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COMPARATIVE STUDY OF THE FLUORESCENCE YIELD AND OF THE C550 ABSORPTION CHANGE AT ROOM TEMPERATURE

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Summary

The C550 absorption change and the fluorescence yield were studied at room temperature in chloroplasts in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea, and under conditions in which contributions of *P*-700 and of the electrochromic effect were negligible.

1. The C550 difference spectrum is a typical band shift with an isobestic point close to 550 nm.

2. The maximum amplitude of C550 absorption change is reached upon the first flash of a series of saturating flashes, unlike the maximum fluorescence yield which is attained after several flashes.

3. The comparison of the induction curves of the C550 change and the fluorescence yield in weak light shows that the fluorescence yield is controlled by two quenchers: one of them (Q_1), the redox state of which C550 is a probe, is responsible for the major part of the quenching; the other one (Q_2), which is less concentrated and less efficient becomes predominant at the end of the fluorescence induction.

4. Quencher Q_2 back-reacts faster than quencher Q_1.

5. Two alternative models are discussed in which Q_1 and Q_2 belong either to the same Photosystem II center or to two different photocenters.

Introduction

It is now generally admitted that the fluorescence yield of chlorophyll (Chl) *a* in vivo depends upon the redox states of both primary donor Chl and

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PS I, Photosystem I; PS II, Photosystem II; Chl, chlorophyll.

acceptor Q of Photosystem II [1,2]. Among the possible forms of the photocenters, only Chl-Q^- has a low quenching efficiency. Excitation energy migrates freely within a domaine including at least four photocenters and about a thousand chlorophyll molecules [3—5]. The interpretation of the fluorescence induction curve is then complicated, since the fluorescence yield is a non-linear function of the concentration of the quenchers. However, the preceding hypotheses do not completely explain the complex fluorescence rise observed when the reoxidation of the primary electron acceptor Q is blocked either by the action of inhibitor as 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) or by low temperature.

The fluorescence rise always shows at least two phases [4,6,7,9]. Their relative amplitude depends upon several parameters, such as temperature, the number of oxidized equivalents stored on the donor side [6,10], the pH [7,8]. Different interpretations which are not mutually exclusive have been proposed. (a) Two electron acceptors, quenchers in their oxidized form, are associated to each photocenter [6,11,12]. We have recently suggested [13] that a fraction of the PS II centers can undergo a double photoreaction during the course of a few microseconds flash. (b) There exist two types of centers, each of which has a different quenching efficiency [8]. The results of Diner [14] which are compatible with hypotheses (a) or (b) show that only one of the two electron acceptors is connected to the electron transfer chain. (c) The size of the photosynthetic unit, i.e. the ratio between the number of photocenters, is not constant [4]. This hypothesis is supported by structural experiments of Staehelin et al. [15] which showed that the size of the particles attributed to the System II antenna varies within the same photosynthetic membrane.

Two absorption changes respectively characterized in the ultraviolet region (X320) [16] and in the green region (C550) [17] of the spectrum have been ascribed to the reduction of the Photosystem II electron acceptor [18]. The C550 absorption change studied mainly at low temperature, is an indirect indicator of the presence of a negative charge on the PS II acceptor side [19,20]. Nevertheless, in order to simplify our presentation, we will consider C550 as an electron acceptor.

In this paper, we investigated at room temperature the relationship between the C550 absorption change and the fluorescence in isolated spinach chloroplasts. These experiments were performed in the presence of DCMU which prevents the reoxidation of the Photosystem II electron acceptor by the electron transfer chain.

Materials and Methods

Spectrophotometric measurements were performed using an apparatus first described in [21] and improved by Joliot, Béal and Frilley. The sensitivity of the method is $\Delta I/I \approx 3 \cdot 10^{-5}$. The biological signals we measured are generally 3—4 times higher than those reported in the literature. This is due to the large optical path of the measuring cuvette (18 mm) and to the efficient collection of scattered light. The absorption of the material is sampled by 2-μs monochromatic (4 nm bandwidth) flashes which induce a negligible actinic effect.

Fluorescence measurements were performed using the same apparatus where

the photocell was replaced by a XP1002 Radiotechnique photomultiplier protected by two red filters (Ulano Rubylith and Kodak Wratten No. 70). Fluorescence yield was sampled by the same detecting flashes as the ones used in spectrophotometric measurements. Both sides of the 8 mm thick measuring cuvette are illuminated by two synchronized actinic Xenon flashes (General Radio Stroboslave 1539A) which permits a homogeneous illumination of the sample. Saturating actinic flashes were either red (Kodak Wratten No. 29) or blue (Corning 4-96); non-saturating actinic flashes were green (2 Kodak Wratten No. 57). In most experiments, the absorption or the fluorescence is sampled 5 ms after each actinic flash.

