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COOPERATIVITY BETWEEN PHOTOSYSTEM II CENTERS AT THE LEVEL OF PRIMARY ELECTRON TRANSFER

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SUMMARY

1 Spinach chloroplasts, but not whole *Chlorella* cells, show an acceleration of the Photosystem II turnover time when excited by non-saturating flashes (exciting 25 % of centers) or when excited by saturating flashes for 85–95 % inhibition by 3-(3,4-dichlorophenyl)-1,1-dimethylurea Following dark adaptation, the turnover is accelerated after a non-saturating flash, preceded by none or several saturating flashes, and primarily after a first saturating flash for 3-(3,4-dichlorophenyl)-1,1-dimethylurea inhibition A rapid phase ($t_{\frac{1}{2}}$ approx 0 75 s) is observed for the deactivation of State S_2 in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea

2 These accelerated relaxations suggest that centers of Photosystem II are interconnected at the level of the primary electron transfer and compete for primary oxidizing equivalents in a saturating flash The model in best agreement with the experimental data consists of a paired interconnection of centers

3 Under the conditions mentioned above, an accelerated turnover may be observed following a flash for centers in S_0, S_1 or S_2 prior to the flash This acceleration is interpreted in terms of a shift of the rate-limiting steps of Photosystem II turnover from the acceptor to the donor side

INTRODUCTION

Photosynthetic units of Photosystem II are arranged in an ensemble sufficiently compact to permit the transfer of energy between adjacent units Despite this close proximity, no convincing evidence has as yet been presented to indicate that any interaction occurs between adjacent reaction centers at the primary level of electron transfer Stiehl and Witt [1] have proposed a paired model for Photosystem II in which a pair of reaction center chlorophylls reduce a pair of plastoquinones in the primary photoreaction The latter then dismutate in about a millisecond and transfer a pair of electrons to the pool This model however is not consistent with the data of

Abbreviations DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea, CMU 3-(*p*-chlorophenyl)-1,1-dimethylurea

Bouges-Bocquet [2] and Velthuys and Amesz [3] which demonstrate that no electron exchange occurs between primary or secondary electron acceptors of adjacent centers

Furthermore, Kok et al [4] have demonstrated, using spinach chloroplasts and *Chlorella*, that oxidizing equivalents are successively accumulated on the oxidizing side of the Photosystem II reaction center, until four such equivalents give rise to a molecule of O_2 No equivalents are shared by adjacent centers, each acts independently of its neighbors

However, Joliot, A [5] through measurements of the O_2 gush at varying 3-(*p*-chlorophenyl)-1,1-dimethylurea (CMU) concentrations, and Siggel et al [6] through measurements, at varying 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) concentrations, of the reduction of P_{700} following illumination, have shown that an exchange of electrons occurs between adjacent photosynthetic chains at the level of the plastoquinone pool The latter authors suggest that as many as ten pools of ten equivalents each may freely exchange electrons

In the following paper evidence is presented for a cooperative interaction at the level of the primary photoreaction of Photosystem II in spinach chloroplasts This phenomenon was demonstrated by a study of the turnover times for centers in States S_0, S_1 and S_2 The turnover time is the dark period required, following excitation of a Photosystem II center, before that center may effectively utilize a second photon These turnover times were first characterized by Kok et al [4] and later, in more detail by Bouges-Bocquet [7], and are here shown to be dependent on the fraction of centers that undergo charge separation during a light flash These turnover measurements alone, however, could not localize the rate-limiting step to the donor or acceptor side However, independent measurements of various reducing-side phenomena show relaxation kinetics similar to those measured by oxygen methods [8–12] Dependence of the turnover times on the redox state of the pool has also been demonstrated [13] The models, proposed to explain the cooperative interaction, are consistent with the idea that the relaxations $S'_0 \rightarrow S_1$, $S'_1 \rightarrow S_2$ and $S'_2 \rightarrow S_3$* occur at least partially on the acceptor side for the experimental conditions of Kok et al [4] and Bouges-Bocquet [7] It is proposed that for the experimental conditions explored here, the rate-limiting steps for these relaxations are shifted to the donor side Collectively, the data of Joliot et al [14], Zankel [15] and Bouges-Bocquet [7] show that $S'_3 \rightarrow S_0$ is normally limited by the donor side

MATERIALS AND METHODS

Chloroplasts were prepared from market spinach according to the method of Avron and stored at −70 °C in 0 05 M Tris–HCl buffer (pH 7 8), containing 0 01 M NaCl, 0 4 M sucrose and 5 % dimethylsulfoxide When used for O_2 measurements, the chloroplasts were diluted to a chlorophyll concentration of 300 μg/ml using the same buffer as above, containing, in addition, 0 1 M KCl but without dimethylsulfoxide The results, to be described, were observed for both fresh and previously frozen chloroplasts, and with and without KCl

* The notation $S'_n \rightarrow S_{n+1}$ used in this paper, refers to the dark transition between intermediate state S'_n, formed by flash excitation of a center in state S_n, and the successive, more oxidized state S_{n+1} This dark transition includes reactions on both the donor and acceptor sites of Photosystem II

Chlorella pyrenoidosa were grown on Knop medium containing Arnon's trace elements A_6 and B_6 Prior to use, cells were suspended in 0 05 M phosphate buffer (pH 6 4) containing 0 1 M KCl at the same chlorophyll concentration as used for chloroplasts

