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NEW EVIDENCE SUPPORTING ENERGY TRANSFER BETWEEN PHOTOSYNTHETIC UNITS

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SUMMARY

The non-exponential character of the fluorescence induction observed in presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea or at low temperature has been previously interpreted in terms of energy transfer between photosynthetic units. Alternative hypotheses have been recently proposed and this problem is discussed on the basis of new experimental results.

Several independent methods are used to decrease the concentration of photoactive centers. If the inactive centres are blocked in a non-quenching form, one observes that the number of photons collected per active center increases up to a factor of 3 as the concentration of active centres decreases. However, if the inactive centres are blocked in a quenching form, the number of photons collected per active center remains independent of the concentration of the active centers.

From the experiments described in this paper, one can conclude:

1. Each center includes only one photoactive chlorophyll.
2. Energy transfer occurs between three and probably more connected photosynthetic units.
3. One must assume that the photosynthetic units are not identical. This heterogeneity may be due to their size or some structural features.

INTRODUCTION

The fluorescence yield of chlorophyll *in vivo* depends upon the concentration of one or more quenchers which are directly associated with System II photocenters. Duysens *et al.*[1] interpreted the change of fluorescence yield as the variation of the concentration of quencher Q, the oxidized form of the primary electron acceptor of Photosystem II. Though this theory qualitatively describes the features of the fluorescence rise curves, recent experiments[2–4] have shown that the interpretation of the quenching process requires a more complex hypothesis. First, the fluorescence properties depend upon both the redox state of the electron acceptor and the number of oxidizing equivalents stored on the donor side of Photosystem II. Second, the fluorescence rise curves observed at low temperature cannot be interpreted by the destruction of a single quencher, Q.

Abbreviation: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

To simplify the kinetic analysis of the photodestruction of the quenchers, one chooses experimental conditions in which the regeneration of the quencher is slow compared to its photodestruction:

(a) Inhibitors such as phenylurethane, *o*-phenanthroline[5] and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU)[6] block the reoxidation of the quencher Q by the pool A (plastoquinone).

(b) Lowering the temperature to −70 °C decreases the rate of dark regeneration of the quencher, while the photochemical rate for quencher destruction remains unchanged[2,4,7,8].

(c) The light intensity of the actinic beam can be increased to obtain a large photochemical rate compared to the dark rate[9,10].

These three experimental processes lead to very similar results: the fluorescence rise curves are not exponential and present a characteristic lag followed by an inflexion point.

We interpreted[11] these non-exponential rise curves by the intervention of energy transfer between the photosynthetic units. In this theory, the light energy absorbed by a unit containing a closed trap can be transferred to another unit with a probability p. A simple mathematical analysis of this hypothesis leads to an unique relation between the fluorescence F and the normalized concentration q of a quencher Q:

$$F = kI\left(1 - \frac{q}{-p + pq}\right) \tag{1}$$

k, photochemical rate constant; I, light intensity.

This simple theory does not agree with the results reported by several authors[12,13]. They observed that the fluorescence rise curves measured after a preillumination and partial regeneration of the quencher in the dark were much faster than the rise curves observed with dark-adapted material (Fig. 1). This result means that the same fluorescence levels are shown by different concentrations of the quencher. This problem has been discussed by Lavorel and Joliot[14]. The main criticism of the simple model proposed in ref. 11 is that the light energy absorbed by a closed unit is assumed to be transferred to any other unit with equal probability. Taking into account the fact that the energy can only be transferred to a neighboring unit, significant deviations from the initially proposed model have been predicted. Nevertheless, these deviations are not sufficient to explain the data in refs 12 and 13.

Recently, Doschek and Kok[13] and Lavorel[15] proposed a different interpretation for the non-exponential fluorescence rise curves observed in presence of DCMU: no energy transfer is assumed between units, but each center can undergo two photoreactions. Such a model was previously proposed by Morin[9]. Due to the complexity of the quenching processes discussed in ref. 4, it becomes necessary to reconsider this problem from an experimental point of view. This study confirms the existence of energy transfer between units.