Chloroplasts were isolated from market spinach according to the method of Avron [22] and stored at −70°C in the presence of 5% Me_2SO. Prior to use, chloroplasts were suspended in 0.05 M phosphate buffer (pH 6.4) with 0.4 M sucrose, 0.1 mM methylviologen, 0.1 M KCl, 0.5 μM gramicidin D and 7% ficoll. All the experiments were performed at 18°C, in the presence of 10 μM DCMU added to dark-adapted chloroplasts. Subsequent addition of 0.2 mM ferricyanide oxidized cytochrome *f* and partially *P*-700. It is essential to add ferricyanide after DCMU in order to avoid an Ikegami and Katoh effect [23]. We verified that under our experimental conditions, the addition of ferricyanide does not induce any increase in the concentration of the quenchers as defined by the ratio S/F_v, in which S is the area bound between the fluorescence induction curve and its asymptote and F_v the variable fluorescence (see Ref. 23).

Chloroplasts were dark adapted in a helic-shaped reservoir. The sample in the measuring cuvette can be automatically renewed and recycled. In order to maintain *P*-700 mainly oxidized, chloroplasts were exposed alternately to illumination (by one or several actinic flashes) and to a dark period (2.1—36 s). The same sample thus undergoes five to ten light plus dark cycles to improve the signal to noise ratio; no measurement is performed during the first cycle which differs from the other in that the chloroplasts were not preilluminated.

Results

Difference spectrum of C550 component at room temperature

The C550 difference spectrum has been measured using chloroplasts incubated for times longer than 15 min in 0.2 mM ferricyanide and in the presence of gramicidin D. Chloroplasts were illuminated by a series of five flashes given 300 ms apart and separated by 9 s of darkness. Each sample is exposed to ten successive light and dark cycles. Under these conditions, a large fraction of the PS II electron acceptors are in the oxidized form at the end of each 9 s dark period.

In the 540—560 nm range, several absorption changes can contribute to the signal in addition to the C550 absorption change. (a) The electrochromic effect [24]. As shown by Junge and Witt [24], the addition of gramicidin and KCl drastically decreases the lifetime of the transmembrane potential. Under these conditions, we observed a biphasic decay of the 515 nm absorption change induced by a saturating actinic flash: a fast phase (85—90% of the total amplitude) terminated in 2 ms is followed by a slow phase which lasts several

seconds. The significance of this slow phase which is insensitive to gramicidin, is not yet understood. The eliminate the electrochromic effect, our measurements were performed in the presence of gramicidin D and KCl for times equal to or longer than 5 ms after the actinic flash. As for all previous measurements of the C550 absorption change we cannot estimate the contribution of the gramicidin-resistent 515 nm change in the 540—560 nm range. Preliminary experiments suggest that the difference spectrum of this absorption change is relatively flat. We therefore do not expect any contribution of this phenomenon to the differential signal $\Delta I/I$ (544 nm) $-$ $\Delta I/I$ (552 nm). (b) *P*-700 [25]: After pre-illumination in the presence of DCMU, *P*-700 reduction appears as a slow process even in the absence of ferricyanide. In the 540—560 nm region, the difference spectrum ($P\text{-}700^{+} - P\text{-}700$) is relatively flat and shows a positive absorption band [26]. We estimate the rate of reduction of *P*-700 by measuring the light-induced absorption change at 550 nm (isobestic point in Fig. 1). We observed a slow reduction of *P*-700 ($t_{1/2} \approx 60$ s); after infinite dark adaptation we estimate that the fraction of *P*-700 reduced does not exceed 20—25%, by comparative measurements on chloroplasts in the absence of ferricyanide. After the 9 s dark period, the fraction of reduced *P*-700 is not measurable and is certainly less than 2—3%. This estimation of the rate of *P*-700 reduction is confirmed by measurements of the rate of methylviologen reduction performed with a modulated electrode (unpublished results). We conclude that the spectrum (Fig. 1) does not include any light-induced component linked to System I activity. (c) Cytochrome *f* and cytochrome *b*-559: as shown by