O_2 was detected by the polarographic method described by Joliot and Joliot [16] Flash excitation was provided by Stroboslaves (General Radio, Type 1539-A) employing xenon flash lamps (4 μs width at half-height, with a tail amounting to 10 % of peak intensity at 10 μs, 4 % at 20 μs, and 1 % at 50 μs)

Measurements of the carotenoid band shift at 520 nm were made using the flash-detector differential spectrophotometer described by Joliot and Delosme [17] Chloroplasts were suspended in the same buffer mixture used for O_2 measurements plus 7 % Ficoll, but without 0 1 M KCl The chlorophyll concentration was 60 μg/ml

All experiments were performed at 22 °C except where indicated

RESULTS

Spinach chloroplasts, totally deactivated for 15 min in the dark, were exposed to a sequence of short saturating flashes separated by 320 ms (Fig 1) Following flash excitation, these chloroplasts were again permitted to deactivate for 15 min, at which time they were equilibrated with 1 5 10^{-6} M DCMU (85 % inhibition in the steady state) over an additional 15-min period Steady-state inhibition varied with the chloroplast batch Between 85 and 95 % inhibition was usually observed for this concentration of DCMU A flash sequence was then given identical to the first A comparison of the two shows that in the presence of DCMU the second-flash yield is 60 % of the third whereas under uninhibited conditions, the second is less than 10 % of the third In addition, in the presence of DCMU, the oscillations following the third flash, while of diminished amplitude, were nonetheless in phase with those in the absence of DCMU The relative increase in the second-flash yield and the diminished amplitude of the later oscillations of the sequence suggest the existence of appreciable double hitting (e g Photosystem II reaction centers transferring two equivalents during a flash) This double hitting must have occurred primarily on the first or second flash to explain both the fact that the sequence remains in phase with that for uninhibited chloroplasts and that the second flash shows an elevated yield

Two possible mechanisms for this double hitting early in the sequence are 1 instantaneous double-electron transfer per functional center during a flash or 2 an acceleration of the turnover time, permitting a center to function twice during the flash lifetime Double hitting normally (in the absence of DCMU) arises from an overlap of the turnover kinetics with the flash lifetime Any acceleration of the turnover would increase that overlap Because the flash tail is a decreasing function with time, a halving of the turnover time would more than double the double hitting per flash A third possibility that the elevated second-flash yield stems from incomplete deactivation is eliminated by the protocol used for the experiment of Fig 1 The chloroplasts were allowed to completely deactivate for 15 min in the dark (see sequence in absence of DCMU) prior to the dark equilibration with 1 5 10^{-6} M DCMU

The second possibility was confirmed by actual measurements of the turnover time in the presence of 1 5 10^{-6} M DCMU (Fig 2, Table I) These experiments

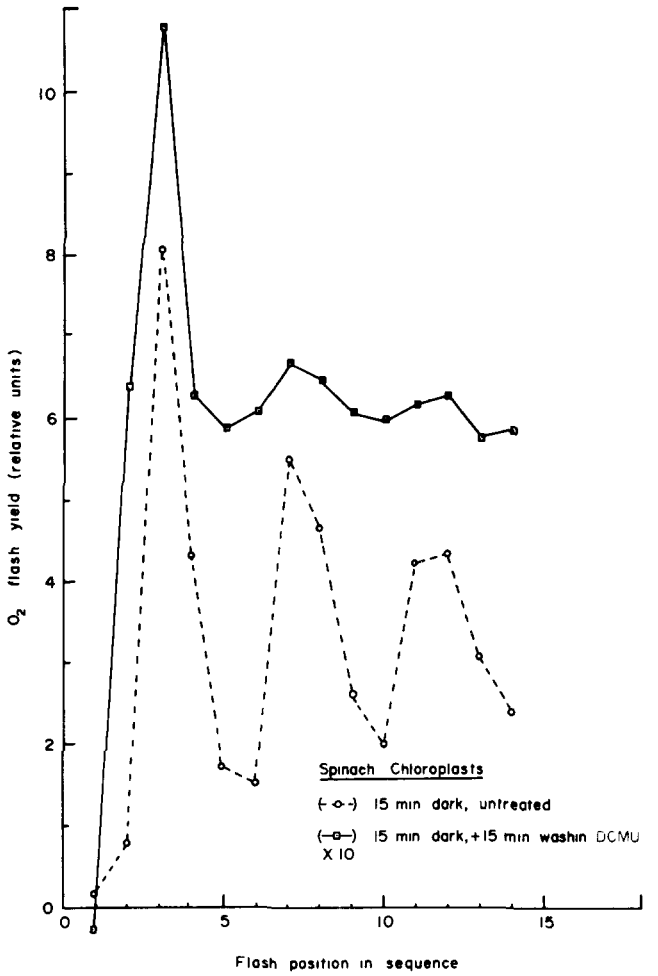

Fig 1 Flash sequence of dark-adapted spinach chloroplasts in the presence (□) and absence (○) of 1 5 10^{-6} M DCMU Untreated chloroplasts were dark-adapted for 15 min before flashing These same chloroplasts were again, dark-adapted for 15 min, then equilibrated for an additional 15 min with 1 5 10^{-6} M DCMU in the dark before flashing Flashes were spaced 320 ms apart The DCMU curve has been multiplied by a factor of 10

were performed on dark-adapted chloroplasts, with centers primarily in the S_1 state by varying the time between the first and second ($S'_1 \rightarrow S_2$) or between the second and third ($S'_2 \rightarrow S_3$) flashes of a sequence and detecting O_2 produced on the third For centers in the S_0 state in the dark, O_2 was detected on the third plus the fourth flashes and the time varied between the first and second ($S'_0 \rightarrow S_1$, Table I), second and third ($S'_1 \rightarrow S_2$, Table I) and third and fourth ($S'_2 \rightarrow S_3$, Fig 2)

