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A study on the fluorescence induction curve of the DCMU-poisoned chloroplast

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The fluorescence induction curve of the DCMU-poisoned chloroplast can be resolved into three kinetically different phases, a rapid sigmoidal phase (α) followed by two slower exponential phases (β and γ), by using a mathematical analysis method previously described (Hsu, B.D., Lee, Y.S. and Jang, Y.R. (1989) Biochim. Biophys. Acta 975, 44–49). Evidence is given which suggests that the three phases originate from different types of Photosystem II (PS II). In order to disclose the nature of the three phases, fluorescence inductions are measured with chloroplasts subjected to various modifying treatments. It is found that only the rapid α-phase responds to the depletion of Mg^{2+}, the addition of ferricyanide and the variation in excitation light intensity. On the other hand, the slower β- and γ-phases are accelerated by adding electron donors like hydroxylamine. Inhibition of oxygen evolution by alkaline pH shift or Tris wash results in a partial conversion of the α-phase to the β- and γ-phases. The results are explained by the hypothesis that the α-phase represents the major 'normal' PS II centers, while the β- and γ-phases represent two minor groups of 'abnormal' PS II centers with low quantum efficiencies and which are rate-limited by their slow electron donation systems.

Introduction

The fluorescence induction arisen from DCMU-poisoned chloroplasts is thought to reflect the reduction of the PS II primary acceptor, Q_A [1]. The induction apparently is not generated by a single first-order photochemical event. A method for analysis of the kinetics of the induction curve has been worked out by Melis and Homann [2,3]. It involves the calculation of the growth of the complementary area, defined by the induction curve and the line of the maximum fluorescence level (F_m), with time. The semilogarithmic plot of such an analysis reveals two kinetically different phases: a rapid sigmoidal phase followed by a slow exponential phase.

The nature of these two phases has been widely studied. One of the explanations is that the heterogeneity is due to incomplete DCMU blocking of some PS II centers [4,5]. It has also been suggested that the two phases could originate from different degrees of PS II–LHC interaction [6]. However, most of the data point to the interpretation that the biphasicity can be attributed to the two forms of PS II, termed PS II_α and PS II_β, respectively [7–14]. They differ, among other things, in their location on thylakoid membranes [7], the antenna size and the composition [8,9] as well as the responses to herbicides and Mg^{2+} level [10,11]. The differences in their corresponding primary acceptor Q_A have also been reported. Unlike PS II_α, Q_A of PS II_β has a higher midpoint potential [12] and is not associated with the two-electron gate [13]. The lack of connection between PS II_β and the plastoquinone pool has also been suggested [14].

Nevertheless, the determination of the complementary area during kinetic analysis, though straightforward, has a pitfall, the asymptotic level of F_m. Bell and Hipkins [15] showed that an increase as small as 2% in the ratio of the maximum variable fluorescence to the maximum total fluorescence resulted in an increase in the area of over 150%. Sinclair and Spence [16], using theoretical curve, reached a similar conclusion. Therefore, they both stressed the importance of using long period of illumination (approx. 20 s) when performing

Abbreviations: Chl, chlorophyll; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethyl urea; F_m, maximum fluorescence yield; F_v, variable fluorescence yield; LHC, light harvesting chlorophyll protein complex; PS II, Photosystem II; Q_A, primary quinone acceptor of PS II; Q_B, secondary quinone acceptor of PS II.

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fluorescence induction measurement. Their objective was to obtain a value as close to the 'correct' value of F_m as possible.

However, using long period of illumination may still have flaws. It faces the problems of finding an adequate illumination time [16], and sometimes the presence of very slow fluorescence rise or quench observed over a time-scale of about 1 min [15,17,18], of which the causes are still not clear. These complicated variations in fluorescence make the F_m determination after long-term illumination undependable.

