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FLUORESCENCE YIELD KINETICS IN THE MICROSECOND-RANGE IN *CHLORELLA PYRENOIDOSA* AND SPINACH CHLOROPLASTS IN THE PRESENCE OF HYDROXYLAMINE

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SUMMARY

The kinetics of fluorescence yield in *Chlorella pyrenoidosa* and spinach chloroplasts were studied in the time range of 0 5 μs to several hundreds of microseconds in the presence of hydroxylamine Fluorescence was excited with a just-saturat ng xenon flash with a halfwidth of 13 μs ($\lambda = 420$ nm) The fast rise of the fluorescence yield which was limited by the rate of light influx, was, in the presence of 10^{-3}–10^{-2} M hydroxylamine, replaced by a slow component which had a half risetime of 25 μs in essence independent of light intensity This slow fluorescence yield increase reflects a dark reaction on the watersplitting side of Photosystem II Simultaneous oxygen evolution measurements suggested that a fast fluorescence component is only present in organisms with intact O_2-evolving system, whereas a slow rise predominantly occurs in organisms with the watersplitting system irreversibly inhibited by hydroxylamine

The results can be explained by the following hypotheses (a) The primary donor of Photosystem II in its oxidized state, P^+, is a fluorescence quencher (b) Hydroxylamine prevents the secondary electron donor Z from reducing the oxidized reaction center pigment P^+ rapidly This inhibition is dependent on hydroxylamine concentration and is complete at a concentration of 10^{-2} M (c) A second donor (not transporting electrons from water) transfers electrons to P^+ with a half time of roughly 25 μs

INTRODUCTION

Reactions in Photosystem II of green plants are accompanied by changes in the yield of chlorophyll a_2 fluorescence Duysens and Sweers [1] concluded that the fluorescence was quenched when the primary acceptor of Photosystem II, Q, was in the oxidized state but not when in the reduced state, Q^-

Recently, a light-induced quenching of fluorescence, ascribed to a photo-

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

reaction $S \xrightarrow{h\nu} T$, in which the quencher T presumably is a carotenoid triplet, has been reported [2, 3] From fluorescence experiments at 77 °K, Okayama and Butler [4] concluded that the oxidized primary donor of Photosystem II, P^+ may be a fluorescence quencher Fluorescense yield measurements at 77 °K in the μs range made clear that the fluorescence yield rise following a saturating flash was complete after about 40 μs [5] From absorbance measurements, however, the reduction of P^+ at 77 °K was estimated to occur with a half time of 4 5 ms [6]

From the results mentioned above it could not be concluded with any certainty whether P^+ quenches fluorescence It would be very interesting though to know whether or not P^+ is a fluorescence quencher, because the fast fluorescence rise following a short flash, may monitor the reduction of P^+ formed during the flash P^+ will be accumulated in the reaction centers if the electron donation from the secondary donor Z to P^+ is inhibited Z is the donor related to the watersplitting system An inhibitor acting close to the watersplitting side of the reaction center of system II is hydroxylamine [7, 8]

In this paper we will present evidence supporting the hypothesis that P^+ is a quencher of chlorophyll a_2 fluorescence, that hydroxylamine prevents the reaction $ZP^+ \rightarrow Z^+P$, and that instead P^+ is reduced by another donor D with a half time of about 25 μs

MATERIALS AND METHODS

Algae (*C pyrenoidosa* Chick, strain Emerson 3) were grown as described previously [9], then centrifuged, suspended in fresh growth medium and brought to an extinction at 680 nm (corrected for scattering at 720 nm) of 0 1 in a 1 mm cuvette The algae, pretreated with hydroxylamine, were incubated in dark with 10^{-2} hydroxylamine for 10 min, then washed 4 times by centrifugation and suspended in fresh growth medium

Spinach chloroplasts were prepared from market spinach, suspended in Tricine buffer, (0 05 M *N*-tris) (hydroxymethyl) methylglycine, 0 01 M KCl, 0 002 M $MgCl_2$ and 0 4 M sucrose, pH 7 8 and diluted to an extinction of 0 1 in 1 mm For fluorescence measurements the suspensions were contained in a 1 mm perspex cuvette

Oxygen measurements were performed as described earlier [10] with suspensions compressed to a thin layer in a polarograph The conditions were held constant by a continuous flow of growth medium, enriched with 0 1 M KCl

For excitation a xenon flash was used Flash tube FT 230 (General Electric), $C = 40$ μF (or 18 μF), $V = 1500$ volts The flash was provided with the filter combination Calflex-C, Balzer K1, Schott BG 18/2, which transmitted a band with maximum at 420 nm In a flash series, the repetition rate was one flash per 2 56 s Fluorescence was measured at $\lambda = 681$ nm with the filter combination Schott AL 681 and Schott RG 8/2 The intensity of the flash was adjusted with neutral density filters Without additional neutral density filter the intensity is called 100 percent An intensity of 3 5 % (40 μF, 1500 volts) was sufficient to saturate oxygen evolution in the flash The photomultiplier was a S-20 type (EMI 9558 extended) Fluorescence yield plots were obtained as described earlier [2, 5] For measurements of the yield 200 μs after the flash (table 1) a weak measuring flash was used FT 230 (General Electric), $C = 0\,3$ μF, 1000 volts, $\lambda = 420$ nm, halfwidth of spectral band 3 nm