$S'_2 \rightarrow S_3$ was also measured by varying the time between the fourth and fifth flashes and detecting on the fifth The $S'_2 \rightarrow S_3$ is the only turnover which may be readily measured in the steady state Judging from the sequence of Fig 1, (oscillation amplitude small), this latter turnover time is measured, in Fig 2, for a mixture of

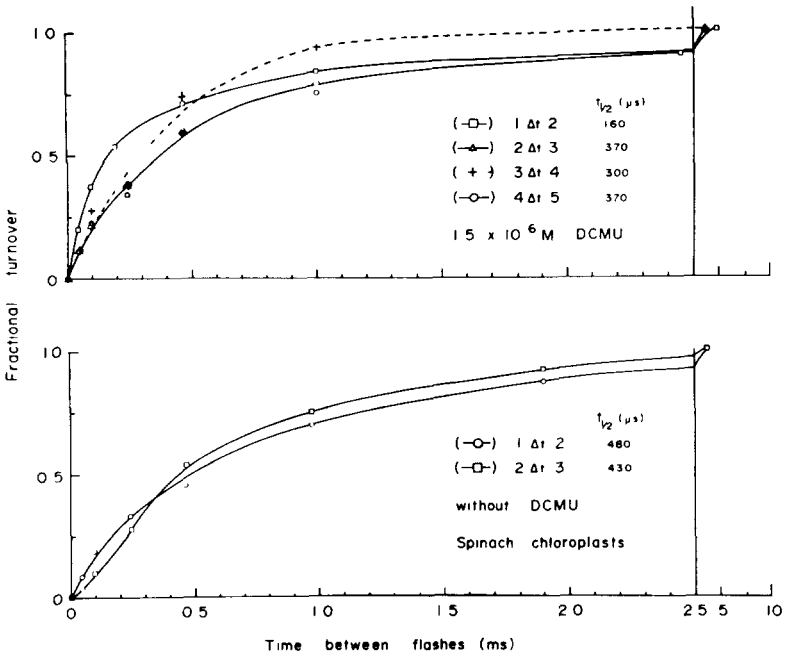

Fig 2 Turnover time measured by O_2 emission in the presence (upper) and absence (lower) of 1 5 10^{-6} M DCMU Chloroplasts were dark adapted for 8 min (−DCMU) or 6 min (−DCMU) (n) Δt (n+1) signifies that the time was varied between the (n)th and (n−1)th flash of a sequence with the interval indicated on the abscissa The time between the remaining flashes of the sequence was 160 ms for DCMU-treated chloroplasts and 320 ms for untreated chloroplasts both many times longer than the turnover In the case of 1 Δt 2, O_2 was detected on the third flash In all other cases, O_2 evolved was detected on the second member of the pair The ordinate indicates the fraction of centers which have recovered in a given time

TABLE I

HALF TURNOVER TIMES FOR $S'_0 \rightarrow S_1$, $S'_1 \rightarrow S_2$ AND $S'_2 \rightarrow S_3$ FOR CENTERS IN STATES S_0 AND S_1 IN THE DARK

These times were measured in the presence and absence of 1 5 10^{-6} M DCMU

State of origin	Relaxation (μs)		
	$S'_0 \rightarrow S_1$	$S'_1 \rightarrow S_2$	$S'_2 \rightarrow S_3$
S_0 (−DCMU)	500	400	—
S_0 (+DCMU)	210	400	—
S_1 (−DCMU)	—	480	430
S_1 (+DCMU)	—	160	370

centers, not far from a steady-state distribution of states These turnover times (Fig 2) are compared with $S'_1 \rightarrow S_2$ and $S'_2 \rightarrow S_3$ (for centers in S_1 in the dark) in the absence of DCMU The DCMU curves show an acceleration which is most marked following the first flash $S'_1 \rightarrow S_2$ for centers initially in the S_1 state and $S'_0 \rightarrow S_1$ for centers initially in the S_0 state showing, respectively, a $t_{\frac{1}{2}}$ of 160 μs instead of 480 μs and

210 μs instead of 500 μs The turnover, following the second flash in the presence of DCMU, is much less accelerated relative to the uninhibited curves For centers in S_1 in the dark, the half-time for $S'_2 \rightarrow S_3$ was 430 μs and 370 μs before and after addition of DCMU, respectively For centers in S_0 in the dark, the half turnover time for $S'_1 \rightarrow S_2$ was approx 400 μs in both cases Thus at least some of the double hitting, observed in the flash sequence (Fig 1) is attributable to the rapid turnover during the first flash of the sequence

To obtain some indication of the amount of instantaneous double hitting occurring during the light flash in 1 5 10^{-6} M DCMU, chloroplasts were cooled to 2 °C, which slows the initial turnover rate by a factor of 3 from that observed at 22 °C Over this 20 °C temperature range the ratio of the O_2 yields of the second and third flashes decreased only slightly from an average of 0 55 to 0 45 Thus the double hitting observed in Fig 1 arises from two phenomena an accelerated turnover occurring primarily on the first flash and an instantaneous double hitting occurring on either the first and second flash or on both