On the assumption that the last part of the fluorescence induction curve was exponential, we solved this problem in a previous report [19], by using mathematical analysis and curve fitting. It enabled us to determine the F_m level from a fluorescence curve obtained after a relative short period of illumination ($\leqslant 2$ s). Such an analysis revealed that the fluorescence induction curve of the DCMU-poisoned chloroplast consisted of three instead of two phases, a major rapid sigmoidal phase followed by two minor slower exponential phases. A similar finding was obtained by Strasser [20] and Sinclair and Spence [16]. It thus appears that the existing hypotheses dealing with only two phases have to be re-examined.

In this report, the fluorescence inductions were studied with DCMU-poisoned chloroplasts subjected to various modifying treatments, with the object of finding the nature of these three phases.

Materials and Methods

Spinach was purchased from a farmer nearby. To avoid deterioration of chloroplasts, they were isolated within 2 hours after harvesting as previously described [19]. Chloroplasts were finally suspended in a medium containing 50 mM Tricine-NaOH (pH 7.8), 10 mM NaCl, 5 mM $MgCl_2$ and 0.3 M sucrose. Chlorophyll concentration was estimated according to Arnon [21]. All steps were performed at 4° C under dim light.

In the experiment studying the effect of Mg^{2+}, chloroplasts were incubated in the suspension medium without $MgCl_2$. For alkaline pH shift to pH 8.4 or 9.3, chloroplasts were incubated in the suspension medium with buffer replaced by 50 mM Tris-HCl at pH 8.4 or 9.3. These incubations all lasted for at least 10 min before measurements, For Tris treatment, chloroplasts (0.4 mg Chl/ml) were incubated in the presence of 0.8 M Tris-HCl (pH 8.5) for 15 min at room temperature under room light. Following the incubation, chloroplasts were washed twice in the suspension medium. Such treatment resulted in complete loss of the oxygen evolution activity of chloroplasts.

Fluorescence induction was carried out at room temperature, using a homemade apparatus essentially as previously described [19]. Chloroplasts were dark-adapted for at least 30 min. Chlorophyll and DCMU concentrations for the measurement normally were 2 μg Chl/ml and 20 μM, respectively. The intensity of blue actinic light ($\lambda_{max} = 440$ nm) was set at 1.5 W/m^2. For measurement involving Chl *b* excitation, the 440 nm interference filter which guarded the actinic light was replaced by a 475 nm filter. The light intensity was adjusted so that the F_m level remained unchanged. The fluorescence induction, started by the opening of a shutter (opening time 2 ms), was collected at 90° by a photomultiplier (R928F, Hamamatsu) shielded by a 685 nm interference filter. The fluorescence signal from the photomultiplier was amplified and digitized by an A/D converter (12 bit resolution, 4 μs conversion time). The data were then stored and processed by a microcomputer (IBM PC/AT compatible). 6000 data points were collected in a 2 s period of measurement.

The fluorescence induction curves were analyzed according to a method described in Ref. 19. This method was specially designed for determination of the F_m level through mathematical calculation and curve fitting. After finding the F_m level, the growth of the complementary area with time can be calculated and the rate constants of different phases can be determined from the semilogarithmic plots, using the method of Melis and Homann [2,3].

Results

Following the analysis procedures described in Ref. 19, it can be found that the fluorescence induction curve of DCMU-poisoned chloroplasts consists of three kinetically different phases, a rapid sigmoidal phase followed by two slower exponential phases. Here, we designate them as α-, β- and γ-phases, respectively. Under our experimental conditions, their rate constants (the initial rising rate of the sigmoidal α-phase) are 27.8, 10.2 and 1.31 s^{-1}. Their relative contributions to the total complementary area are 61.6%, 13.4% and 25%, respectively.

One of the explanations for the heterogeneous nature of the fluorescence rise is that it simply arises as a consequence of incomplete DCMU blocking of some PS II centers [4,5]. We have performed DCMU titration on the fluorescence induction curve. Table I shows that, at a low concentration of DCMU, fluorescence rises slowly, which results in very large complementary area. With increasing DCMU concentration, the α-phase appears first. The complementary area corresponding to the α-phase (A_α) reaches a steady value at the DCMU concentration around 0.15 μM. The β-phase appears later at a concentration around 1 μM. As for the slowest phase, with increasing DCMU concentration, its rate constant (K_γ) increases, while the corresponding complementary area (A_γ) decreases. A_γ reaches a steady value at about 5 μM DCMU, indicating that DCMU makes a complete blocking beyond this concentration.