METHODS AND MATERIALS

Fluorescence measurements are performed at room temperature with the method Bennoun previously described[12] and at low temperature with the method described in ref. 4. Oxygen measurements are performed with the modulated oxygen polarograph[16].

Spinach chloroplasts prepared according to Avron[17] are suspended in 0.05 M Tris buffer (pH 7.5) with 0.4 M sucrose and 0.1 M NaCl *plus* 50% (v/v) glycerol for low-temperature experiments.

The mutants and wild-type strains of *Chlamydomonas reinhardti* are grown under conditions described by Gorman and Levine[18]. Strains F34SU1 and F34SU2 are double mutants (Bennoun, P., unpublished): the original strain F34, selected by the method described in[19] has no System II activity, no variable fluorescence, and the stationary fluorescence yield is high. This strain does not grow autotrophically. A second mutation, which partially restores the photosynthetic activity, is induced by ultraviolet irradiation. The double mutants are selected by their ability to grow slowly on minimum medium. These mutants display some variable fluorescence and O_2 evolution.

RESULTS

Low-temperature fluorescence rise curves

In a previous paper[4], we described the fluorescence rise curves observed at low temperature. In Fig. 1, Curve 1 represents the typical fluorescence rise curve with dark-adapted chloroplasts. Curve 2 is obtained when a saturating flash is added at the onset of continuous illumination. This and similar experiments (see ref. 4) demonstrate the existence of two quenching processes; one of them, Q_F, can be destroyed by a single flash, the second one, Q_S, is slowly destroyed by continuous light. When the chloroplasts are preilluminated for 5 s at −43 °C and kept in the dark for 1 min, we observe that a small fraction of the quencher is restored. It is clear that the rise time of Curve 1′ is much faster (3 times) than the overall rise time of Curve 1. However, a comparison can be criticized on the grounds that fluorescence rise is due to the simultaneous destruction of two quenchers of different properties. The tail of Curve

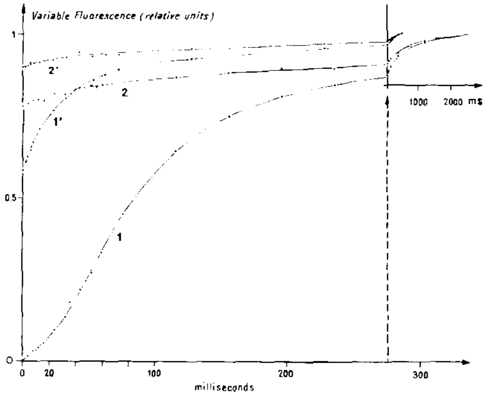


Fig. 1. Fluorescence rises on dark-adapted and preilluminated material. $T = -43$ °C. Curve 1, dark-adapted material; Curve 2, one flash added 4 ms before the onset of the detecting flash. Curve 1′, 5 s continuous preillumination at −43 °C (same intensity as detecting beam) *plus* 1 min dark period; Curve 2′, same as Curve 1′ *plus* one flash added 4 ms before the onset of the detecting light.

1 is mainly due to the destruction of the slow quenching Q_S, while Curve 1′ reveals essentially the destruction of the fast quenching Q_F.

It is more significant to consider only the quenching Q_F after elimination of the slow process Q_S. In Fig. 2, the difference ΔQ_F (ordinate) is plotted as a function of time. Even after the elimination of the slow quenching Q_S, the overall half-time of Curve 1 (dark-adapted chloroplasts) is 3.6 times longer than the half-time of Curve 1′ (after partial regeneration). Curve 1″ is obtained by translating Curve 1′ along the abscissa to compare it with the tail of Curve 1. The difference between the kinetics of these two curves is not predicted by the simple theory [11].