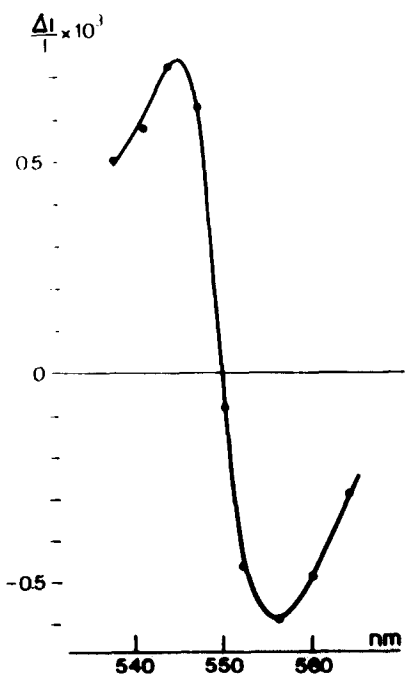

Fig. 1. C550 light minus dark difference spectrum at room temperature. Chlorophyll concentration 84 μg/ml. Measurements were performed 5 ms after the first actinic flash of a series of five flashes.

Horton and Cramer [27], these two cytochromes are oxidized by ferricyanide. Moreover, the fact that *P*-700 is oxidized excludes any light-induced absorption change linked to cytochrome *f*. (d) Cytochrome b_6: the potential of cytochrome b_6 is close to 0 mV [29] and this component is certainly oxidized by ferricyanide; this cytochrome is generally associated with Photosystem I (see a review by Cramer and Whitmarsh [29]), which excludes its contribution in our experimental conditions.

Then we can assume that Fig. 1 gives the difference spectrum of C550. The same type of measurement was performed in the absence of ferricyanide and for 36 s dark adaptation, i.e. when the concentration of reduced *P*-700 is relatively high. We measured the signal at 544 and 552 nm both in the presence and in the absence of Photosystem II active centers (Table I). When the Photosystem II reaction is blocked, the C550 shift disappears and there remains only a signal due to *P*-700. This result confirms that the C550 shift is associated to Photosystem II reaction.

The difference spectrum we obtained for C550 is displaced about 5 nm towards longer wavelengths compared to the spectrum obtained by Katoh [30] using digitonin particles and Van Gorkom [31] using deoxycholate particles. This difference can be due to the fact that the environment of the System II centers is modified by the methods of preparation of the particles. Also, the use of a 4-nm bandwidth interference filter significantly widens the spectrum of Fig. 1. Nevertheless, this difference remains difficult to explain. We cannot definitely exclude the contribution of an unknown component, directly linked to Photosystem II reaction centers. We must stress that this component cannot be due to photoreduction of cytochrome b_6. Such a reduction would induce a positive band at 563 nm, which would displace the negative peak of the difference spectrum in Fig. 1 towards shorter wavelengths.

In the experiments reported below, the C550 component is characterized by the difference $\Delta I/I$ (544 nm) $-$ $\Delta I/I$ (552 nm). All our experiments were performed in the presence of ferricyanide on chloroplasts first preilluminated and then kept in the dark from 2 to 36 s; under these conditions, the concentration of *P*-700 is maintained at a very low level and its contribution to the signal ($\Delta I/I$ (544 nm) $-$ $\Delta I/I$ (552 nm)) remains negligible (less than 2%). Our experimental process differs from the one followed by Ben-Hayyim and Malkin [32]. In their experiments, the chloroplasts, in the absence of ferricyanide, were preilluminated and then kept in the dark for 90 s. Under these

TABLE I

ABSORPTION CHANGES AT 544 nm AND 552 nm IN THE PRESENCE OR IN THE ABSENCE OF PHOTOSYSTEM II ACTIVE CENTERS

No ferricyanide is added. Chlorophyll concentration: 57 μg/ml. Chloroplasts are exposed to a series of five light and dark cycles, including 10 saturating red actinic flashes given 166 ms apart followed by a 36 s dark period.

	$\Delta I/I$ (544 nm)	$\Delta I/I$ (552 nm)	$\Delta I/I$ (544 nm)— —$\Delta I/I$ (552 nm)
Control	578	26	552
+ 0.5 mM hydroxylamine	267	226	41

conditions, cytochrome *f* is maintained mainly oxidized but a large fraction of *P*-700 is already reduced, and its contribution to the signal 550 nm versus 540 nm is important. This could explain the complex kinetics reported in Ref. 32 for the C550 absorption change, and their dependence upon far red illumination.

C550 decay

In experiment shown in Fig. 2, chloroplasts were exposed to six light and dark cycles, each of which included a saturating flash followed by 32 s dark period. This procedure eliminates the tail of the decay for times longer than 32 s; we estimate that 82% of the C550 is reoxidized in 32 s. The total reoxidation of C550 requires several minutes. We also observe this very slow phase in the fluorescence decay (Fig. 5, compare the initial fluorescence levels of curves 2 and 3). A possible interpretation of this slow phase is the very long lifetime of S_1Q^- (refering to Kok's S states [33] which results from illumination of centers in the S_0Q state). The half-time of the fast phase of C550 reoxidation is about 3.2 s and is significantly longer than the half-time of the fast phase in the reoxidation of the quenchers.