The intensity of the measuring flash was set to less than 1 percent of a just-saturating flash with neutral density filters

Hydroxylamine (pH adjusted to the same value as the pH of the growth medium or the Tricine buffer) was added to dark-adapted samples and incubated for 10 min (unless indicated otherwise) prior to the measurements All experiments were done at room temperature (18–22°C)

RESULTS

In the presence of 10^{-5} M DCMU, which slows down the reoxidation of Q^-, the fluorescence yield is high in continuous background light The high fluorescence yield is due to the presence of the state PQ^- under these conditions [1] The light-induced quenching in a light flash (Fig 1) is due to the formation of the quencher T [2] In the presence of hydroxylamine the kinetics are the same as in its absence (Fig 1), showing that hydroxylamine does not markedly affect the formation and/or decay of state T

In a 10 min dark-adapted sample without additions a fast fluorescence rise occurs, with a rise time limited by the light influx (Fig 2b) If hydroxylamine is present, a concentration-dependent inhibition of the fast fluorescence rise is seen At hydroxylamine concentrations of 10^{-2} M and 10^{-3} M the fast rise disappears completely, however, a slow component is left, due to a dark reaction (Figs 2b, 3a) Although low light intensities were used, some light-induced quencher T [2, 3] was formed, which causes a slight dip in the fluorescence yield especially at the maximum intensity of the excitation pulse ($t = 7$ μs) (Figs 2b, 3a) Measurements at $t = 40$ μs and $t = 80$ μs following a saturating flash, revealed that the fluorescence yield in the presence of 10^{-2} M hydroxylamine had risen to 70 % and to 80–90 % respectively of the yield in the control experiment From the slope of the fluorescence rise we estimate that the half time of the fluorescence rise is roughly 25 μs, if we assume an

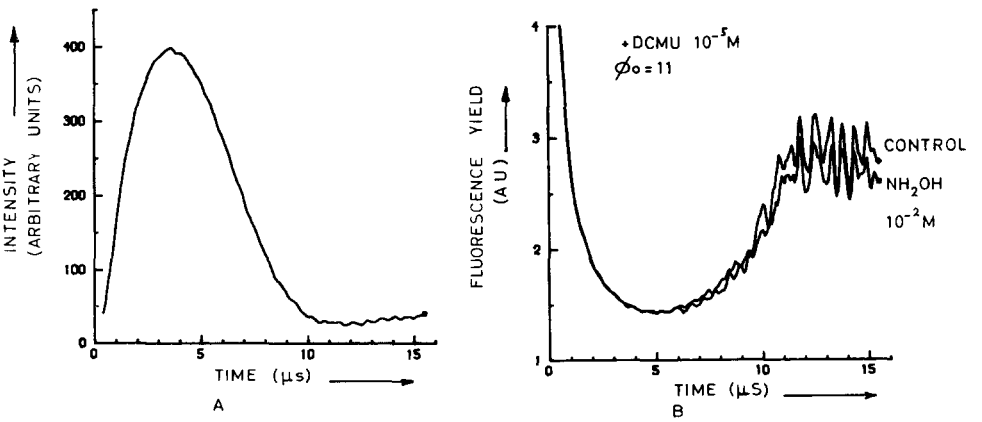

Fig 1 (a) Relative intensity $I(t)$ of exitation flash $C = 18$ μF, 1500 volts Halfwidth 5 6 μs $\lambda =$ 420 nm Supersaturating (20 times) A disturbance due to the ignition pulse of the flash tube made it impossible to measure the shape of the flash in the time range 0–0 5 μs (b) Fluorescence yield changes in *Chlorella pyrenoidosa* during the flash in (a) Conditions $+10^{-5}$ M DCMU, blue background light ($\lambda = 420$ nm) Same conditions for the second lower curve, except for extra addition of 10^{-2} M NH_2OH Incubation in the dark for 10 min

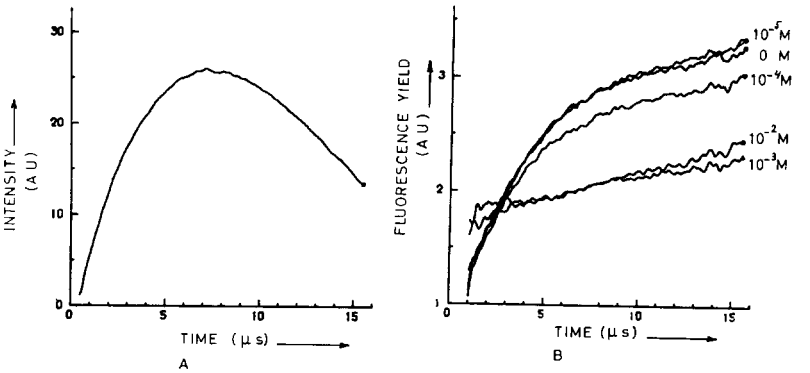

Fig 2 (a) As in Fig 1a except for $C = 40\,\mu F$, 1500 volts Halfwidth 13 μs This flash was used in all of the following experiments (b) Fluorescence yield during the seventh flash in a series after 10 min dark, with various concentrations of hydroxylamine (as indicated), in *Chlorella pyrenoidosa* in growth medium Flash intensity 7 % (see Methods)