The dependence of the double-hit phenomena on the DCMU concentration is shown in Fig 3a In this experiment, an increased second to third flash ratio was used as an indicator A comparison of this ratio with the inhibition of O_2 production in the steady state, indicates that the double hitting requires five times higher DCMU concentration than the steady-state inhibition The acceleration of the turnover time

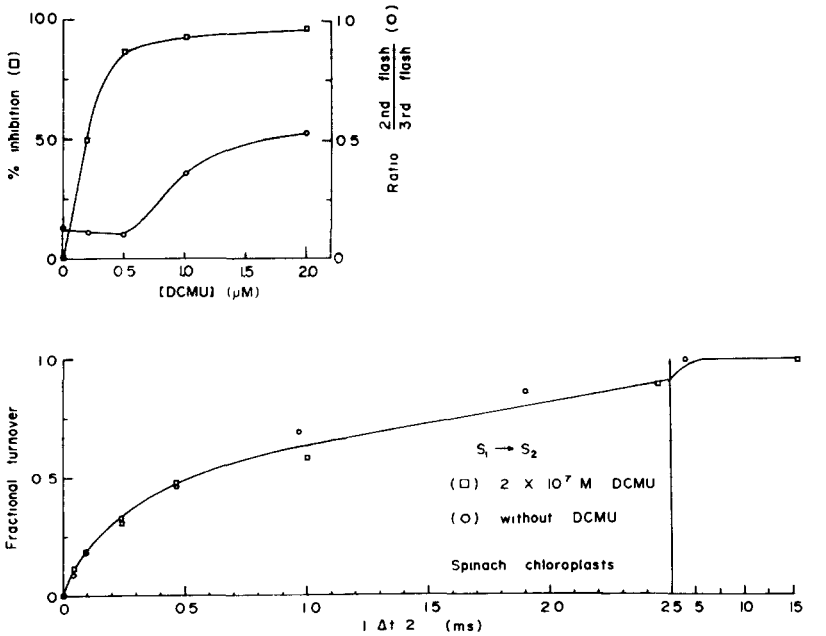

Fig 3 Dependence of double hitting on DCMU concentration Chloroplasts were dark adapted for 10 min (a) or 8 min (b) In (a) the percent inhibition (□) of the steady-state O_2 yield and ratio of the second to third flash (○) of a flash sequence are plotted as a function of DCMU concentration Flashes were spaced 320 ms apart In (b) the $S'_1 \rightarrow S_2$ turnover in the presence and absence of 2 10^{-7} M DCMU, was measured by varying the time between the first and second flashes and detecting O_2 320 ms later on the third flash

also followed this double-hitting dependence No acceleration of the $S'_1 \rightarrow S_2$ turnover was observed at 2 10^{-7} M DCMU (Fig 3b) which inhibits 50 % of the Photosystem II centers Thus, the instantaneous double hitting and the accelerated turnover both require high DCMU concentration

An accelerated relaxation of $S'_1 \rightarrow S_2$ was observed in the absence of DCMU by giving a flash, exciting 25 % of the centers (as measured by steady-state O_2 yield), followed a variable time later by a second saturating flash O_2 was detected 320 ms later by a third, saturating flash For $S'_2 \rightarrow S_3$, a first saturating flash was followed 320 ms later by a flash exciting 25 % of the centers O_2 was detected by a third, saturating flash, given at variable times after the second The kinetics for saturating and non-saturating flashes are shown in Fig 4 for chloroplasts $S'_1 \rightarrow S_2$ and $S'_2 \rightarrow S_3$ following non-saturating flashes show half-recovery times of 250 μs and 210 μs, respectively These are compared to the appreciably longer recovery times of 500 μs and 450 μs following saturating flashes The recovery time for $S'_2 \rightarrow S_3$ was also measured for a non-saturating flash in the steady state An equipartition of S states was achieved through a series of eight flashes spaced 320 ms apart, each exciting 25 % of the centers This experiment also showed an accelerated recovery time of