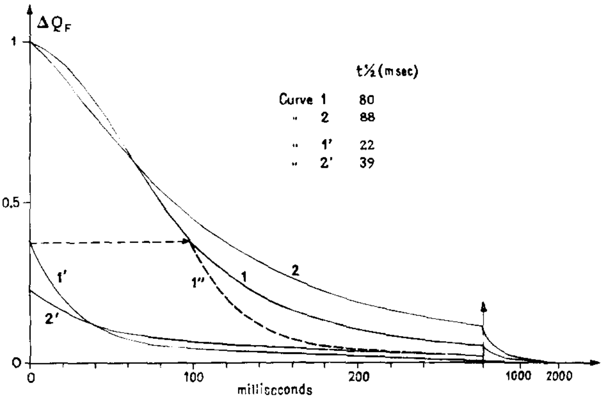


Fig. 2. $\Delta Q_F = f(t)$ in the absence or presence of 0.4 mM dinitrobenzene. Difference ΔQ_F (ordinate) between the rise curves observed with and without low-temperature preilluminating flash. Same conditions as Fig. 1. The amplitude of variable fluorescence is normalized to 1. Curve 1, Curve 2 *minus* Curve 1 of Fig. 1; Curve 1′, Curve 2′ *minus* Curve 1′ of Fig. 1; Curve 1″, same as Curve 1′ translated along the abscissa. Curves 2 and 2′, same as Curves 1 and 1′ in the presence of 0.4 mM dinitrobenzene.

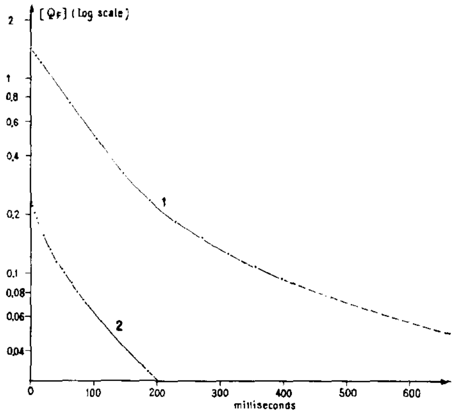
As the fluorescence yield and the rate of the photochemical reaction are complementary[20,21], ΔQ_F is proportional to the rate of the photochemical reaction:

$$[Q_F]_t = k\int_{t=0}^{t=\infty} \Delta Q_F dt \qquad (2)$$

In Fig. 3, the logarithm of $[Q_F]$ is plotted as a function of time. The destruction of Q_F is definitely not exponential. In dark-adapted chloroplasts (Curve 1) the slope increases, then decreases markedly. The initial slope of Curve 2 is about 2.5 times greater than that of Curve 1, this indicates the same as the change in the half-time of Curves 1 and 1′ (Fig. 2). We must point out that at −43 °C, the rate of Q_F regeneration is negligible compared to the rate of its photodestruction. In the experiment reported by Doschek and Kok[13] at room temperature in presence of DCMU, and in the experiment of Fig. 5, the rate of the back reaction is certainly not negligible compared to the photochemical rate.

Effect of m-dinitrobenzene

Lavorel and Joliot[14] previously reported that in the presence of dinitrobenzene, the variable part of the fluorescence induction curve is specifically decreased while the



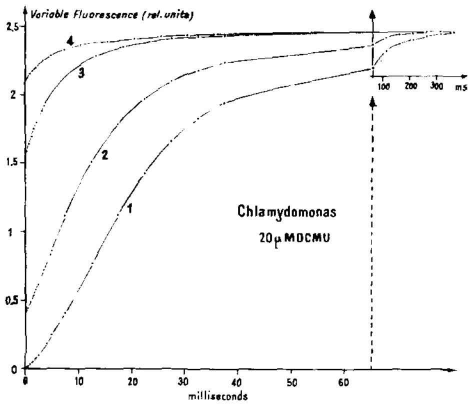


Fig. 3. Logarithmic plot of $[Q_F]$ as a function of illumination time (see text). Curve 1, computed from Fig. 2, Curve 1; Curve 2, computed from Fig. 2, Curve 1′.