exponential rise curve More accurate estimations can be made when DCMU, which prevents a fast fluorescence yield decay, is added to the sample (see below) Fig 3b shows the fluorescence yield during saturating flashes in a sample pretreated with 10^{-2} M hydroxylamine and washed afterwards It appears that the slow fluorescence rise does not depend on the presence of hydroxylamine, but that the behaviour with background illumination is different (Compare Figs 3a and 3b)

In the pretreated sample and in the sample with hydroxylamine a fluorescence yield decrease occurs, due to the light-induced quencher T In the sample with 10^{-2} M hydroxylamine a fluorescence increase is superimposed on this decrease (Fig 3a) In samples with 0 5 10^{-3} M hydroxylamine the fluorescence yield increase tends to become less pronounced after a large number of flashes (Fig 4a) or during continuous illumination This phenomenon was especially seen if the sample was preilluminated and kept in dark for some time following the preillumination (Fig 4b)

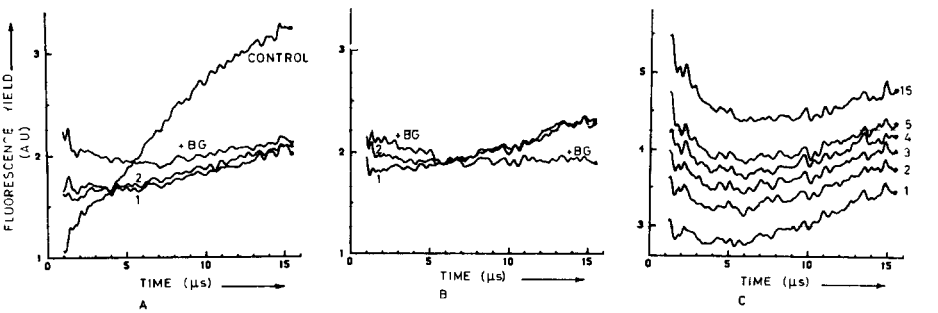

Fig 3 Fluorescence yield in *Chlorella pyrenoidosa* Flash intensity 3 5 % (a) In the presence of 10^{-2} M NH_2OH, lower curves during first two flashes after dark Upper curve BG during flash superimposed on continuous background illumination, $\lambda = 420$ nm, 1 3 mW/cm² (b) As under (a) but with algae pretreated with 10^{-2} M NH_2OH and washed afterwards (c) Algae pretreated with 10^{-2} M NH_2OH and washed in the presence of 10^{-5} M DCMU Flash numbers after dark as indicated

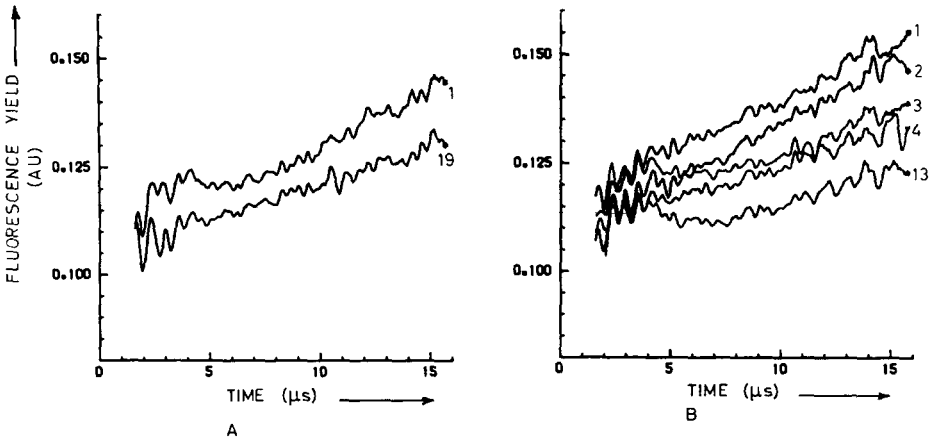

Fig 4 Fluorescence yield in *Chlorella pyrenoidosa* in the presence of 5 10^{-4} M NH_2OH Flash intensity 3 5 % (a) First flash and nineteenth flash after 30 min incubation in dark (b) After 15 min following the 9th flash in (a) Flash numbers as indicated