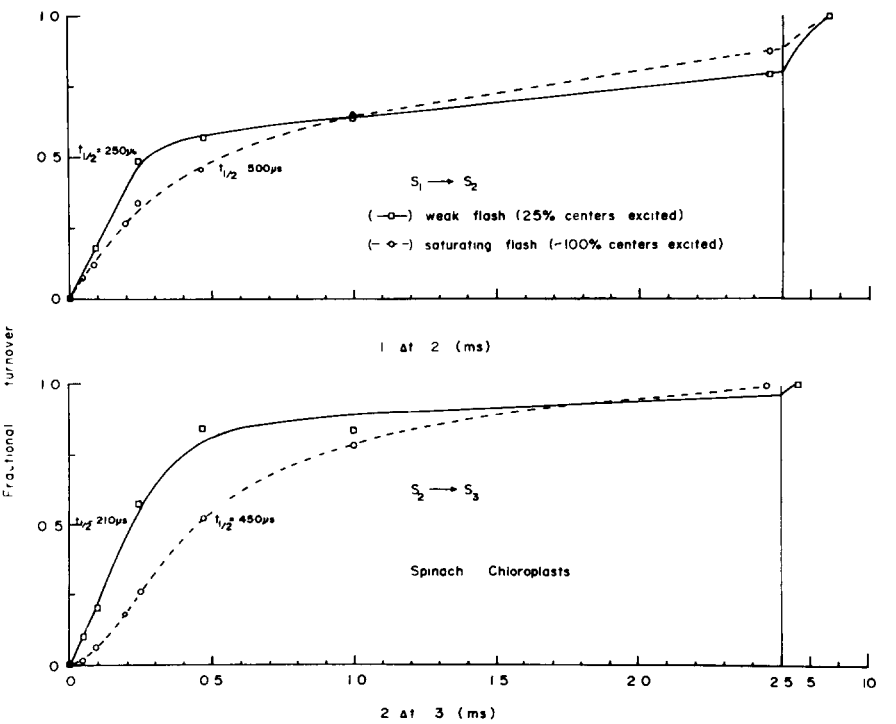

Fig 4 Turnover time as measured by O_2 detection, following a non-saturating or saturating flash Chloroplasts were dark adapted 8 min before flashing For $S'_1 \rightarrow S_2$ (a) the first flash of the sequence excited either 25 % or 100 % of the centers, followed a variable time later (abscissa) by a saturating flash O_2 was detected 320 ms later by a saturating flash For $S'_2 \rightarrow S_3$ (b) a first saturating flash was given, followed 320 ms later by a flash exciting either 25 % or 100 % of the centers O_2 was detected a variable time later (abscissa) by a saturating flash

300 μs Another non-chemical method exists as well for inducing the accelerated turnover Chloroplasts, left on the polarized O_2 electrode for 1 5 h undergo a loss in activity of about 50 % of the Photosystem II units Inactive units appear to be blocked in a non-quenching form in that a non-saturating flash excites a greater fraction of active centers (based on the steady-state O_2 yield) after the aging process than before Thus a blocked center can transfer its photon energy to an adjacent center, effectively increasing the optical cross-section of a functional center Under these conditions, an accelerated turnover $S'_1 \rightarrow S_2$ is observed ($t_{\frac{1}{2}} = 370\,\mu s$) following a saturating flash

Characteristic of both the non-saturating-flash and aged-chloroplast experiments is the fact that the accelerated turnover occurs for conditions where only a fraction of the centers undergo a light-induced charge separation

These results suggest that an interaction occurs between centers at the level of the electron transfer components responsible for the turnover time This interaction, however, does not exist in *Chlorella* as shown in Fig 5 and in which neither DCMU (inhibiting 90 % of the centers) nor flashes exciting 20 % of the centers induce an acceleration of the turnover time

Another phenomenon, also indicative of a cooperation between Photosystem II centers is the acceleration of deactivation in the presence of non-saturating concentrations of DCMU As demonstrated by Bennoun [18], DCMU induces a recombination of oxidizing and reducing equivalents generated in the primary photoact ($t_{\frac{1}{2}}$ -

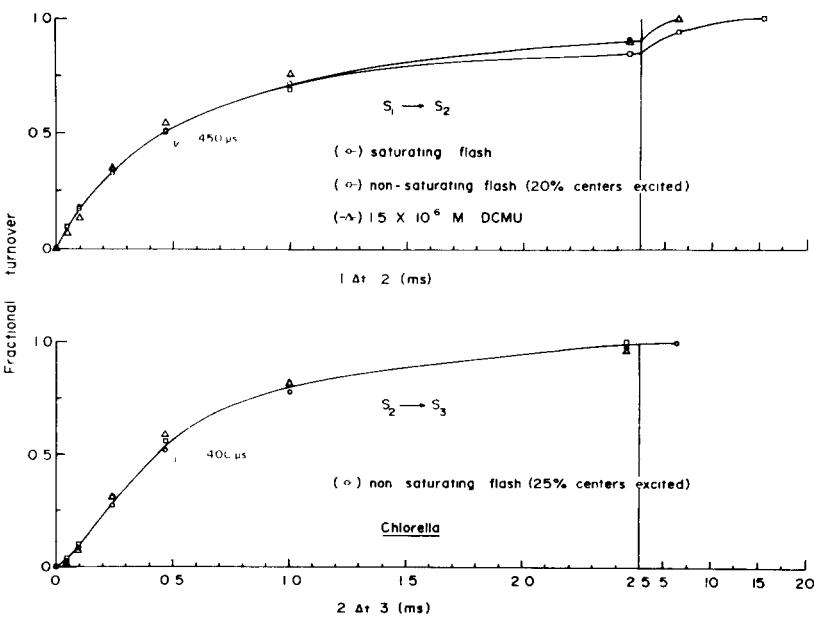

Fig 5 Turnover time, in *Chlorella*, as measured by O_2 detection following a non-saturating flash or a saturating flash in the presence and absence of 1 5 10^{-6} M DCMU *Chlorella* cells were dark adapted for 6 min The conditions for the non-saturating flash experiments were the same as for Fig 4 with the exception that for $S'_1 \rightarrow S_2$ the non-saturating flash excited 20 % instead of 25 % of the centers The conditions for the turnover measurement, following a saturating flash in the presence of DCMU, are as in Fig 2 except that the time interval between flashes other than the flash pair was 320 ms for all experiments