Correlation between C550 and the 515 nm absorption change

According to Junge and Witt [24], the 515 nm absorption increase induced by a flash is linearly related to the number of charges which crossed the thyl-

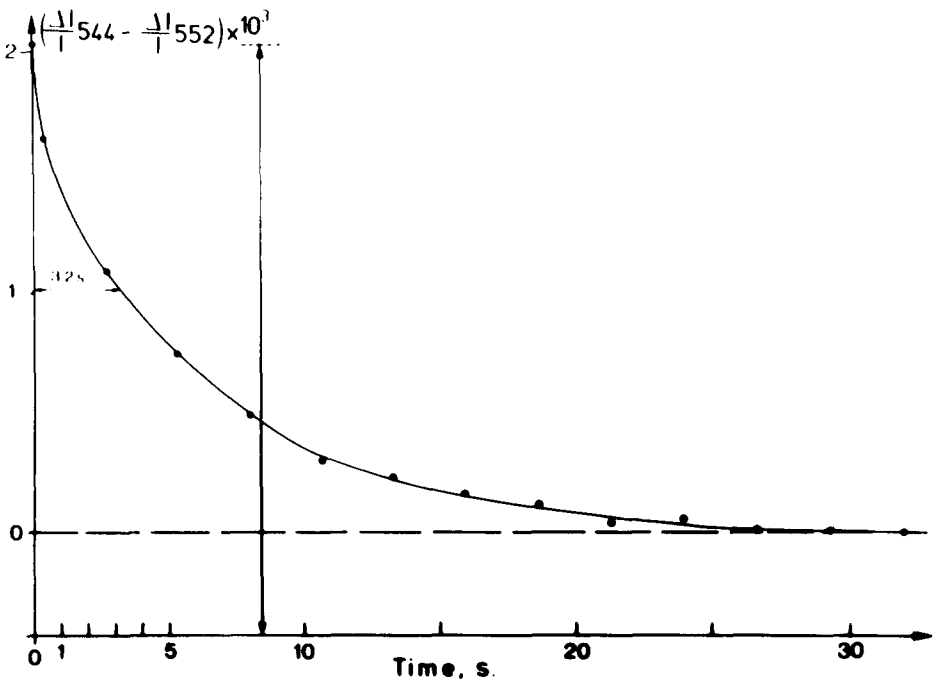

Fig. 2. Decay of the C550 absorption change induced by a single saturating red actinic flash. Chlorophyll concentration 78 μg/ml. The arrow indicates the total amount of C550 in fully dark-adapted chloroplasts. This value was computed after correction for the contribution of *P*-700.

akoid membrane. In the experiment (Fig. 3), performed in the absence of KCl and gramicidin, we measured the number of charges transferred only by Photosystem II reaction. The contribution of PS I was eliminated as follows: dark-adapted chloroplasts were preilluminated for 15 s by far red continuous light ($\lambda > 705$ nm) which fully oxidized *P*-700 and reduced about 20% of the Photosystem II electron acceptors. In a subsequent 15 s dark period, the major part of Photosystem II centers hit during the preillumination are reactivated while practically no $P\text{-}700^+$ is reduced. The 515 nm absorption increased was then measured 80 μs after a single actinic flash of variable energy. In a similar experiment, the C550 absorption change was measured either in the presence or in the absence of gramicidin and KCl. We concluded that the 515 nm absorption increase is linearly related to the C550 absorption change within the experimental error.

Correlation between C550 and the fluorescence yield

Fig. 4 shows the fluorescence (Fig. 4A) and the C550 absorption change (Fig. 4B) measured 5 ms after each flash of a series of saturating flashes given 166 ms apart. As first shown by Doschek and Kok [11] a single saturating flash is not sufficient to destroy all the quenchers. For curves 1, chloroplasts were submitted to five cycles including 35 saturating flashes followed by a 36 s dark period. Curve 2 was obtained with dark-adapted chloroplasts. One can observe that C550 reaches its maximum value after the first flash while the fluorescence yield increases slowly during the sequence. This slow increase is not associated with the C550 reduction. We checked using a control that in the conditions of the experiment of Fig. 4, there is no significant drift in the absence of actinic flashes.