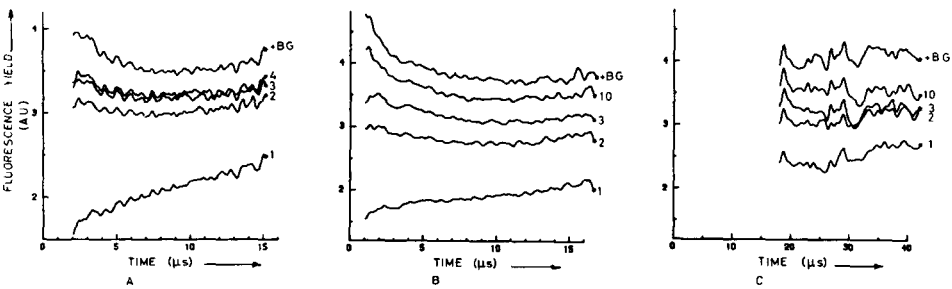

Fig 5 Fluorescence yield in *Chlorella pyrenoidosa* after 10 min dark incubation with 10^{-2} M NH_2OH and 10^{-5} M DCMU Flash numbers as indicated Upper curves during background illumination (a) Intensity 7 % (b) and (c) Intensity 1 9 % Batch of algae in (b) and (c) different from batch used in (a)

Dark-adapted *Chlorella* with hydroxylamine had a higher initial fluorescence yield than normal dark-adapted *Chlorella* This increase was larger at high hydroxylamine concentrations and was also present in pretreated algae (Figs 2b and 3)

Samples with 10^{-5} M DCMU

Samples with DCMU show a fast rate of fluorescence rise which is limited by the rate of light influx (Figs 6b, 7a) In spinach chloroplasts we see an additional slow increase between 25 and 75 μs (Fig 6c), which is most pronounced following the first flash This increase can be distinguished from the restoration of the quenching state T, because in this experiment the formation of state T is not very pronounced (see upper curves in Fig 6b, c, d, e) This slow component of fluorescence yield increase with a half time of the order of 45 μs seems to be due to a dark reaction Zankel [3] who measured the fluorescence kinetics in the region beyond 80 μs,

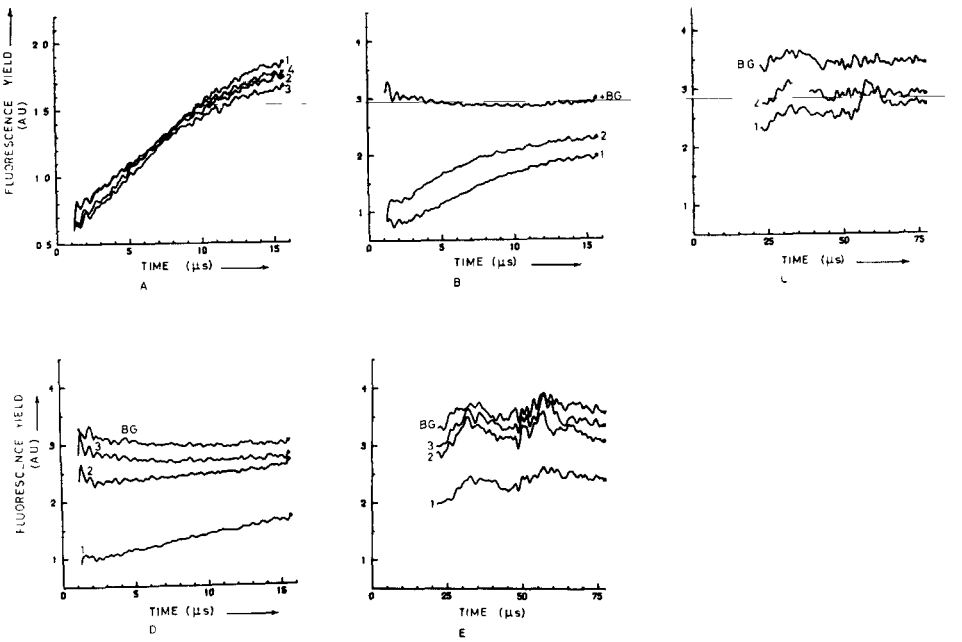

Fig 6 Fluorescence yield in spinach chloroplasts after 10 min dark Flash intensity 3 5 %, Flash numbers as indicated (a) Control (b) With 10^{-5} M DCMU Upper curve with background illumination (c) See (b) Time interval 25–80 μs (d) With 10^{-5} M DCMU and 10^{-2} M NH_2OH (e) See (d) Time interval 25–80 μs

observed a fluorescence increase between 80–100 μs which he attributed to a component with a life time of 35 μs

Samples with DCMU and hydroxylamine

Figs 5a, b, c, 7b, c, d, and 6d, e show the fluorescence yield rise in a flash series in *C pyrenoidosa* and spinach chloroplasts respectively in the presence of 10^{-5} M DCMU and several concentrations of hydroxylamine In these experiments the fluorescence increase is irreversible even in the time range of several seconds and more This confirms the results of Bennoun [11] At low hydroxylamine concentration (about 50 μM) however, the fluorescence yield increase becomes reversible again after the sample has been illuminated for several seconds (Fig 7d, Table 1) The longer the continuous illumination, the larger the reversible fraction of the fluorescence yield The fluorescence yield decay takes several seconds, comparable to the decay time in a sample with only DCMU added In samples with DCMU and a high concentration of hydroxylamine, e g, 10^{-3}–10^{-2} M, the fluorescence decay could only be restored slightly or not at all even after prolonged illumination