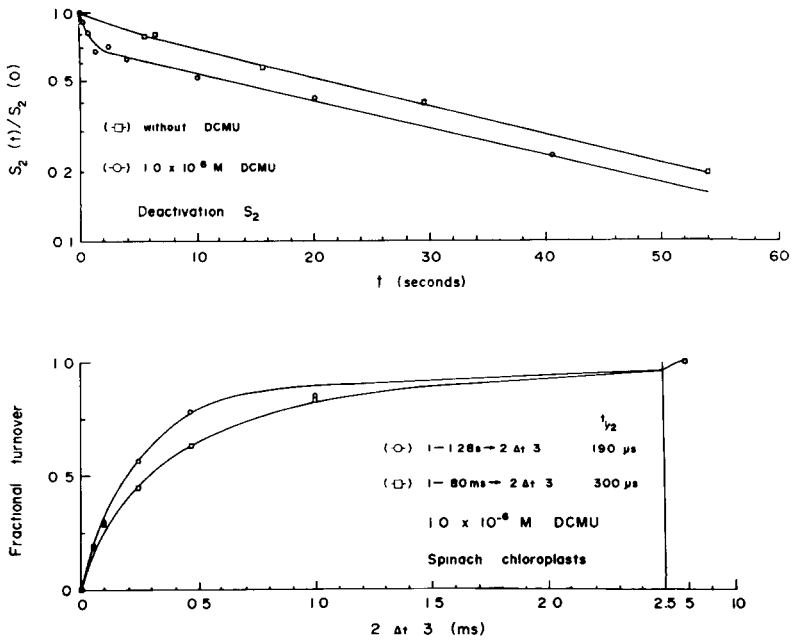

Fig 6 Deactivation of S_2 in the presence and absence of DCMU and turnover measured at two different deactivation times Chloroplasts were dark adapted 8 min in the absence of and 6 min in the presence of 1 0 10^{-6} M DCMU For deactivation of S_2 (a), a saturating flash was followed a variable time (t) later (abscissa) by a flash pair given 320 ms apart (—DCMU) or 80 ms apart (+DCMU) O_2 emitted following the third flash measures the amount of S_2 remaining at the end of the time interval between the first and second flashes The deactivation of S_2 is expressed as a ratio (ordinate) of S_2 remaining at time t to that at zero time The turnover $S'_2 \rightarrow S_3$ (b) was measured at either 80 ms or 1 28 s following a first flash by varying the time between the second and third flashes and detecting O_2 on the third

0 7 s in *Chlorella*, 2 s in Spinach chloroplasts) The source of the reducing equivalent is the primary acceptor Q

As shown in Fig 6, S_2 decays in a biphasic manner in 10^{-6} M DCMU The rapid phase of the deactivation occurs with a half-time of 0 75 s and accounts for 30 % of the decay The remainder shows a half-time of 35 s, about the same rate as in the absence of DCMU

This acceleration by DCMU is to be distinguished from the phenomenon which occurs in *Chlorella* [19] in which a DCMU molecule becomes bound to a Photosystem II center with a half-time of 12 s for a DCMU concentration of 10^{-6} M Because the DCMU fixation time is approximately the same in chloroplasts, (Bennoun, P , personal communication), the rapid 0 75-s decay must have occurred during a period in which there was essentially no turnover of DCMU Thus the rapid deactivation must have occurred by charge recombination and not by additional binding of DCMU to the centers Because an unblocked center should decay with the characteristic decay time shown in the absence of DCMU, an additional unstable reducing equivalent must be postulated to explain this acceleration The most likely candidate for this equivalent is the reduced acceptor of an adjacent blocked center, particularly

in that the time course for the rapid deactivation phase occurs at close to the same back-reaction rate observed by Bennoun [18] Thus both the turnover and deactivation reactions show cooperative properties in chloroplasts but not in *Chlorella*

Turnover measurements for $S'_2 \rightarrow S_3$, performed at the end of the rapid deactivation phase (1 3 s) show an accelerated turnover ($t_{\frac{1}{2}} = 190\ \mu s$), resembling that observed for $S'_1 \rightarrow S_2$ (Fig 2), following the first flash in dark-adapted, DCMU-treated chloroplasts Turnover measurements before appreciable deactivation (80 ms) show the much slower recovery kinetics characterized earlier ($> 300\ \mu s$) The recovery of the acceleration is thus coincident with the rapid phase of deactivation In addition, the fact that an accelerated turnover may be observed for centers in the S_2 state as well as for those in States S_0 and S_1 indicates that the acceleration is independent of the S state

DISCUSSION

The fact that the Photosystem II turnover time depends on the number of centers functioning during a light flash, indicates that an interaction occurs between centers That random excitation of 25 % of the centers shows a 2-fold acceleration of the turnover indicates that this interaction is a general phenomenon shown by most centers Because the number of functional centers determines the number of electrons transferred, it is most likely that the interaction occurs at the level of electron transfer The possibility that the acceleration stems from a more global phenomenon, such as the contribution of an electric field across the membrane, can be eliminated by the following arguments The 520-nm carotenoid band shift is linearly related to the strength of the electric field across the thylakoid membrane [25] Also at short times following a flash (< 1 ms) this band shift is a linear indicator of the number of centers that underwent a charge separation during a flash [12, 25] A measurement of the 520-nm absorbance change, 120 μs after a saturating flash given to dark-adapted chloroplasts, indicates a diminution of the field produced by only 15–20 % due to $1\ 75\ \ 10^{-6}$ M DCMU A diminution of smaller magnitude (3–7 %) is observed in the presence of 10 μM DCMU in *Chlorella* [17]

The most important acceleration of the turnover is observed following the first flash in the presence of DCMU Thus, for chloroplasts, DCMU has induced an important change in the turnover time with little effect on the electric field generated across the membrane Furthermore, the fact that neither a weak flash nor $1\ 5\ \ 10^{-6}$ M DCMU, in *Chlorella*, induces any change in the turnover time further supports the claim that the accelerated turnover and the electric-field effect are independent phenomena