In the experiments shown in Fig. 5, chloroplasts were illuminated by a series

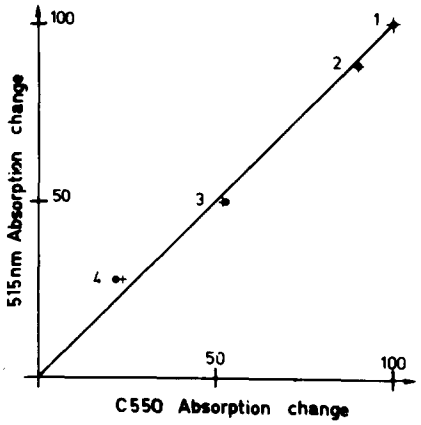

Fig. 3. Correlation between C550 and 515 nm absorption changes. Maximum values of both changes obtained 100 ms (C550) or 80 μs (515 nm) after a saturating flash were normalized to 100. For C550, 100 corresponds to ($\Delta I/I$ (544 nm) — $\Delta I/I$ (522 nm)) $\approx 1.49 \cdot 10^{-3}$ and for 515 nm, 100 corresponds to $\Delta I/I \approx 7.02 \cdot 10^{-3}$. Chlorophyll concentration: 42μg/ml. +, C550 and 515 nm changes measured without KCl nor gramicidin; •, C550 measured in the presence of 0.1 M KCl and 0.5 μM gramicidin. The numbers refer to the energy of the actinic flash. 1, 100%; 2, 44%; 3, 17.5%; 4, 7.8%.

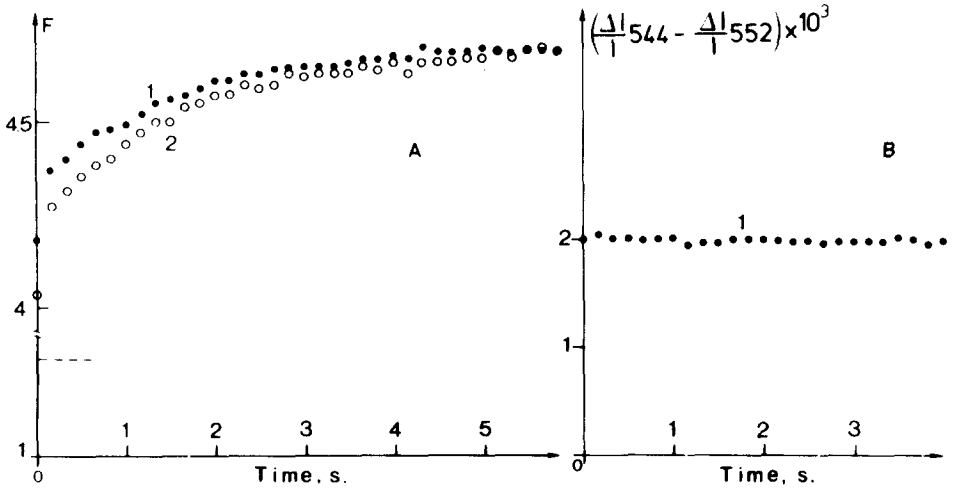

Fig. 4. Fluorescence yield (A) and C550 absorption change (B) in chloroplasts exposed to a series of saturating actinic flashes. Chlorophyll concentration 68 μg/ml. Curves 1: 36 s dark adaptation. ------, initial level of fluorescence after 36 s dark adaptation. Curve 2: Chloroplasts dark adapted for more than 6 min.

of weak green actinic flashes given 20 ms apart; green light was choosen to obtain an homogeneous illumination of the sample. In Fig. 5A, the fluorescence yield is plotted for different periods of dark adaptation. The same experiments were performed for the C550 absorption change (Fig. 5B) for 36 s or 2.1 s dark adaptation. Due to the low energy of the actinic flashes, the fluorescence induction curves are identical to the ones which would be observed in continuous weak light. It has been shown [34,35] that fluorescence yield is linearly related to the rate of Photosystem II reaction. This linear relation is not perfectly satisfied during the final part of the fluorescence induction, when the efficiency of charge stabilization is significantly lower than 1. If one admits the validity of this linear relation, one can compute the concentration of the

