. A, in the absence of Mg^{2+}; B, in the presence of 5 mM Mg^{2+}; taken from Fig. 2B. The curves are analyzed in terms of the fluorescence due to energy transfer from the Photosystem II reaction centers to Photosystem I, $\beta\varphi_{TII}\varphi_{t(II\to I)}$; the fluorescence due to variable and constant parts of the energy transferred from the antenna chlorophyll of Photosystem II to Photosystem I, $\beta\varphi_{T(II\to I)(V)}$ and $\beta\varphi_{T(II\to I)(o)}$; and the fluorescence due to the direct excitation of Photosystem I, α, according to the calculations indicated in the text.

If we assume that what we calculated previously as a k_{dII} process is actually a $k_{t(II\to I)}$ process and that $\varphi_{t(II\to I)}$ changes from 0.25 in the absence of Mg^{2+} to 0.10 in the presence of divalent cation, we can estimate the relative contributions of the two types of energy transfer. At the maximum level of fluorescence ($A_{II} = 0$), the fluorescence of variable yield at 730 nm due to energy transfer from the reaction centers of Photosystem II will be $\beta\varphi_{TII}\varphi_{t(II\to I)}$ while that due to the variable component (see ref. 3) of the energy transfer from the antenna chlorophyll will be:

$$\beta\varphi_{T(II\to I)(V)} = \beta\varphi_{T(II\to I)(o)} \frac{\varphi_{TII}\varphi_{tII}}{1-\varphi_{TII}\varphi_{tII}}$$

where $\varphi_{TII}\varphi_{tII} = (F_V/F_M)_{690}$. In the absence of Mg^{2+}:

$$\beta^-\varphi^-_{TII}\varphi^-_{t(II\to I)} = \beta^-(0.77)(0.25) = 0.19\beta^-$$

$$\beta^-\varphi^-_{T(II\to I)(V)} = \beta^-(0.116)(0.58/0.42) = 0.16\beta^-$$

(The value of 0.116 for $\varphi^-_{T(II\to I)(o)}$ was taken from our previous calculation.) In the presence of Mg^{2+}:

$$\beta^+\varphi^+_{TII}\varphi^+_{t(II\to I)} = \beta^+(0.80)(0.10) = 0.08\beta^+$$

$$\beta^+\varphi^+_{T(II\to I)(V)} = \beta^+(0.065)(0.72/0.28) = 0.17\beta^+$$

Thus, the relative contributions of the two types of energy transfer can be estimated. New values of $\beta\varphi_{T(II\to I)(o)}$ can be calculated from the new values of $\beta\varphi_{T(II\to I)(V)}$ by the same procedure that was used previously and, therefore, new values of α can be determined (Fig. 3). The ratio $k^-_{T(II\to I)}/k^+_{T(II\to I)}$ can also be recalculated using the new values of $\beta\varphi_{T(II\to I)(o)}$ and the old values of $\varphi_{F690(o)}$. On the basis of these new values, α^-/α^+ is 1.3 (instead of 1.2 calculated previously) and $k^-_{T(II\to I)}/k^+_{T(II\to I)}$ is 1.3 (instead of 1.9) and to compensate for the smaller control of energy transfer by $k_{T(II\to I)}$, the ratio of $k^-_{t(II\to I)}/k^+_{t(II\to I)}$ is 3.0.

We have calculated the degree by which Mg^{2+} control the distribution of excitation energy by α, $k_{T(II\to I)}$ and $k_{t(II\to I)}$ for the two extreme cases with regard to the control by $k_{t(II\to I)}$. It would seem likely that actual cases should be someplace between these two extremes and that energy transfer from Photosystem II to Photosystem I should involve both the $k_{T(II\to I)}$ and the $k_{t(II\to I)}$ types of energy transfer.

The energy transfer process

To make a sharp physical distinction between the $k_{T(II\to I)}$ and $k_{t(II\to I)}$ types of energy transfer is probably misleading. Both types are probably different manifestations of the same type of energy transfer process which originates in the closed Photosystem II reaction centers and passes through chlorophyll a_{II} and/or the light-harvesting complex on the way to Photosystem I. However, the effect of a direct transfer of excitation energy from Photosystem II reaction centers to Photosystem I (the $k_{t(II\to I)}$ process) to specifically quench F_V may be simulated by a spacial distribution of energy transfer out of the closed centers such that some excitons (those transferred to the proximity of Photosystem I) have a higher probability of transfer to Photosystem I. Such transfer will simulate a $k_{t(II\to I)}$ process since these excitons will contribute less to the fluorescence of variable yield.