Kok et al [4] have shown that the Photosystem II centers accumulate oxidizing equivalents independently, i e that no electron transfer occurs at the level of secondary donors Bouges-Bocquet [2] and Velthuys and Amesz [3] have shown that no electron exchange occurs at the level of the primary or secondary acceptors These criteria eliminate a large number of cooperative models, except for the following type, illustrated here for a pair of centers

In this type of model, the cooperativity occurs at the level of oxidation or reduction by the reaction-center chlorophyll

Aside from these primary reactions no electron exchange occurs between adjacent units In the oxidizing-side model, a reaction-center chlorophyll may oxidize its own donor Y, or that of an adjacent center with equal probability In the reducing-side model, both its own acceptor Q and that of an adjacent center are equally available for reduction Both of these models explain the observed accelerations of the non-saturating flash experiments in that only one of the two centers is excited A second flash a short time later finds the other center or Q untouched and available for charge separation In both cases, the need to await reoxidation of Q is obviated

There is now considerable evidence that reduction of Chl^+ by Y occurs in 35 μs [15, 26] This time is not observed in the turnover measurements performed here Von Gorkom and Donze [27] have proposed, on the basis of luminescence measurements of Zankel [15], that $Chl^+ Q^-$, generated in the presence of oxidized donor, Y^+, undergoes a charge recombination in 200 μs If the acceptor side is no longer rate limiting in the non-saturating flash experiments, then the donor side becomes rate limiting Because charge recombination occurs for as long as Y^+ exists, a second effective excitation of a Photosystem II center must await $Z \rightarrow Y$ electron transfer We propose then, that this is the rate-limiting step observed, following a non-saturating flash, and occurring with a half-time of 200 μs

If DCMU were to block one of the two centers shown in the oxidizing-side model then one would predict an unacceptable damping of the flash sequence for partially inhibiting concentrations of DCMU Siggel et al [20] have proposed that DCMU blocks two centers at a time If the connections proposed above occurred in a paired manner for most centers, and DCMU blocked both members simultaneously, then such damping would not be observed Arguments that such a paired connection probably exists will be presented in the next section

Action of DCMU

The behavior of DCMU in these experiments poses several problems The fact that higher concentrations of DCMU are required to obtain acceleration than are required for steady-state inhibition of O_2 production (Fig 3), might suggest that Photosystem II centers are inhomogeneous with respect to their turnover times It would have to be argued that DCMU preferentially blocks slow centers, leaving the fast ones to be observed in turnover experiments The strongest argument against this possibility is the observation of accelerated turnover following a non-saturating flash and for aged chloroplasts Thus, a rapidly recovering center would also have to have a large optical cross-section and be resistant to aging a highly unlikely combination It is more likely that the acceleration in the presence of DCMU arises from an interaction between homogeneous centers in a manner analogous to the non-saturating flash condition

The major distinction of the DCMU-induced effect is the acceleration primarily in the first flash, whereas an acceleration occurs for every non-saturating flash in untreated chloroplasts This observation indicated that in the presence of DCMU the cooperativity between centers is observed almost exclusively on the first flash By analogy with the non-saturating flash experiments, this would mean that an additional one-electron acceptor is available primarily for the first flash and not

afterwards However, as indicated by the deactivation experiment (Fig 6), the acceleration returns following a waiting period of sufficient length (1 3 s), to allow for its reoxidation This recovery is, however, long relative to the 160 ms between flashes, used for the turnover experiments of Fig 2 The fact that the acceleration disappears after one flash and then reappears following a one-electron deactivation, indicates that the additional acceptor is a one-electron species and that there is only one per functional center

Further support for the existence of a one-equivalent additional acceptor comes from flash experiments of Bouges-Bocquet [2, 21] in which were demonstrated an oscillation of period two, with pairs of electrons entering the plastoquinone pool on even-numbered flashes This observation, supported by Velthuys and Amesz [3], indicated that two electrons had to be accumulated on the reducing side of Photosystem II before being donated to the pool A consequence of an additional one-equivalent acceptor, at the high DCMU concentrations used here would be a 180 phase change for this oscillation Initially, three equivalents would be accumulated before the transfer to the pool occurred Thereafter, electrons would be transferred two by two as long as the additional acceptor remained reduced Bouges-Bocquet [21] has shown that in fact such an inversion occurs in going from 3 10^{-7} M to 10^{-6} M DCMU or in approximately the same concentration range as observed for the phenomenon of acceleration described here (Fig 3) At 10^{-6} M DCMU electrons are transferred from Photosystem II to the pool on odd-numbered flashes

There remains the problem of compatibility of an additional one-electron acceptor, in the presence of DCMU with the non-saturating-flash and aged-chloroplast experiments The latter experiments suggest that the additional acceptor is the primary acceptor of an adjacent center The existence of an acceleration primarily on the first flash of a sequence in DCMU and of the 180° phase change in the oscillation of Period 2 implies that the centers of Photosystem II are arranged in pairs If a greater number of centers were linked, then an accelerated recovery should occur after each of several flashes This model however poses the problem of explaining how a DCMU-fixed center can both undergo a charge separation, following a dark incubation period and have its primary acceptor available to the adjacent center For the reducing-side model, it is possible that these DCMU-fixed centers are the approx 30–40 % which, 120 μs after a saturating flash, show no 520-nm absorbance change in 1 75 10^{-6} M DCMU (15–20% of 520 nm signal, 40% of which is attributed to Photosystem II), and thus do no charge separation From fluorescence measurements at 20 μM DCMU, Joliot and Joliot [22] observed a similar fraction of centers in a non-quenching form While not excluding this possibility, it is more likely that the latter are centers in which Q is reduced by DCMU-induced transfer of an electron from B^- to Q [3] For such centers, Q would not be available as an additional acceptor