TABLE I

The effect of DCMU concentration on various parameters of the fluorescence induction curve

DCMU concentration (μM)	Rate constant (s^{-1})			Complementary area (relative units)			F_0	F_m
	α	β	γ	α	β	γ		
0.06	25.4	–	0.17	57.6	–	19905	686	4207
0.15	25.3	–	0.15	78.1	–	9263	704	4060
0.4	25.2	12.4	0.44	84.0	23.1	644	738	3195
1	26.7	11.0	0.95	84.2	17.1	209	807	3150
2	26.5	11.3	1.1	82.9	16.9	76	870	3216
5	26.8	9.4	1.4	83.1	18.4	41	885	3197
10	27.0	9.8	1.33	80.4	17.0	34	909	3208
20	27.8	10.2	1.31	81.7	17.8	33	893	3201

Thus, it appears that, although the three phases have different sensitivity to DCMU, their presentations in the fluorescence rise (measured at 20 μM DCMU) are not due to incomplete blocking by DCMU.

Complete DCMU blocking is also demonstrated in the fluorescence measurement with methyl viologen (see Table II). With the presence of this efficient PS I acceptor, the plastoquinone pool should stay oxidized. Therefore, if any one of the three phases is due to incomplete DCMU inhibition, then the corresponding complementary area should become very large or the entire phase should be suppressed. However, the result shows that the three phases are still present after the addition of methyl viologen.

Another interpretation for the heterogeneity of the fluorescence rise is that it could originate from different degrees of PS II–LHC interaction [6]. If this is the case, then the kinetics of the fluorescence rise should be different when excited at 475 nm (a wavelength absorbed mainly by Chl *b* of LHC) and 440 nm (absorbed mainly by Chl *a* of LHC and PS II). Such a difference, however, was not observed (see Table II).

Therefore, the heterogeneity is likely to arise from PS II of different types as suggested by Melis and Homann [3]. In order to understand the nature of different types of PS II, the fluorescence inductions were measured with DCMU-poisoned chloroplasts subjected to various modifying treatments.

The effects of divalent cations (especially those of Mg^{2+}) on chloroplast have been widely investigated [22]. The most pronounced changes in the absence of Mg^{2+} are the unfolding of the grana stacking, and the lowering of the fluorescence yield of PS II. The effect of Mg^{2+} on the fluorescence induction is presented in Table II. After removing Mg^{2+} from the medium, the variable part of fluorescence decreases to about 1/3. The kinetic analysis reveals that only the α-phase is seriously affected. Its rate constant is lowered by about 20%, and the corresponding complementary area decreases to 1/4 of the area in the presence of Mg^{2+}. This result generally agrees with that of Melis and Ow [11], suggesting that only the fluorescence yield of PS II_α is lowered by removal of Mg^{2+}. Since the main changes brought about by Mg^{2+} are believed to be through the aggregation of LHC in the grana region of thylakoid membranes, the absence of any effect of Mg^{2+} on the β- and γ-phases seems to suggest that these two types of PS II may not have close interactions with the granal LHC as does PS II_α.

A PS II electron acceptor Q_{400} (midpoint potential

TABLE II

The parameters of fluorescence induction curves measured under various conditions

The final concentration of methyl viologen was 200 μM. When Chl *b* was to be excited, the 440 nm interference filter which guarded the excitaiton light was replaced by a 475 nm filter. To measure fluorescence induction in the absence of Mg^{2+}, chloroplasts were incubated in the suspension medium without $MgCl_2$ for at least 10 min before measurements. All samples contained 20 μM DCMU.