Because with DCMU the fluorescence yield increase is irreversible, following the first flash, it is easier to determine the rise time of the yield From Figs 5 and 6d, e we conclude that with 10^{-2} M hydroxylamine and 10^{-5} M DCMU the half time of the fluorescence rise in *Chlorella* and spinach chloroplasts is 25–30 μs and 18 μs

TABLE I

Fluorescence yield in flashes of various intensities of *Chlorella pyrenoidosa* in the presence of 10^{-5} M DCMU and of various concentrations of NH_2OH after 10 min incubation $E = 0.1$ in 1 mm Exciting flash FT 230, 40 μF, 1500 volts Total energy of detecting flash 0.15 % of 3.5 % exciting flash % $\Delta\phi$ and % ϕ is 100 % in background light

Extra addition	ϕ_0	ϕ 200 μs after one saturating flash	% I_ϕ after one saturating flash	ϕ during background illumination	ϕ after 10 s background light and 3 s dark	% decay of ϕ after 10 s background and 3 s dark	Flash intensity %
0 M NH_2OH	1.6	4.5	83	5.1	2.2	57	1.9
10^{-4} M	2.0	3.5	47	5.2	4.1	21	
$3\ 10^{-4}$ M	2.1	3.4	40	5.4	4.8	11	
10^{-3} M	2.1	3.6	45	5.4	5.0	7	
10^{-2} M	2.3	4.2	62	5.4	5.3	2	
0 M NH_2OH	1.6	5.0	100	5.0	2.2	56	3.5
10^{-4} M	2.0	3.8	56	5.2	4.2	19	
$3\ 10^{-4}$ M	2.1	3.9	54	5.4	4.8	11	
10^{-3} M	2.4	4.0	53	5.4	5.0	7	
10^{-2} M	2.1	4.5	72	5.4	5.0	7	
0 M NH_2OH	1.5	5.0	100	4.9	2.0	59	7
10^{-4} M	1.8	3.5	55	4.9	4.0	18	
$3\ 10^{-4}$ M	2.1	4.0	56	5.5	4.8	13	
10^{-3} M	2.4	4.3	59	5.6	5.4	4	
10^{-2} M	2.1	4.8	82	5.4	5.1	6	

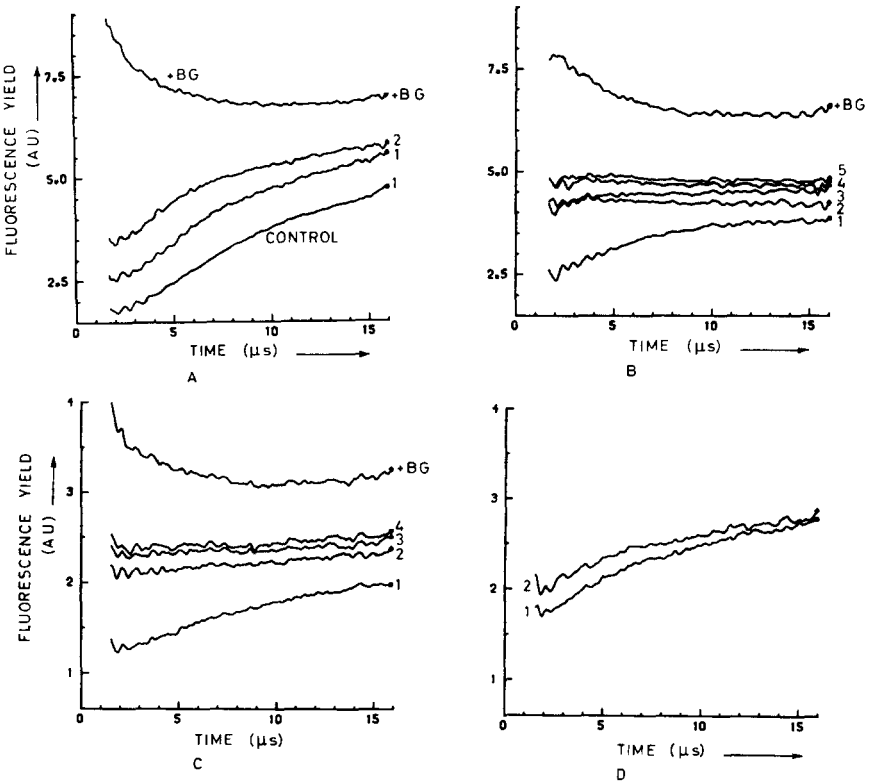

Fig 7 Fluorescence yield in *Chlorella pyrenoidosa* after 10 min dark Flash numbers after dark as indicated Flash intensity 3 5 % (a) Control (lower curve) and $+10^{-5}$ M DCMU Upper curve with background (b) $+10^{-5}$ M DCMU and 10^{-4} M NH_2OH (c) $+10^{-5}$ M DCMU and 50 μM NH_2OH (d) As under (c) Two consecutive flashes after 20 s continuous background illumination ($\lambda = 420$ nm) 1 3 mW cm^{-2} Batch of algae used for (c) and (d) was different from the batch used for (a) and(b)

respectively It should be noted that the results were very reproducible in *Chlorella*, in contradistinction to spinach chloroplasts in which the results varied with the batch