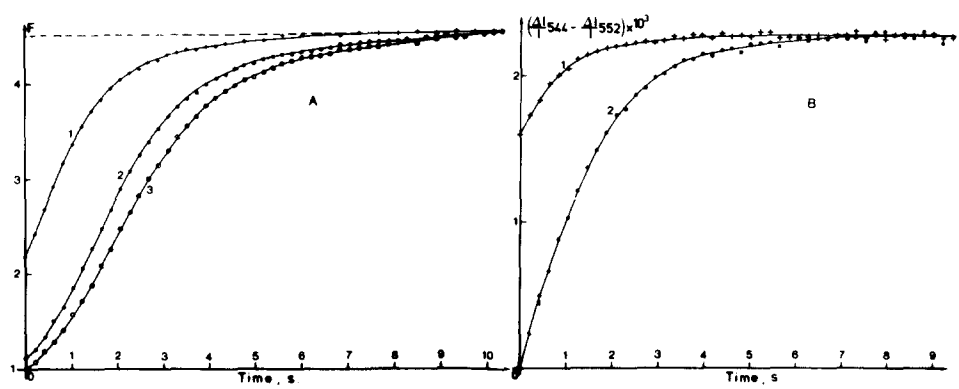

Fig. 5. Fluorescence induction curves (A) and C550 absorption change (B) induced by a series of weak green actinic flashes. Chlorophyll concentration 68 μg/ml. Curves 1: chloroplasts exposed to six cycles including 55 weak actinic flashes given 20 ms apart followed by a 2.1 s dark period. Curves 2: as curves 1, except that the dark period is 36 s. Curve 3: dark-adapted chloroplasts.

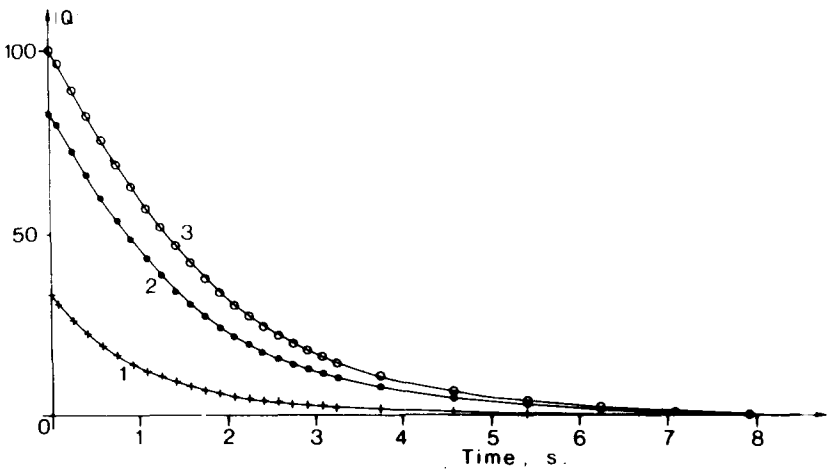

Fig. 6. Concentration of the quenchers as a function of time. Curves 1, 2, 3 were computed respectively from curves 1, 2, 3 (Fig. 5A).

quenchers from the area bound by the induction curve and its asymptote. Fig. 6 shows the concentration of the quenchers as a function of time for different durations of dark adaptation.

Discussion

In Fig. 7 are plotted the variable fluorescence as a function of the concentration of the reduced quenchers (curves 1) or as a function of the concentration

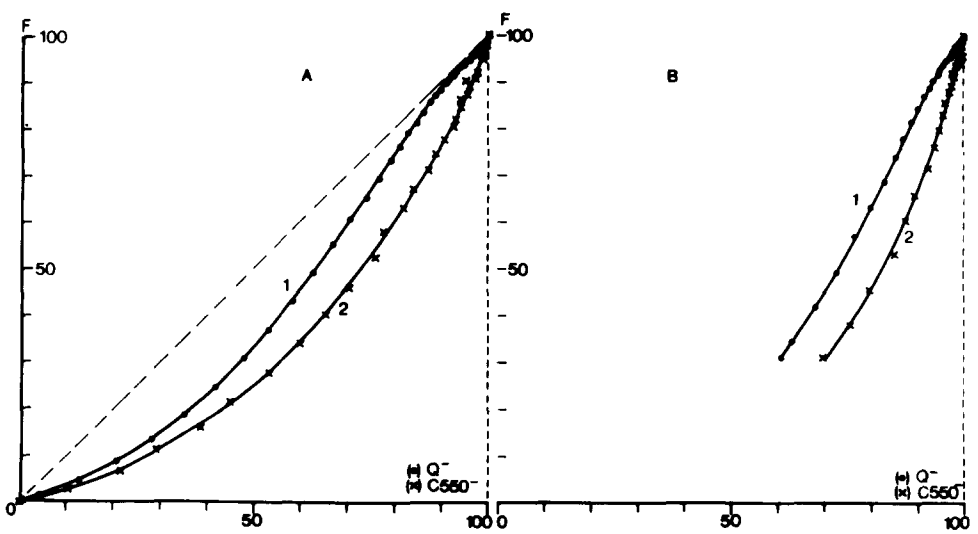

Fig. 7. (A) Concentration of the reduced quenchers (curve 1, •) and concentration of C550 in the reduced form (curve 2, ×) as a function of the variable fluorescence. These curves were computed from curve 2 (Fig. 5A), curve 2 (Fig. 5B), and curve 2 (Fig. 6). 36 s dark adaptation. The concentration of the quenchers, the concentration of C550 and the variable fluorescence were normalized to 100. (B) Same as (A), but for 2.1 s dark adaptation. The same normalization as in (A) was used.