Fig. 4. Fluorescence induction for *Chlamydomonas* in the presence of 20 μM DCMU. Room temperature. Curve 1, dark-adapted material; Curve 2, 5 s preillumination *plus* 1 s dark period; Curve 3, *plus* 262 ms dark period; Curve 4, *plus* 46 ms dark period.

rise curve changes from sigmoidal to exponential. However, the addition of up to 1 mM dinitrobenzene does not appreciably inhibit the rate of O_2 evolution measured in weak light. In Fig. 2, the effect of 0.4 mM dinitrobenzene on the kinetics of Q_F destruction is shown. The amplitude of variable fluorescence (before normalization to 1 in Fig. 2) was 3.8 times smaller in presence of dinitrobenzene (Curve 2) than in its absence (Curve 1). Because chloroplasts are less sensitive to dinitrobenzene than Chlorella[14], the lag phase is not completely suppressed in these experiments. Comparing Curves 1 and 2, one can observe that, though the addition of dinitrobenzene does not affect particularly the half-times, it markedly slows down the destruction of Q_F after partial regeneration of the quencher (Curves 1′ and 2′).

Fluorescence and O_2 measurements on Chlamydomonas

Fluorescence rise curves in the presence of DCMU were studied at room temperature on dark-adapted material (Curve 1, Fig. 4) and after partial regeneration of the quencher (curves 2, 3, 4). One observes that the smaller the fraction of quencher regenerated, the shorter the half-time of the rise. The ratio of the longest to the shortest reaches 4.3 (see Curves 1 and 4).

Chlamydomonas mutants F34SU1 and F34SU2 which have a normal chlorophyll content per cell, but a small number of System II photoactive centers, show fluorescence induction curves characterized by a high initial level and a small variable part. We observe that the fluorescence rise in the presence of DCMU is 3.4 times faster for the mutant F34SU2 than for the wild type. Thus, the number of light-collecting chlorophylls per center is greater in the mutant than in the wild type. Secondly, the rise curve for the mutant more closely resembles an exponential than that of the wild type (log plot in the insert Fig. 5). We checked at low temperature that the fluorescence rise curves of this mutant depend on the number of oxidizing equivalents stored in a way similar to the wild type.

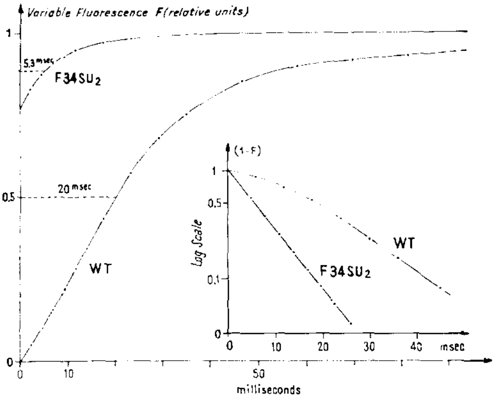


Fig. 5. Fluorescence induction in the presence of 10 μM DCMU with dark-adapted *Chlamydomonas* (wild type and mutant F34SU2). Room temperature. Insert: log plot of $(1-F)$ for both curves.

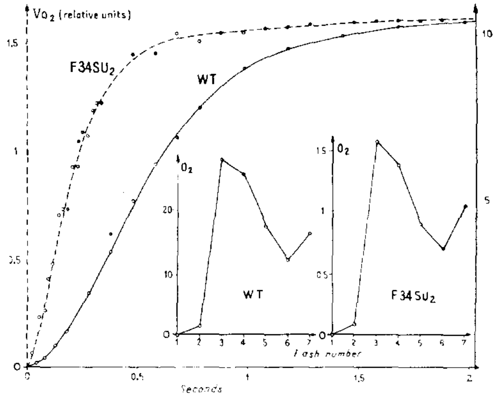


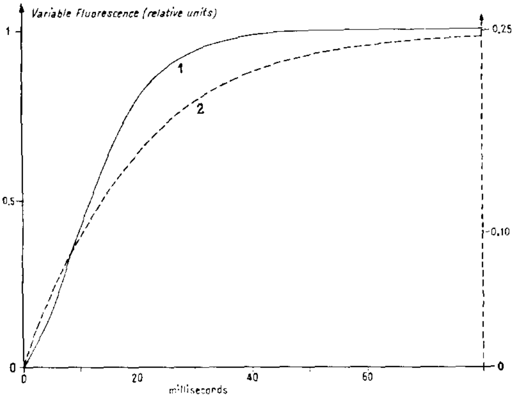
Fig. 6. Rate of O_2 evolution with dark-adapted *Chlamydomonas* (wild type and mutant F34SU2). Room temperature. The maximum rates of O_2 evolution are normalized. Insert: yield of O_2 evolution under a series of short saturating flashes with *Chlamydomonas* Wild type and mutant F34SU2.