At lower hydroxylamine concentrations with 10^{-5} M DCMU a fast fluorescence rise occurs, just like in samples with low concentrations of hydroxylamine without DCMU In a flash series in the presence of DCMU only the first flash of the series is efficient in raising the fluorescence yield when a low concentration of hydroxylamine (50–100 μM) is present (Figs 7b and 7c)

With 10^{-2} M hydroxylamine the first flash causes a fluorescence yield increase at a low initial rate The level reached in the dark following a saturating flash is roughly 70 % of the maximum fluorescence level (Fig 5a, Table I) Also the flashes following the first one are relatively efficient (Fig 5a, b) Several flashes were needed to reach the final value of the fluorescence yield When samples pretreated with 10^{-2} M hydroxylamine and washed after incubation were used, the efficiency was lower and more flashes were necessary to reach the final fluorescence yield (Fig 3c) With continuous background illumination a final fluorescence yield, which proved to be rather independent of hydroxylamine concentration, was reached (see Table I).

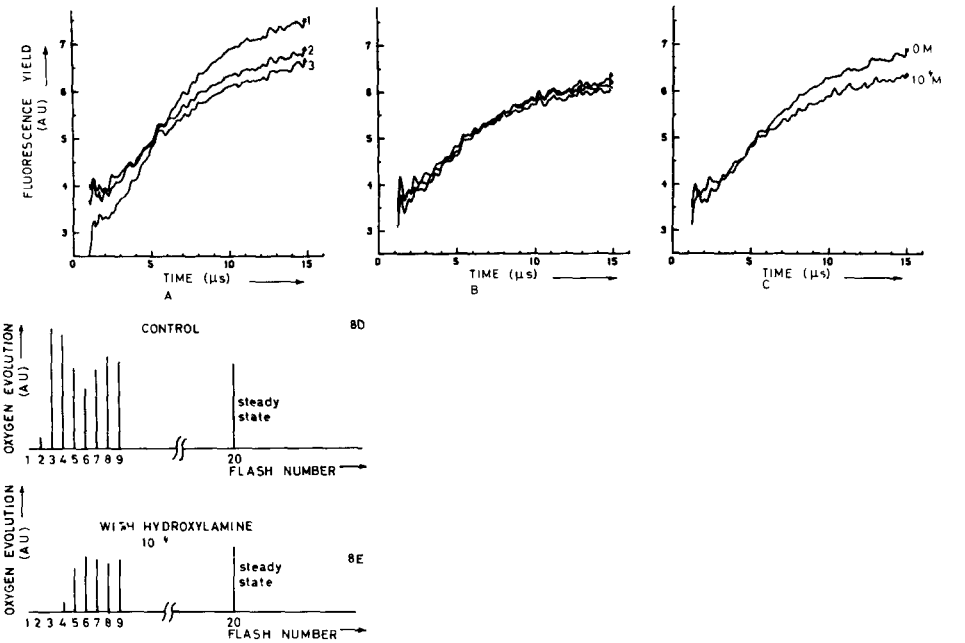

Fig 8 Fluorescence yield in *Chlorella pyrenoidosa* with simultaneous recording of oxygen evolution in the flash series Intensity 3 5 % (a) Control 3 flashes after 10 min dark Simultaneous oxygen evolution shown in (d) (b) With 100 μM NH_2OH, 3 flashes after dark Simultaneous oxygen evolution shown in (e) (c) As under (a) and (b) During the steady state (20th flash)

Simultaneous measurements of oxygen evolution and fluorescence yield increase

In Fig 8 results are plotted from measurements in a flash series of fluorescence yield increase during the flashes and of oxygen evolution due to these flashes We confirm [12, 13] the delay in oxygen evolution by two flashes in the presence of hydroxylamine We further notice that in the steady state in the presence of 10^{-4} M hydroxylamine an 18 % inhibition of the amplitude of the fast fluorescence yield increase is accompanied by a 25 % decrease in oxygen evolution, in addition a strong damping of the oscillations occurs in both the fluorescence yield and oxygen pulses Oscillations in the fluorescence yield as described by Delosme [14] are unobservable with hydroxylamine Algae treated with 10^{-4} M hydroxylamine and washed after incubation showed oscillations in the fluorescence yield again after they had been preilluminated by several flashes

DISCUSSION

At low concentrations (50–100 μM) the effects of hydroxylamine are complicated and difficult to interpret We will therefore first discuss the effects at high concentrations (10^{-2} M)