Condition	Rate constant (s^{-1})			Complementary area (relative units)			F_0	F_m
	α	β	γ	α	β	γ		
Control	27.8	10.2	1.31	81.7	17.8	33.1	893	3201
+ Methyl viologen	27.9	9.8	1.53	87.3	21.3	24.8	905	3265
Chl *b* excitation	28.5	10.1	1.25	76.7	15.7	34.5	903	3211
No Mg^{2+}	22.1	11.5	1.15	20.8	19.3	33.7	737	1475

TABLE III

The effect of ferricyanide on various parameters of the fluorescence induction curve

Potassium ferricyanide was added 5 min before DCMU. The excitation light intensity normally was 1.5 W/m^2. The high intensity was 2.98 W/m^2. All samples contained 20 μM DCMU.

Ferricyanide concentrations (mM)	Rate constant (s^{-1})			Complementary area (relative units)			F_0	F_m
	α	β	γ	α	β	γ		
Control	27.8	10.2	1.31	81.7	17.8	33.1	893	3201
0.1	23.8	9.8	1.36	62.6	26.6	30.5	726	2476
0.3	18.9	9.2	1.41	56.2	34.8	31.1	612	2157
1.0	–	9.4	1.45	–	90.5	32.3	318	1176
1 mM + high intensity light	20.1	8.9	1.47	67.7	28.7	32.1	623	2303

400 mV at pH 7) has been described [23,24]. It has been identified as the iron closely associated with Q_A and Q_B [23]. The Fe^{2+} can be oxidized by ferricyanide added before DCMU. Under this condition, the fluorescence rise is significantly slowed down, which results in an increase in the complementary area [24]. The effect of ferricyanide on the fluorescence induction is shown in Table III. It is found that only the rate constant of the α-phase is slowed down. At 1 mM ferricyanide, K_α becomes indistinguishable from K_β. However, PS II_β is not PS II_α with its Q_{400} in the oxidized state as indicated by the light intensity measurement shown below (see Table III). Thus, it seems that the quinone-iron complex is present in PS II_α only, or the complexes, though existing in PS II_β and PS II_γ, are not accessible to ferricyanide.

In the studies on fluorescence induction, Melis and Homann [2,3] have suggested that, under low excitation light intensity, the rate-limiting step is the number of photon absorbed/s. Therefore, the rate constant of each phase should reflect the size of the light-harvesting antenna associated with each type of PS II. If this is the case, then the rate constants should also be linearly proportional to the excitation light intensity, provided that the light intensity is well below that which causes saturation of photon absorption. Table IV shows the result of the analysis of the induction curves measured at three different light intensities. It is found that only K_α varies with light intensity. This result generally agrees with that of Melis and Homann [2], who have found that the rate constant of the slow phase is much less sensitive to light intensity than that of the fast phase. Since K_β and K_γ are smaller than K_α, they are supposed to correspond to PS II centers with smaller antenna than PS II_α. Their insensitivity to light intensity therefore cannot be explained by the saturation of photon absorption. The slow β- and γ-phases then must be rate-limited by something other than light absorption. Note that the corresponding complementary areas of the three phases do not vary with light intensity.

Since K_α and K_β have different sensitivity to light intensity, this property then can be used to test whether the α- and β-phases are really identical in the presence of 1 mM ferricyanide (see Table III). It is found that the α- and β-phases, which are indistinguishable at low light intensity, become kinetically separable at higher light intensity. It thus indicates that PS II_β cannot be identified as PS II_α with Q_{400} in the oxidized state.

One possible cause of the low rates in PS II_β and PS II_γ might be the slow turnovers in the electron transport in these PS II, which are independent of the incoming excitation light intensity. They are thus PS II centers of low quantum efficiencies. This is in accordance with the reports which show that single saturating flash removes

TABLE IV

The effect of light intensity on various parameters of the fluorescence induction curve

The excitation light intensity of control was 1.5 W/m^2. The low intensity was 1.05 W/m^2 and the high intensity was 2.3 W/m^2. All samples contained 20 μM DCMU.