Oxygen evolution in weak modulated light and in flashing light has been studied for mutant F34SU2 and wild type. The O_2 yield per flash (insert Fig. 6) for the mutant is 0.055 of that of the wild type, while the oscillatory pattern remains identical: this result indicates that the number of System II photoactive centers for the mutant is 0.055 of that of the wild type, while the relative initial concentrations S_0 and S_1 are identical[22]. However, the stationary rate of the mutant measured in weak light is 0.155 relative to that of the wild type (Fig. 6). One can thus conclude that the number of photons collected by each System II photoactive center is about $0.155/0.055 = 2.8$ larger in the mutant than in wild type. The same conclusion can be drawn from the comparison of the oxygen activation measured for both the mutant and wild type. The duration of the activation is inversely proportional to the number of photons collected per active center per unit time[23]. The activation process is also 2.8 times faster for the mutant than for the wild type.

In another mutant, F34SU1, the number of active centers is 25% of the wild type, while the stationary rate in weak light is 50% of the wild type. The activation process is about 1.7 times faster for this mutant. A comparison of mutants F34SU2 and F34SU1 indicates that the fewer the number of centers, the higher the cross-section of each center.

Effect of ultraviolet irradiation

It has been reported[24,25] that ultraviolet irradiation decreases the number of active centers and the variable part of fluorescence, while it does not modify the initial F_0 level. Fig. 7 shows a fluorescence rise curve observed in presence of DCMU before (Curve 1) and after (Curve 2) partial ultraviolet irradiation. The variable fluorescence, which is 4 times smaller for the ultraviolet-irradiated cells, has been normalized to 1. The treated cells display an approximately exponential rise curve,



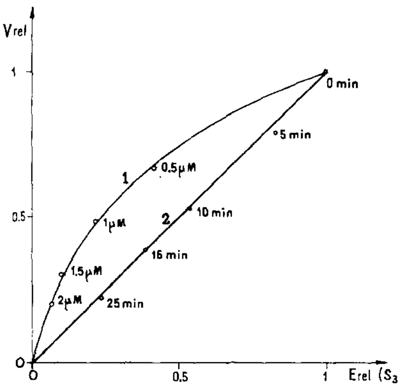


Fig. 7. Fluorescence rises in presence of 10 μM DCMU with dark-adapted *Chlorella*. Room temperature. Curve 1, control; Curve 2, algae were first irradiated with ultraviolet light from a germicidal lamp for 3 min. DCMU was added after the irradiation: the fixed fluorescence was unchanged, but the amplitude of the variable part is reduced by a factor of 4.

Fig. 8. Relation between stationary rate of O_2 evolution in weak far-red light and O_2 produced by a superimposed short saturating flash. Room temperature. Curve 1, algae were treated with the indicated concentrations of DCMU; Curve 2, algae were exposed to ultraviolet irradiation for the indicated times.

similar to that reported in ref. 14 for the effect of dinitrobenzene on *Chlorella*. Fig. 8 represents the relation between the stationary rate V_{O_2} of O_2 evolution measured in weak light *versus* the amount E of O_2 evolved by a saturating flash superimposed on the weak illumination. E is proportional to the concentration of the System II photoactive centers (see Discussion). The concentration of active centers is decreased either by increasing concentration of DCMU, as in ref. 26 (Curve 1), or by exposure to ultraviolet irradiation. As reported in ref. 25, we observed that in the presence of DCMU and in weak light, *i.e.* when the inhibited centers are in a non-quenching state, the ratio V_{O_2}/E increases as E decreases. On the contrary, for ultraviolet-irradiated cells, *i.e.* when the inhibited centers are blocked in a quenching form, the ratio V_{O_2}/E remains constant as E varies. In another experiment, we checked that the time course of the activation process is not modified by ultraviolet irradiation. We conclude that partial inhibition by ultraviolet irradiation does not modify the cross-section of the photocenter.