of reduced C550 (curves 2). The more likely hypothesis for the interpretation of these curves is that the fluorescence yield is controlled by two types of electron acceptors, Q_1 and Q_2, each of which has different quenching properties. One of them, Q_1, the redox state of which C550 absorption change is a probe, is responsible for the major part of the quenching. The other one, Q_2, which is less efficient, becomes predominant at the end of the fluorescence induction and its reduction does not induce a significant absorption change in the 540—560 nm range. We observed that the reduction of C550 attains its maximum level after the first saturating actinic flash of a series, unlike the fluorescence yield (Fig. 4). This result shows that after the charge separation which led to the C550 reduction, there is a very efficient charge stabilization, i.e. a very fast reduction of Chl^+. On the contrary, several flashes are required to reach the maximum level of fluorescence; therefore, the charge stabilization is less efficient after the photoreduction of Q_2.

Two models can take into account these results, depending on whether the two electron acceptors belong either to the same photocenter (model 1) or to two independent photocenters (model 2).

(1) Model 1: This model has been discussed in detail in Ref. 13 where we assumed that each photoactive chlorophyll was associated to two electron acceptors (Q_1 and Q_2) and to two electron donors (Y and D). Upon illumination by a 2 μs saturating flash, the folllowing sequence of reactions occurs starting form state S_1 [33] and where Z is the final donor involved in the water-splitting reaction:

$$\overset{D}{Z^+}\overset{}{Y}\overset{}{Chl}^{Q_1}_{Q_2} \xrightarrow{h\nu} \overset{D}{Z^+}\overset{}{Y}\overset{}{Chl}^{+Q_1^-}_{Q_2} \xrightarrow{<1\,\mu s} \overset{D}{Z^+}\overset{}{Y^+}\overset{}{Chl}^{Q_1^-}_{Q_2} \underset{\approx 100\ \mu s}{\overset{h\nu}{\rightleftharpoons}} \overset{D}{Z^+}\overset{}{Y^+}\overset{}{Chl}^{+Q_1^-}_{Q_2^-} \rightarrow \overset{D^+}{Z^{++}}\overset{}{Y}\overset{}{Chl}^{Q_1^-}_{Q_2^-}$$

The first hit reduces Q_1 which is the more efficient quencher: the efficiency of charge stabilization is equal to 1 due to the very fast reduction of Chl^+ by the secondary donor Y. The properties of Q_1 coincide with the properties we observed here for C550. The second hit reduces Q_2; the efficiency of charge stabilization is significantly lower than 1, due to the slower reduction of Chl^+ by the auxiliary donor D. This explains why the maximum fluorescence yield cannot be obtained after a single short saturating flash illumination. In this model, we assume that there are two types of centers, those including Q_1 and Q_2 in the oxidized form, and others containing only Q_1. It is possible that all centers include Q_2, either in the oxidized or in the reduced form.

(2) Model 2: In this model, C550 and Q_2 belong to independent photocenters. This type of model has been discussed by Melis and Homann [8] and more recently by Diner [14] and Pulles [36]. To make this model consistent with our data we must assume that for the centers including Q_1, the electron transfer between the secondary donor and Chl^+ is extremely fast. For the centers including Q_2, the electron transfer between the secondary donor and Chl^+ would be slower. In addition, one must also assume that Q_1 and Q_2 are included in the same antenna and compete for the capture of excitation energy.

Relative concentrations of C550 and Q_2

In model 1, and if no centers including a reduced C550 and an oxidized Q_2 are present, only C550 is reduced at the onset of a weak actinic illumination;

one can then normalize curves 2 in Figs. 5B and 6 to the same initial slope (Fig. 8). The difference between curves 1 and 2 of Fig. 8 (curve 3) gives the kinetics of reduction of Q_2. The time course of curve 3 compared to that of curve 1 indicates that Q_2 is a relatively inefficient quencher. The maximum amplitude of curve 3 is proportional to the concentration of oxidized Q_2 at time $t = 0$: at this time, 28% of the centers included both acceptors in the oxidized form. In previous experiments [13] we were also led to conclude that all of Q_2 is not in the oxidized form in dark-adapted chloroplasts. This value is close to the one obtained by Diner [14] using Tris-washed chloroplasts.