Light intensity	Rate constant (s^{-1})			Complementary area (relative units)			F_0	F_m
	α	β	γ	α	β	γ		
Low	19.6	9.4	1.34	76.5	18.1	28.6	595	2077
Control	27.8	10.2	1.31	81.7	17.8	33.1	893	3201
High	39.8	10.1	1.37	88.6	16.5	35.6	1358	4548

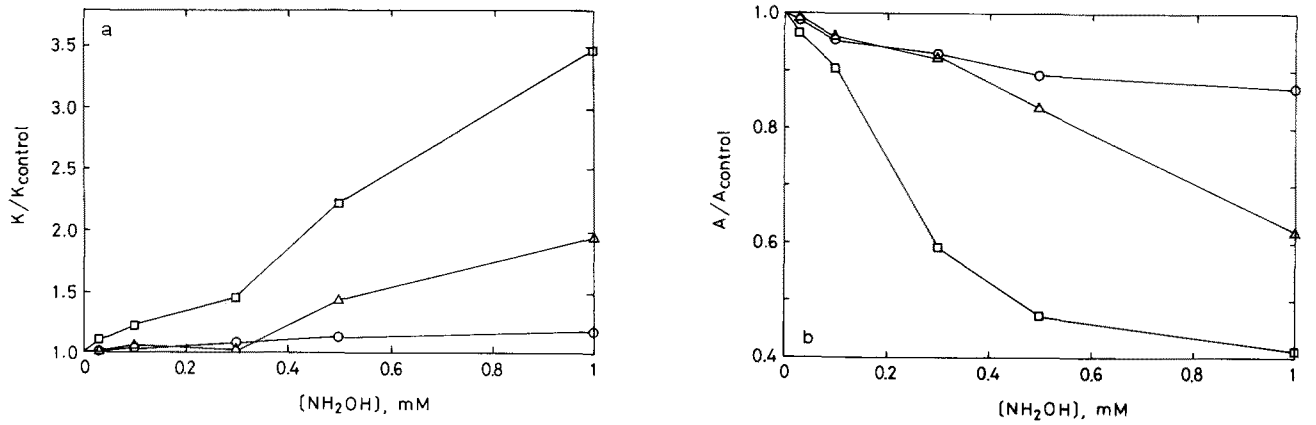

Fig. 1. The effects of hydroxylamine on (a) rate constants and (b) corresponding complementary areas of the three phases of the fluorescence induction curve. $K/K_{control}$ and $A/A_{control}$ denote the ratio of the rate constant and the complementary area in the presence of hydroxylamine to those in the absence, respectively. The α-phase (○), the β-phase (△) and the γ-phase (□). Chloroplasts were incubated with the presence of hydroxylamine for 5 min before measurement.

proportionally much less the complementary area of the slow phase than that of the fast phase [25,8]. The following results support this proposal.

Hydroxylamine is known to be able to suppress the back reaction by keeping the endogenous donors of PS II in the reduced state. Fig. 1 shows the effects of hydroxylamine on the kinetics of the fluorescence rise. Chloroplasts were incubated with various concentrations of hydroxylamine for about 5 min before measurements. It is found that at 1 mM hydroxylamine, K_β and K_γ increase nealry 2- and 3.5-fold, respectively, while K_α remains almost unchanged (see Fig. 1a). The corresponding complementary areas of PS II_β and PS II_γ decrease with increasing concentration of hydroxylamine (see Fig. 1b), suggesting that there are increases in the quantum efficiencies of photochemistry in these two types of PS II. The result seems to indicate that the slow rates of PS II_β and PS II_γ might be due to slow electron donation to these reaction centers, which may undergo many turnovers via back reaction under continuous excitation, until their reduced primary acceptors are stabilized by the electron donation into the systems. This situation can be alleviated by the addition of an extrinsic electron donor such as hydroxylamine, which increases the rate of electron donation as well as the quantum efficiency. A similar result was obtained with the addition of another donor, Mn^{2+}, though the effect was less (data not shown).