In the absence of hydroxylamine and when allowance for the effect of the quencher T is made, the increase in fluorescence yield is an increasing function of the number of absorbed light quanta only, in essence independent of the light intensity

of the flashes used In the presence of 10^{-2} M hydroxylamine, the rate of fluorescence yield increase caused by saturating flashes is essentially due to a dark reaction and thus not markedly influenced by the number of absorbed quanta The increase occurs with a half time of about 25 μs, the final level reached is only little (about 10–20 percent) below the maximum level obtained without hydroxylamine In the presence of 10^{-2} M hydroxylamine no oxygen evolution occurs The experiments can be explained by assuming that in the light, simultaneous with the reduction of Q^- a quencher is formed, which disappears with a half time of 25 μs The simplest hypothesis (earlier proposed by Okayama and Butler [4]) is that the oxidized primary donor P^+ is a quencher of chlorophyll a_2 fluorescence Hydroxylamine then prevents the reaction of P^+ with the secondary donor Z, which is involved in oxygen evolution, we further assume that P oxidizes another donor D in 25 μs, in which time half of P^+ and thus of the quenching, is removed The slow fluorescence component is also present if hydroxylamine is removed from the algae by washing (Fig 3b), so it seems unlikely that the oxidized form of hydroxylamine generated by illumination [15], quenches fluorescence [16] The rapid increase in fluorescence yield observed without hydroxylamine then implies that P^+ reacts in a few microseconds or less with the normal donor Z

The observation that even after a 30-times supersaturating flash, the level of fluorescence yield obtained with hydroxylamine is only 80–90 percent of the maximum level obtained without hydroxylamine suggests that part of Q^- reacts back with P^+ The half time of this reaction is then roughly 45–100 μs, if first order kinetics are assumed The kinetics after the first flash are similar if, in addition to 10^{-2} M hydroxylamine, 10 μM DCMU is added In accordance with the findings of Bennoun [11], the fluorescence yield does not markedly decrease after the flash Apparently D^+ just like Z^+ at low hydroxylamine concentrations does not reoxidize Q^-, but rather oxidizes hydroxylamine In the absence of hydroxylamine (pretreated sample), D^+ may reoxidize Q^- (see Fig 3c), just like the normal donor Z does in several seconds in the absence of hydroxylamine [11] *Chlorella* pretreated with 10^{-2} M hydroxylamine and washed, shows different fluorescence yield kinetics in a flash superimposed on continuous background light, than when 10^{-2} M hydroxylamine is present (Fig 3a, b) The increase due to the reaction $DP^+Q^- \rightarrow D^+PQ^-$ is more pronounced in the presence of hydroxylamine, which indicates that hydroxylamine lowers the concentration of D^+ in the steady state, which is understandable, since hydroxylamine is an electron donor to Photosystem II Also in accordance with this is the fact that samples pretreated with hydroxylamine and removed by washing, show a less efficient increase in fluorescence yield in a flash series in the presence of 10 μM DCMU (Fig 3c) compared to samples with 10 μM DCMU in which hydroxylamine is present (Fig 5) Apparently hydroxylamine prevents partially the back reaction $D^+PQ^- \rightarrow DPQ$, by reacting rapidly with D^+

Low concentrations of hydroxylamine

At low hydroxylamine concentrations (50–100 μM the inhibition of the reaction $ZP^+ \rightarrow Z^+P$ is only partial and oxygen can be evolved (Fig 8, [12, 13, 17]) In a flash series, both oxygen evolution and the fast fluorescence component are partly inhibited in the steady state (Fig 8) We conclude that, in the steady state, an 18 percent inhibition of the fast fluorescence component is accompanied by a 25 percent

decrease in oxygen evolution The 18 percent inhibition of the fast fluorescence component represents an even smaller percentage of inhibited reaction centers if we take energy transfer among units [18] into account Since the lowering of the oxygen pulses is much more than 18 percent, we have to assume that the large decrease in oxygen evolution is not only due to an inhibition of the reaction $ZP^+ \rightarrow Z^+P$ but also to an increased number of "misses" [19, 20] in the units with intact O_2 evolving systems This loss apparently occurs after the flash in the oxidized donor This may be due to an increased probability for loosing oxidized equivalents due to the presence of hydroxylamine

This is confirmed by the strong damping of the oscillation of oxygen pulses in the presence of hydroxylamine We agree with Bennoun and Bouges that the two equivalents produced by the first two flashes are lost completely, but the further delay in oxygen production and the lower steady state (Fig 8, also observed by Bennoun and Bouges [12, 13]) can be explained by the above stated hypothesis

In *Chlorella* with 10^{-5} M DCMU the fluorescence yield reaches a final value with continuous background illumination This value is also reached following a saturating flash applied to dark-adapted *Chlorella* with DCMU (Table I) In samples with hydroxylamine and DCMU the final yield with background illumination is slightly higher and the final yield cannot be reached with one saturating flash, given to a dark adapted sample (Table I), due to the back reaction $P^+Q^- \rightarrow PQ$ At low hydroxylamine concentrations and 10^{-5} M DCMU the fast fluorescence component is present (Fig 7b) though less pronounced than in samples with DCMU (Fig 7a) or hydroxylamine (Fig 2b) only The inhibition of the fast component in the presence of both hydroxylamine and DCMU seems to be stronger than with only hydroxylamine present, which indicates that the actions of DCMU and hydroxylamine are not independent