DISCUSSION

In the preceding paper[4] we distinguished two types of quenching, Q_F and Q_S: Q_F is destroyed by a saturating flash, and the remaining quenching Q_S can be destroyed by low efficiency photochemical process. With dark-adapted material at low temperature, Q_F is responsible for the major part of the quenching. Similar behavior is described by Doschek *et al.*[13] at room temperature and in presence of DCMU. Thus, the function $-\Delta Q_F = f(t)$ (Fig. 2) does not differ essentially from the fluorescence rise curve itself, except for the final phase. Both are far from exponential functions and present a well-developed lag.

To interpret these results, several hypotheses are proposed:

Hypothesis I. The destruction of Q_F involves only one photoreaction, achieved during a short saturating flash. Q_F thus accepts only one electron. The light energy absorbed by a unit including a closed trap is transferred with high efficiency to other units[11]. Clayton[27] proposed an alternative hypothesis in which he associates n photocenters with their collecting pigments in a "domain" where excitons can migrate freely. Delosme[10] had previously considered the particular case $n=\infty$. All these models lead to a similar mathematical analysis which accounts for the characteristic lag phase of the fluorescence rise. The problem of energy transfer has been recently discussed from a theoretical point of view by Paillotin[28]. This author supposes that the photosynthetic units are separated ones from the others by an energy barrier linked to the heterogeneity of the collecting pigments.

Hypothesis I′. One can consider a particular case of Clayton's model, where a domain includes two photocenters, each of them being an independent entity for the information of an O_2 molecule.

Hypothesis II. Destruction of Q_F involves two photoreactions occurring either in series on one quencher able to store successively two negative charges, or in parallel on two quenchers[9]. In this case, no energy transfer is required to interpret the lag phase. As two photoreactions may occur during a short saturating flash, one must assume that each center includes two photoactive chlorophylls, or that the turnover of the photoactive chlorophyll is faster than the duration of the flash (1 μs). This last assumption is inconsistent with the data of Döring *et al.*[29].

Doschek *et al.*[13] and Lavorel[15] recently developed arguments which favor Hypothesis I′ or II. We now attempt to make a choice between these three hypotheses, considering the results of oxygen and fluorescence experiments.

Hypothesis II implies that two charges can be transferred during one flash and is thus contradictory to the model of Kok *et al.*[30] where only one positive charge is stored per photoreaction. Because the model of Kok *et al.*[30] describes correctly the pattern of oxygen evolution under both flashing light and continuous light, the possibility of high-efficiency double-hit events is ruled out, even on the first flash of the sequence. One might thus assume that the photocenters react in a different way at low temperature or in the presence of DCMU.

Oxygen measurements

According to Kok *et al.*[30], the rate V_{O_2} of O_2 evolution is equal to the product of the number of centers in the S_3 photoactive state times the number N of photons absorbed per active center per unit time:

$$V_{O_2} = N[S_3] \qquad (3)$$

The concentration of S_3 is measured by the amount E of O_2 evolved by a saturating flash. In weak light, the steady state rate is limited by photochemical processes. The concentration of the four photoactive states S_n are equal (*i.e.* S_3 is 0.25 of the total concentration of centers[30]). In the presence of a non-saturating concentration of DCMU, Kok *et al.*[29] observed that the non-inhibited fraction of the centers evolved O_2 exactly as they do in absence of DCMU. (For example, the amount of O_2 evolved per flash is decreased while the flash yield pattern is not modified.) Thus, in steady-state conditions, the relative distribution of the photoactive states is unchanged.

$$[S_3] = 0.25\Sigma[S_n] \quad (4)$$

Fig. 8, Curve 1, which reproduces an experiment of Joliot[26], shows that when the degree of inhibition, *i.e.* the number of inactivated centers increases, the ratio V_{O_2}/S_3 increases by as much as a factor of 3. This increase can only be interpreted by the increase of the frequency N of absorbed photons per center. As in steady-state conditions, the inhibited centers are in the non-quenched form, one must assume that the collecting pigments associated with these centers transfer the light energy to other centers. A similar conclusion can be drawn, even if the concentrations of the different S_n states are not equal. The increase by a factor of 3 of V_{O_2}/S_3 indicates that at least three photosynthetic units and probably more are connected. In terms of the model of Clayton, a "domain" includes at least three centers.