We have also tried to perturb the physiological donor of PS II by inactivating oxygen evolution. Alkaline condition is known to inhibit oxygen evolution by release of bound Mn [26]. The effect of high pH on the fluorescence induction is shown in Table V. With pH increasing from 7.8 to 9.3, K_α increases a little (13%), while A_α decreases by 22%. On the other hand, K_γ decreases by 25%, while A_γ dramatically increases about 3-fold. The β-phase does not seem to be affected. Note that Sinclair and Spence [16] observed similar variations in the complementary areas after illuminating chloroplasts at 6 $\mu E \cdot m^{-2} \cdot s^{-1}$ for 30 min.

Similar but more pronounced changes have been observed on Tris-washed chloroplasts (see Table VI). The fluorescence induction curve, though it is dramatically changed due to the inhibition of oxygen evolution by Tris wash, still can be resolved into three phases. The responses of these three phases to Mg^{2+}, ferricyanide and light intensity have been checked (data not shown). Their reactions are similar to those of control chloro-

TABLE V

The effect of pH on various parameters of the fluorescence induction curve

Chloroplasts were incubated in the mediums at various pH for at least 10 min before measurement. All samples contained 20 μM DCMU.

pH	Rate constant (s^{-1})			Complementary area (relative units)			F_0	F_m
	α	β	γ	α	β	γ		
7.8	27.8	10.2	1.31	81.7	17.8	33.1	893	3201
8.4	29.5	10.5	1.10	72.4	17.4	59.7	999	3173
9.3	31.4	10.0	0.98	63.5	16.1	100.1	1073	3056

TABLE VI

The effect of a Tris wash on various parameters of the fluorescence induction curve

Tris wash was carried out as described in Materials and Methods. The final concentration of $MnCl_2$ was 0.5 mM. All samples contained 20 μM DCMU.

Conditions	Rate constant (s^{-1})			(Complementary area (relative units)			F_0	F_m
	α	β	γ	α	β	γ		
Control	27.8	10.2	1.31	81.7	17.8	33.1	893	3201
Tris-washed	24.1	9.6	0.97	51.5	64.4	411.5	990	3265
Tris-washed + $MnCl_2$	24.3	10.1	0.99	62.0	30.1	180.9	997	3285

plasts, suggesting that the corresponding phases in Tris-washed and control chloroplasts are identical. As shown in Table VI, Tris wash decreases K_γ by 26%, while increases the corresponding complementary area 12-fold. K_β is not affected, but A_β is also increased 3.6-fold. On the other hand, the rate constant of the α-phase decreases a little (13%), while A_α also decreases by 37%. These changes in the complementary areas can be partially reversed by the addition of electron donor Mn^{2+} (Table VI). A_β and A_γ decrease by 53% and 56%, respectively, whereas A_α increases by 20%. The rate constants of the three phases, however, are not affected by adding Mn^{2+}.

Since the inhibition of oxygen evolution by alkaline pH shift or Tris wash does not significantly change the rate constants, it is unlikely that the quantum efficiencies of the three types of PS II would be greatly altered. Thus, the dramatic increases in A_β (in the case of Tris wash) and A_γ, and a substantial decrease in A_α suggest that the numbers of PS II_β and PS II_γ are considerably increased by the inhibition of oxygen evolution at the expense of PS II_α. This is in accordance with our proposal that the β- and γ-phases are PS II centers with slow electron donation systems, to which PS II_α might switch (to PS II_γ only in the case of alkaline pH shift) after inactivation of the physiological donors. Addition of an extrinsic electron donor seems to be able to partially reverse this switch.

Discussion

Using the analysis method described in Ref. 19, we can resolve the fluorescence induction curve of DCMU-poisoned chloroplasts into three kinetically different phases. The major phase is rapid and sigmoidal, which is then followed by two slower exponential phases.