If there are centers including reduced C550 and oxidized Q_2, the rate of reduction of Q_2 at the onset of illumination is not negligible. The value of 28% becomes a minimum estimation of the fraction of the centers which included an oxidized Q_2 at time $t = 0$.

In model 2, the rate of reduction of Q_2 at the onset of illumination is never negligible and depends upon the relative quenching efficiency of C550 and Q_2. A simplified theoretical analysis shows that the centers including Q_1 are 2--3 times more concentrated that the centers including Q_2.

Back reactions of C550 and Q_2

Curves 1 and 2 (Fig. 5B) show that the concentration of C550 reoxidized in 2.1 s dark period is 30% of that reoxidized in 36 s. On the other hand, under these same conditions, 40% of the total concentration of the quenchers (C550 and Q_2) are reoxidized (curves 1 and 2, Fig. 6). In the case of model 1, one can

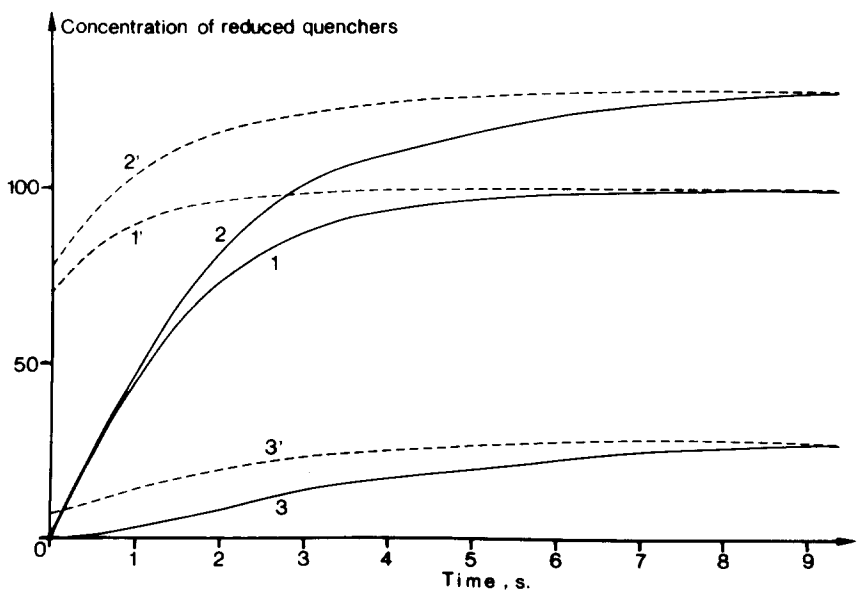

Fig. 8. Concentration of reduced C550 and total concentration of the reduced quenchers as a function of time of illumination. These data were computed from Figs. 5B (C550) and 6 (total quenchers). Curves 1 and 1′: reduction of C550 after 36 s and 2.1 s dark adaptation, respectively. Curves 2 and 2′: reduction of the quenchers after 36 s and 2.1 s dark adaptation, respectively. Curves 1 and 2 were normalized assuming equal initial slopes. The same normalization was used for curves 1′ and 2′ (see text). Curves 3 and 3′: reduction of the quencher Q_2 (difference (curve 2 — curve 1) and (curve 2′ — curve 1′).

compute that 75% of Q_2 and only 30% of C550 are reoxidized in 2.1 s (Fig, 8, curve 3'). The reoxidation of Q_2 is thus much faster than that of C550.

A final conclusion can be drawn from the experiment in Fig. 3, which shows that C550 and 515 nm absorption changes are linearly related. As pointed out in Ref. 12, both quenchers must be reduced upon illumination by a μs flash. Therefore, the linear relation we observed between C550 and 515 nm absorption changes suggests that there is no charge transfer across the thylakoid membrane associated to the photoreduction of Q_2. Renger and Wolff [37] and Malkin [38] have proposed that Photosystem II includes two types of centers on the basis of 515 nm measurements. Our results suggest either that we observed a different type of heterogeneity in the Photosystem II centers or that the precision of the experiment of Fig. 3 is not sufficient to distinguish between the two quenchers.

The qualitative analysis of the results presented here does not permit a choice between the models discussed above. On the other hand, the existence of two electron acceptors of different quenching efficiency is not sufficient to quantitatively explain the 'tail' of the fluorescence induction curves; other types of heterogeneity might be involved as, for instance, a variable size of the antenna [4]. A theoretical analysis of the fluorescence and the C550 induction curves is then required and will be developed in a forthcoming paper.

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