Similar quantitative conclusions are drawn from the study on the highly fluorescent *Chlamydomonas* mutant F34SU2 which includes a small number of active centers (0.055 of the wild type). In this mutant, N is 2.8 times larger than for the wild type. This value is similar to the one computed for a high degree of DCMU inhibition (Fig. 8, Curve 1). Comparison between mutants F34SU1 and F34SU2, which have a different number of active centers, also shows that N decreases when the number of centers increases. Only Hypothesis I can explain the large increase of N (3 times) observed when the number of centers in the quenching state decreases.

Different behavior is observed when the centers are blocked in the quenching form, for example by ultraviolet irradiation (Fig. 8). In this case, the ratio V_{O_2}/S_3 does not depend upon the degree of inhibition of the centers. This result is easily understandable, the unit including an inhibited center but an open trap is unable to transfer the light energy to other centers.

Fluorescence measurements

The fluorescence rise at low temperature (Fig. 1) or at room temperature in presence of DCMU (Fig. 4) is measured either on dark-adapted material or after preillumination followed by a partial dark regeneration of the quencher. We have pointed out that in the presence of DCMU, the half-time of the partial rise curve is about 4 times shorter than the overall half-time of the initial rise. At low temperature (Figs 1 and 2) the partial rise curve is also faster than the corresponding rise in the initial curve, even after corrections for the slow component Q_S. In the mathematical analysis of Lavorel[15] of Hypotheses I′ and II, the maximum variation of the overall half-times is less than a factor of 2. Thus, Hypotheses I′ and II do not permit an interpretation of the experimental data. The large variation of the overall half-times can be explained by Hypothesis I, assuming a large p factor. Nevertheless, Hypothesis I in its simplest formulation does not take into account the difference between the partial rise curve and the corresponding tail of the initial rise (Fig. 2, Curves 1 and 1″).

The rise curve observed for mutant F34SU2 is similar to the partial rise curve obtained with the wild type. In this case, the half-time rise is about 3 times shorter for the mutant than for the wild type (Fig. 5). More important is the fact that the mutant rise curve does not show any lag and is very close to an exponential function. If the non-exponential rise were an intrinsic property of the centers included in isolated units (Hypothesis II), we would expect only a change in amplitude while the shape is unchanged.

Dinitrobenzene is known to suppress the lag phase of the fluorescence rise[14] without appreciably inhibiting O_2 evolution. The simplest way to interpret its action is to assume that dinitrobenzene is fixed on the centers, which then remain in the quenching form, even when the electron acceptor(s) is (are) reduced. Thus, units that have fixed dinitrobenzene can never transfer the light energy to other centers which explain the exponential character of the rise curve, without a major change in the overall half-time (Fig. 4, Curves 1 and 2). On the other hand, dinitrobenzene markedly slows down (2 times) the fast rise observed after partial regeneration of the quenchers. Thus, the action of dinitrobenzene favors the Hypothesis I, where one assumes that fast rise curves are due to energy transfer from neighboring units.

The effect of ultraviolet irradiation on fluorescence rise is similar to the action of dinitrobenzene and can be interpreted in the same way.

Centers blocked in the quenching form are indistinguishable by fluorescence measurements, whatever their photochemical activity.

Most of the experiments reported here agree with the concept of energy transfer between units. Nevertheless, as pointed out before, the simplest theory of energy transfer (Eqn. 1) does not take into account the abnormally fast rise curves observed after partial regeneration of the quencher or with mutants. In this theory, we did not consider the possible existence of different types of heterogeneity in the system.

(1) Illumination of the chloroplasts can be heterogeneous due to technical imperfections or to the "sieve" effect[31]. This effect, though not negligible, does not appear as essential.