The models presented above may then be elaborated upon to explain the DCMU results The essential point of these models is that they organize Photosystem II centers in pairs and that the acceleration results from the transfer of the rate-limiting step from the acceptor to the donor side

The qualitative models presented below are only meant to be illustrative of the kind of model necessary to explain the DCMU results The data are not sufficient to establish a unique model A vertical bar means blocked electron transfer

In the oxidizing-side model, one of the two Q is blocked by DCMU as is one of the Y The latter is consistent with the action of DCMU on the donor side, as recently proposed by Etienne [23] While most centers must be blocked in pairs by DCMU to explain the absence of damping, a small fraction could be singly blocked and still be compatible with both our results and those of Kok et al [4] In the reducing-side model, one of the Q is blocked as well as a further action of DCMU directing the primary electron transfer of the DCMU-fixed center toward an auxiliary one-electron acceptor Q_{aux} Both of these configurations permit a separation of charge in a light flash for both Chl and thus are compatible with the carotenoid band shift data In addition Q_{aux} must be identical chemically to Q_a in order to satisfy the results of Witt [24] who observed an X-320 signal for dark-adapted chloroplasts in the presence and absence of DCMU To explain the high concentration of DCMU necessary to observe the acceleration, it might be admitted that this concentration is required for blockage on the donor side or the redirection of an electron to Q_{aux} The acceleration on the first flash arises from having both acceptors available in dark-adapted chloroplasts

In the oxidizing-side model one of the Chl^+ generated will receive an electron from Y, the other will undergo a rapid charge recombination regenerating Chl Q in about 200 μs Following the first flash ($>$ 1 ms) 50 % of the operative center pairs will have reduced Q_a These centers will not show an accelerated turnover on the second flash because Chl Q_a^- can only be reoxidized by a slow (0 75 s) back-reaction Centers in which Q_b was reduced on the first flash will recover rapidly on the second because the electron was transferred to B and thus two acceptors are again available for the second flash The turnover on the second flash is thus a mixture of fast and slow centers If after the first flash a 1 3-s waiting period is imposed, then Q_a has sufficient time to back-react ($t_{\frac{1}{2}} = 0$ 75 s, Fig 6), only fast centers remain and a rapid 190-μs turnover is observed for $S'_2 \rightarrow S_3$ (Fig 6) Because in a flash sequence of 320 ms or more rapid between flashes, there is insufficient time to reoxidize Q_a by a back-reaction, it is the first flash which shows the most rapid recovery For a rapid sequence, Q_a effectively stores an electron and the 180° phase change of the oscillation of Period 2 is explained

The oxidizing-side model readily explains the double hits observed in DCMU On the first flash, both centers form $Chl^+ Q^-$, one of which will react with Y to form Y^+ Chl Q^- The other will follow one of two possible routes (a) charge recombination to reform Chl Q in 200 μs [15, 27], or (b) electron transfer from Y to Chl^+ once Y^+ is reduced by Z (200 μs) Because these processes occur in comparable times, appreciable double hitting will occur on the first flash Exactly equivalent times for these two processes would lead to 50 % of the centers double hitting The other 50 % would show an accelerated turnover time, corresponding to the 200-μs $Z \rightarrow Y$ transfer The double hitting would be likely to show only a small temperature depen-

dence in that both $Z \rightarrow Y$ transfer and $Chl^+ Q^-$ recombination would be slowed as the temperature decreased

In the reducing-side model, double hitting would arise from overlap of the tail of the exciting flash with the 35-μs electron transfer time between Y and Chl^+ This overlap would permit formation of $Y^+ Chl^+ Q^-$ which would give rise to double hits as in the oxidizing-side model This double hitting would show a stronger temperature dependence than the oxidizing-side model in that the temperature independence of the flash duration results in a decreased overlap as the temperature is lowered

This paired configuration of Photosystem II is of consequence as well for energy transfer According to these models, the reaction-center chlorophylls are sufficiently close to transfer an electron to either of two Q or to accept an electron from either of two Y The reaction-center chlorophylls are thus necessarily sufficiently close to each other to permit energy transfer or even charge transfer

Finally, there remains the absence, in *Chlorella*, of all of the following accelerated turnover following a non-saturating flash, accelerated turnover following a saturating flash in DCMU and 0 75-s deactivation of S_2

It is unlikely that the pairwise organization proposed here exists in chloroplasts, but not in *Chlorella* More likely, a pair of reaction-center chlorophylls are closely associated in both cases, but the probability of cross transfer is considerably greater in chloroplasts

There are a number of differences already reported between the photosynthetic behavior of *Chlorella* and chloroplasts Aside from gross differences in ability to fix CO_2, there are also differences in the kinetics of Photosystem II charge recombination in the presence and absence of DCMU [18, 19], the blockage of charge separation on the first flash in DCMU and in the kinetics of low-temperature fluorescence induction [22] While these latter differences are relative ones the cooperativity in chloroplasts relative to *Chlorella* appears to be an all or none phenomenon However, it is possible that small differences in the distance or orientation between the reaction-center chlorophyll and the adjacent Q or Y could radically alter the ability to transfer or accept an electron to one or the other Thus, the difference observed here between *Chlorella* and spinach chloroplasts might also be a matter of degree

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