The DCMU titration measurement (Table I) shows that, although the three phases have different sensitivity to DCMU, the presence of the slow phases (measured at 20 μM DCMU) is not due to incomplete blocking of some PS II centers. Complete blocking occurs at around 0.15 μM for the α-phase, 1 μM for the β-phase and 5 μM for the γ-phase. A similar study on this subject by Joliot and Joliot [27] also shows that 20 μM DCMU is enough to suppress entirely the slow phase due to the presence of PS II centers with Q_B in the singly reduced state, which lowers the affinity of DCMU. A DCMU titration has also been performed by Hodges and Barber [5]. Their result shows that the major increase in the rate constant and the decrease in the corresponding complementary area of the slow phase stop before 20 μM DCMU. Further minor changes observed beyond 20 μM are probably due to the selective quench of the slow phase as suggested by Horvath et al. [10]. Furthermore, our light intensity measurement also argues against the explanation of incomplete DCMU blocking, since in this case, the rate of photochemistry of unblocked PS II, and thus the rate constant of the slow phases will still increase with increasing light intensity. However, this was not observed (Table IV). Note that, in the presence of methyl viologen, the complementary area of the γ-phase decreases by about 25% (see Table II). This suggests that there might be PS II centers which are not inhibited by 20 μM DCMU. Even though, they are just a very small population (occupying about 6% of the total complementary area).

There is no difference in the kinetics of fluorescence rise when excited at 440 nm (absorbed mainly by Chl *a*) and 475 nm (Chl *b*, Table II). This does not agree with the argument that the heterogeneity of the fluorescence induction is due to different degrees of PS II–LHC interaction [6]. The result also does not seem to be in accordance with the model that the heterogeneity can be attributed merely to different amounts of associated LHC [8], which should result in different chlorophyll compositions and thus different absorption cross sections of Chl *a* and Chl *b* in different types of PS II.

Various modifying treatments on chloroplast result in different responses among the three phases of the fluorescence induction curve. The results point to the interpretation that the heterogeneity can be attributed to the different types of PS II. The major rapid α-phase seems to originate from 'normal' PS II centers as shown by its responses to Mg^{2+}, ferricyanide and light inten-

sity. The sigmoidicity of the α-phase is best explained by that PS II_α exist in a statistical pigment bed which allow energy transfer between PS II units.

The three treatments, including Tris, alkaline pH and hydroxylamine, though all being able to perturb the physiological donor of PS II, have different effects on the fluorescence induction. The discrepancy may arise from the differences in their perturbation of PS II. Tris-wash completely abolishes any electron donation beyond Z, which brings about the most dramatic changes in the induction curve (Table VI). Under alkaline pH, one or two electron could still be donated by the water-splitting system, thus causing a kind of intermediate effect (Table V). On the other hand, hydroxylamine can effectively donate electrons to PS II at the concentrations used, which then results in the changes opposite to those by Tris-wash and alkaline pH shift (Fig. 1).

Tris wash only partially suppresses the α-phase, suggesting that most of the PS II_α can still stabilize Q_A^-, even in the absence of oxygen evolution. However, a significant amount of A_α (37%) disappears after the Tris wash. In the meantime, A_β and A_γ increase by 3.6- and 12-fold, respectively (Table VI). The size of the complementary area is believed to be a function of the number of PS II centers. It is also inversely proportional to the quantum efficiency of the accumulation of reduced primary acceptors. Since the Tris wash does not significantly change the rate constants of the three types of PS II, it is unlikely that their quantum efficiencies will be greatly modified. The variations in the complementary areas then suggest a partial conversion from PS II_α to PS II_β and PS II_γ. Furthermore, a comparison of the absolute values of the decrement in A_α (30.2) and the increments in A_β (46.6) and A_γ (378.4) reveals that PS II_β and PS II_γ are Photosystems II with lower quantum efficiencies than PS II_α. This is in accordance with our proposal that PS II_β and PS II_γ are rate-limited by slow electron donation to the reaction centers.

The donation systems of the β- and γ-centers are as yet unknown. They are probably different, as shown by their different rate constants and differential responses to hydroxylamine (Fig. 1) and the inhibition of oxygen evolution (Tables V and VI). It is worthy of note that there is a report on the heterogeneity of the oxygen evolution complex of PS II [28]. A group of Photosystems II with a high miss rate has been described. The relationship between this group of Photosystems II and the β- and γ-centers is not known.