(2) The size of the photosynthetic units, *i.e.* the number of collecting pigments associated with a center, is certainly not constant, Recently, Schmid and Gaffron[32] reported large variations in the size of the photosynthetic unit. Thus, for our material, we do not know the degree of homogeneity of the photosynthetic unit.

(3) If a limited number of photosynthetic units are organized in a structure, the probability of transfer for the units placed at the periphery is smaller than for the units inside this structure. The number of associated units can also vary.

(4) Even in a perfectly homogeneous association of photosynthetic units, the distribution of closed and open traps is not random during the time course of the fluorescence rise ("ilot" effect[14]): a quantitative analysis shows that this effect is not negligible but insufficient to interpret the data.

A general consequence of the heterogeneity of the pigments apparatus is that some units are more likely to absorb a photon than others. To simplify the reasoning, we arbitrarily distinguish between "fast" and "slow" units. Fast units disappear first and slow units accumulate for the end of the fluorescence rise. Such a hypothesis is supported by the experiment reported in Fig. 3, which shows that the rate "constant" of the destruction of quencher Q_F decreases during the last phase of the fluorescence rise curve.

There is no reason for the dependence of the probability of dark regeneration of the quencher, which is a biochemical property of the center, upon the size or the position of the unit. Thus, after their total destruction, quenchers included in fast or slow units regenerate with the same time course. Furthermore, the fraction of fast units is greater after partial regeneration than in the tail of the initial fluorescence rise curve. The same reasoning is applied to mutants with low concentration of centers if one assumes that they are randomly distributed among the units.

As an example, we computed from Eqn. 1 the theoretical rise curves assuming a heterogeneity in the size of units (Fig. 9). We suppose the existence of two types of units in equal concentration, one in which the number of collecting pigments is twice that of the others. Therefore, the maximum fluorescence yielded by the large units is twice that of the small units. The probability p of energy transfer between units is taken as 0.75 and to simplify the computation, the two types of units are not mixed.

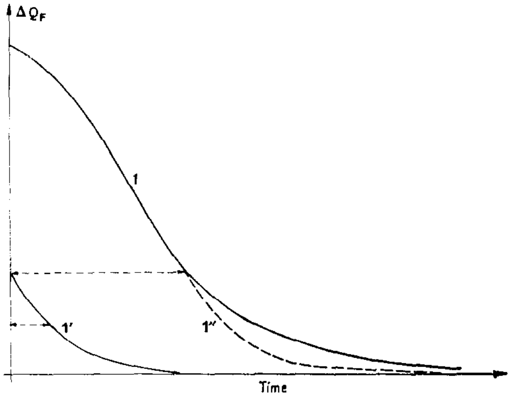


Fig. 9. Theoretical curve computed from Eqn. 1. See text. Curve 1, dark-adapted material, *i.e.* the normalized concentration of the quencher at time zero is equal to 1; Curve 1′, computed when an initial concentration of 0.1 of the quencher is reached after total photodestruction and partial dark regeneration; Curve 1″, same as Curve 1′, translated along the abscissa.

Furthermore, we assume an equal time course for dark regeneration of the quenchers for the two types of units. Although we choose an oversimplified model for the heterogeneity of the units, we obtained a good representation of the experimental data. It is probable that other types of heterogeneity would give an equally good fit to the experimental data; however, their mathematical analysis is more difficult. It is important to point out that a heterogeneous model leads us to choose a larger value for p (0.75) than in a homogeneous model (0.55 (ref. 11)).

In the absence of a more detailed theoretical analysis, we can draw the following basic conclusions:

(1) Only one photoreaction per center occurs during a short saturating flash. This hypothesis is not contradictory to the concept of two quenchers previously reported, if we assume the intervention of one photoactive chlorophyll per center.

(2) Two nearly equivalent models can be proposed to represent the pigment apparatus, depending upon whether we assume that the photosynthetic unit is an actual structural entity or has only a statistical meaning. In the first case energy can migrate from units including a closed trap to neighboring units. The number of connected units is at least equal to 3 and probably higher. In the second case, one must consider according to Clayton[27], a "domain" including at least three centers where the energy can migrate freely.

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