Chlorellae with high hydroxylamine concentration and 10^{-5} M DCMU reach their final fluorescence yield after a few flashes (Fig 5) in contradistinction to samples with low concentrations of hydroxylamine, in which only the first flash causes a considerable increase of fluorescence yield (Fig 7b, c) This may be due to (a) back reaction $P^+Q^- \rightarrow PQ$, due to a poor electron donation to P^+ in the flashes following the first one, and/or (b) back reaction $D^+PQ^- \rightarrow DPQ$ With continuous background illumination the final fluorescence yield is independent of hydroxylamine concentration (Table I)

In *Chlorella* with 5 10^{-4} M hydroxylamine the fluorescence yield increase tends to decrease after a large number of flashes (Fig 4) which presumably is due to the oxidation of D If such a sample is illuminated, kept in the dark for some time and next illuminated by a flash series its behaviour is very strange The first two flashes in the series cause a pronounced fluorescence yield increase which becomes much smaller after consecutive flashes and finally almost completely disappears (Fig 4b) This suggests that only two electrons can be donated to P^+ and that many seconds are required to replace these electrons

Chlorella with low hydroxylamine concentration (50–100 μM) and 10^{-5} M DCMU have a fast fluorescence yield increase during a saturating flash The increase is irreversible (Fig 7b, c), which confirms the results by Bennoun [11] If only 10^{-5}M DCMU is present, a back reaction of Q^-, presumably with the oxidized secondary donor Z^+ [11], occurs in the dark following the flash (Figs 6b, 7a) and the reaction

centers then stay in the state ZPQ^- We find that after continuous illumination for several seconds, the fluorescence yield is not irreversible anymore (Fig 7d) and behaves similarly to *Chlorella* with only DCMU (Fig 7a, Table 1) Apparently the reaction centers are accumulated in the state Z^+PQ^- during continuous illumination, and recombination occurs in the dark after illumination System II reaction centers are presumably not completely inactive with 10^{-5} M DCMU Turnovers may occur during prolonged illumination and bound hydroxylamine can be oxidized and released from the centers [12, 13, 21] This is in accordance with the fact that, in algae treated with 10^{-4} M hydroxylamine and washed after incubation, following preillumination with some flashes, not only the oscillations in oxygen evolution are restored [12, 13] but also the oscillations in the fluorescence yield

Dark-adapted *Chlorella* incubated with hydroxylamine, especially at high concentration, always showed an increased initial fluorescence yield Φ_0, compared to a sample without hydroxylamine This increased yield could not be restored by far-red illumination (730 nm) and thus, presumably, is not due to a reduction of the primary acceptor, but rather to an effect on the watersplitting side of system II or in the trap itself The flash-induced quenching due to T [2] occurs in the increased initial fluorescence level but not in the low Φ_0 level, without hydroxylamine The initial fluorescence yield increased progressively during consecutive periods of illumination and darkness if hydroxylamine was present This may reflect the light-induced destruction of Photosystem II by hydroxylamine as reported by Cheniae and Martin (RIAS report 1969, pp 38–40)

Relation of fluorescence yield kinetics to luminescence decay

The results described in the foregoing suggest that, in the presence of hydroxylamine, the oxidized reaction center pigment P^+ has a longer life time (25 μs) than under normal conditions (several microseconds or less), when P^+ reacts rapidly with the secondary donor Z Hence, in the presence of hydroxylamine, charge recombinations in the primary steps of photosynthesis may be favoured during the presence of P^+ and, according to the recombination hypothesis of luminescence [22], an increased luminescence intensity is expected On basis of the estimated half times for the processes $DP^+ \rightarrow D^+P$ and $P^+Q^- \rightarrow PQ$ in the presence of hydroxylamine, the luminescence components should have half times of 25 μs and about 100 μs, respectively, in *Chlorella*

Zankel reported a luminescence component, which was stimulated by hydroxylamine with a half time of 35 μs in spinach chloroplasts [23] Lavorel measured luminescence components with life times of 50–70 μs and larger than 100 μs respectively in *Chlorella* These components were both stimulated by hydroxylamine [24] A component with half time 5–10 μs decreased upon addition of hydroxylamine, which has been ascribed to an inhibition of the reaction $ZP^+ \rightarrow Z^+P$ [24] These results are qualitatively in agreement with the interpretation of the results of our fluorescence experiments

The probability for the reaction $ZP^+ \rightarrow Z^+P$ to occur may very well depend on the number of positive charges accumulated on the water splitting side of Photosystem II [25] A large number of positive charges may lower the probability for the reaction $ZP^+ \rightarrow Z^+P$ to occur, which in turn may favour the reaction $DP^+ \rightarrow D^+P$ This could explain the periodicity of the 35 μs luminescence component (largest

value after the third flash) in phase with oxygen evolution (largest value after the third flash), as reported by Zankel [23]

N B After this paper was written, a paper by Etienne [26] came to our attention In a footnote the author concluded that some of her experiments on fluorescence kinetics in the time range 0–100 ms could be explained by the assumption that P^+ is a fluorescence quencher

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