If we take the rate constants of the three phases as the estimates of their quantum efficiencies, then after correcting the corresponding complementary areas, we find that about 91% of the PS II centers are PS II_α. The β- and γ-centers take only about 7% and 2%, respectively. After Tris wash, the proportions of the α-, β- and γ-centers become 55%, 27% and 18%, respectively.

Our proposal that the slow β- and γ-phases are rate-limited by their slow electron donation can explain the observation that the slow phase of the fluorescence induction has a high midpoint potential. It has been shown that the removal of the slow phase can be titrated as an $n = 1$ component with a midpoint potential around 120 mV [12]. Hodges and Barber [4] also have shown that it is possible to eliminate the slow phase by partly reducing the system with low concentrations of dithionite, which do not decrease the F_v/F_m ratio, i.e., concentrations which do not result in chemical reduction of PS II primary acceptors. Nevertheless, no such transition has ever been observed in the redox titration of steady-state fluorescence yield [29], absorbance bandshift at 515 nm (an indicator of membrane potential) [30], absorbance bandshift at 550 nm (an indicator of Q_A) [31], cytochrome *b*-559 photooxidation [32], fluorescence lifetime [33] or EPR signal [34]. Our explanation is that a lowering of the redox potential of the system below about 120 mV can probably reduce the endogenous donors, which may greatly accelerate the rate of electron donation to PS II_β and PS II_γ, and thus increase the quantum efficiencies of the two types of PS II, resulting in diminishing in their corresponding complementary areas. The elimination of the slow phases by lowering the redox potential therefore is not accompanied by the reduction of any primary electron acceptor.

It is now well established that Photosystems II of higher plants are not homogenous. Several kinds of PS II heterogeneity have been described [25]. The α and β heterogeneity mentioned above is one of them. It has been recently incorporated into a PS II repair cycle model by Guenther and Melis [35], suggesting a developmental relationship between PS II_β and PS II_α, in which PS II_β serves as a precursor to PS II_α. They also indicates that, in mature, wild-type chloroplasts, PS II_β is structurally and functionally complete except for the absence of the peripheral LHC and the complement of the plastoquinone pool. If this developmental relationship also exists in our three-phase model, then the precursor form(s) of PS II_α (PS II_β and/or PS II_γ) are Photosystems II of low quantum efficiency due to their deficiencies in water-splitting function. The insensitivity of the slow phases to ferricyanide (Table III), which can oxidize the iron of the quinone-iron ocmplex, may suggest that the β- and γ-centers also have defects in their quinone-iron complexes, and thus are unable to reduce plastoquinone. However, our result, which shows that the kinetics of the fluorescence induction is the same when excited at 475 nm (Chl *b*) and 440 nm (Chl *a*), is not in accordance with the model that PS II_α has more chlorophyll *a*/*b* light-harvesting complexes than its precursor.

In summary, the fluorescence induction curve of the DCMU-poisoned chloroplast can be resolved into three

phases. The three phases, though showing different sensitivity to DCMU, are completely blocked at the site after the primary acceptors by DCMU at a concentration higher than 5 μM. The kinetics of the fluorescence induction is the same when excited at 440 nm (Chl *a*) and 475 nm (Chl *b*), suggesting that the heterogeneity cannot be attributed to different degrees of PS II–LHC interaction, nor to different amounts of LHC associated with PS II centers. The three phases therefore are likely to originate from different types of PS II. The fast α-phase is the only phase which responds to the depletion of Mg^{2+}, the addition of ferricyanide and the variation in excitation light intensity. It thus seems to belong to the 'normal' PS II centers. The slower β- and γ-phases are not sensitive to Mg^{2+}, ferricyanide and light intensity, but are accelerated by the addition of electron donors like hydroxylamine. Furthermore, the inhibition of oxygen evolution seems to bring about a partial conversion of the α-phase to the β- and γ-phases. These results suggest that PS II_β and PS II_γ are not rate-limited by the size of their antenna, but rather by slow electron donation to the reaction centers. They are thus PS II of low quantum efficiencies. More research is obviously needed for a further understanding of the nature of the three phases and their physiological roles. Experiments to test our proposal are now in progress.

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