At this point we should consider some of the qualitative aspects of our model for energy transfer. $k_{T(II\to I)}$ and $k_{t(II\to I)}$ are not rigorously defined activities with precise physical meanings. $k_{T(II\to I)}$ and the other rate constants for the antenna chlorophyll of Photosystem II, k_{F690}, k_{DII} and k_{TII}, are averaged values between the rate constants for the light-harvesting complex and chlorophyll a_{II}. Since we have not defined or treated the degree of coupling between the light-harvesting complex and the chlorophyll a_{II} rigorously, these rate constants are not rigorously defined. Such uncertainties are in part a reflection of our inability to treat the consequences of the spacial distribution of excitation energy in a rigorous manner. For instance, excitons in the light-harvesting complex which are close to Photosystem I complexes will have a greater probability of transfer to Photosystem I and therefore will contribute less to the F_V component of the 685 nm emission than excitons which are further away from Photosystem I and closer to Photosystem II. The value of F_M/F_o at 685 nm which reflects an average value of F_V for the light-harvesting complex is lower than the value of chlorophyll a_{II} because of the greater probability for energy transfer to Photosystem I from some regions of the light-harvesting complex. We have considered the spacial distribution of the excitation energy transferred out of the closed Photosystem II reaction centers artificially as a sum of $k_{T(II\to I)}$ and $k_{t(II\to I)}$ effects and $k_{t(II\to I)}$ is used to account

for most of the quenching of F_V. If the $k_{t(II\to I)}$ concept is used, less energy transfer is attributable to the $k_{T(II\to I)}$ process. For many qualitative purposes, however, it may be sufficient to use the simple procedures (line segment measurements from the induction curves) based solely on $k_{T(II\to I)}$ to estimate the effects of different conditions on energy distribution between Photosystem I and Photosystem II. However, the much smaller extent of F_V at 690 nm which is consistently found in the absence of divalent cations indicates that the $k_{t(II\to I)}$ type of process plays an important role in energy transfer from Photosystem II to Photosystem I. The greater manifestation of the $k_{t(II\to I)}$ process in the absence of Mg^{2+} occurs because a larger fraction of the excitons transferred out of the closed Photosystem II reaction centers are transferred on to Photosystem I (presumably because the Photosystem I and Photosystem II complexes are closer together).

The model incorporates a feedback mechanism (an "energy switch" similar to one suggested by Clayton [13]) to regulate the distribution of exitation energy between Photosystem II and Photosystem I. If Photosystem II tends to run faster than Photosystem I, A_{II} will accumulate more in a reduced state. As the Photosystem II reaction centers close, less of the excitation energy is utilized in Photosystem II and more is transferred to Photosystem I. The increased activity of Photosystem I thereby tends to oxidize A^-_{II} and reopen Photosystem II. In essence, the redox state of A_{II} is automatically adjusted to keep the rates of electron flow through the two photosystems in synchrony. For optimum photosynthesis, however, A_{II} should be maintained in a largely oxidized state to maximize the number of functional reaction centers.

In addition to the "energy switch" control, Mg^{2+} via the proton pump (as protons are pumped into the intrathylakoid space Mg^{2+} is pumped out [14, 15]) exerts a slower control on the distribution of excitation energy between the two photosystems so as to keep A_{II} in a largely oxidized state during steady-state photosynthesis. This Mg^{2+} control is the basis of the so-called State 1-State 2 transitions which have been studied in whole cells [16–18] and more recently in chloroplasts [19–22]. We have shown in the present work that Mg^{2+} influences the distribution of excitation energy by exerting a control on both the initial distribution of quanta, α, and the yield of energy transfer from Photosystem II to Photosystem I. Both effects may be consequences of the same conformational change so that an increase of α is accompanied by an increase in the yield of energy transfer from Photosystem II to Photosystem I.

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